PROCEEDINGS

JUSTICE FOR ALL

AAFS 2024

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AMERICAN ACADEMY OF
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of the American Academy of Forensic Sciences 76th Annual Scientific Conference

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PROCEEDINGS

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S1 Oh, the Places You'll Go! Exploring the Pathos to Justice for All

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Program Description: This full-day session will present the opportunity for attendees to learn about the many career paths one can take in the forensic science community and how each play a unique role in promoting justice for all and advancing the field.

Educational Objectives: The Young Forensic Scientists Forum (YFSF) offers students and young professionals the opportunity to interact with professionals in the forensic science community as they begin their careers. This session presents attendees with the chance to learn about the multitude of careers available in forensic science as well as develop professional skills that are essential for the job-hunting process. Our mission is to provide the education, mentorship, and resources necessary to champion students and young forensic scientists to promote interest, membership, and participation with AAFS and the forensic science community for years to come.

Impact on the Forensic Science Community: After attending this session, attendees will have a better understanding of the diversity of roles and career paths in the forensic science community and how each play an integral part in furthering the field on a national and international level. Additionally, attendees will have the opportunity to sharpen their professional skills in resume writing and making impactful introductions.

The overall theme of the 2024 AAFS Conference is *Justice for All.* As AAFS President C. Ken Williams has stated, justice for all is at the core of the AAFS Vision Statement, and this principle reminds us why we all must strive to perform at our best in this field. For this year's YFSF Special Session, the YFSF committee has chosen the theme *Oh, the Places You'll Go! Exploring the Paths to Justice for All* to showcase the various career paths young forensic scientists can choose in order to promote justice for all. A multidisciplinary approach only works to advance the field to achieve this mission.

The speakers for this year's session have a vast range of knowledge and experiences that will shed light on the innumerable ways forensic scientists work and collaborate to bring justice for all. Specifically, our presenters will showcase various disciplines in forensic science, speak about performing research in the field, offer professional development advice as well as offer international perspectives and practices. The YFSF offers this session in an effort to integrate young professionals and students into the field through presentations by professionals with significant experience in the field. Further, the session this year will offer a resume review workshop, so do not forget to bring your updated resume to the session! Also, new to the session this year, we will offer an end-of-the-day mixer for attendees to have an opportunity to meet and speak with the session's presenters as well as other distinguished professionals representing a multitude of forensic science disciplines and expertise. We are once again excited to announce that YFSF has collaborated with The Center for Forensic Science Research and Education to honor and award two outstanding scientists for their contributions to the forensic science community.

YFSF; Global; Student

S2   Interdisciplinary Symposium: With Liberty and Justice for All: The Role of Forensic Science in Truth and Fairness

Claude Roux, PhD, University of Technology Sydney, Ultimo, New South Wales, Australia; Patrick Buzzini, PhD, Sam Houston State University, Huntsville, TX; Jessica Volz, DNP, Adventist HealthCare Shady Grove Medical Center, Rockville, MD; Michelle D. Miranda, PhD, The Center for Criminal Justice Studies-Farmingdale State College (SUNY), Farmingdale, NY; Antonel Olckers, PhD, African Forensic Sciences Academy, Pretoria, Gauteng, South Africa; Bernice B. Donald, JD, Resolute Systems LLC, Eads, TN; Keith Inman, MCrim, California State East Bay, Hayward, CA; Adam Freeman, DDS, Westport, CT; Iain Pretty, BDS, PhD, Manchester Science Park, Manchester, England, United Kingdom; Sarah P. Chu, PhD, Director of Policy and Reform, Perlmutter Center for Legal Justice at Cardozo Law, New York, NY

Program Description: This half-day Interdisciplinary Symposium will discuss the influence of forensic science on the concept of *Justice for All*. Presentations on forensic science's foundational principles, ethics, and evolving definitions will be explored through an in-depth discussion of the Sydney Declaration. The importance of scientific rigor and the role of transparent utilization of knowledge grounded in proper forensic science principles in ensuring the preservation of fair and just judicial and humanitarian outcomes will be highlighted by presenters discussing forensic science policy and context-specific challenges impacting innocence work and wrongful convictions. The last half of the symposium will be focused on understanding similarities and differences in the past and current real-world application and role of forensic sciences to the justice system in the United States, the United Kingdom, and South Africa/Africa. The aspiration of working toward a world where there is *Justice for All* is in the hearts of many forensic scientists. This presentation will discuss the application of forensic science to the basic human right of access to justice and open discussion about the future of the intersection of societal needs, science, and law.

Educational Objectives: Upon completion of this workshop, attendees will: (1) articulate the basic principles outlined in the Sydney Declaration and describe its significance; (2) discuss reasons why scientific rigor and transparent application of science grounded in proper forensic science principles is criticle to ensuring justice; and (3) explain some of the similarities and differences in the application of forensic sciences to justice in the United States, United Kingdom, Australia, and South Africa/Africa.

Impact on the Forensic Science Community: This symposium will impact the forensic science community by providing novice and expert forensic scientists the opportunity to gather, learn, and discuss the role and significance of their practices on the concept of *Justice for All*. Sharing and highlighting knowledge on the foundational principles outlined in the Sydney Declaration, the acknowledgment of forensic science's role in seeking truth, and examination of forensic science application in areas across the globe will lay the foundational knowledge to inspire conversation about the history, current state, and future of forensic science's role in justice in the world. This presentation will underline why it is a critical duty and ethical obligation of forensic scientists to understand the intersection of the principles, limitations, and rigor of science in supporting justice for victims of crime and the accused.

The concept of truth is fundamentally tied to the concept of justice, "the ethical, philosophical idea that people are to be treated impartially, fairly, properly, and reasonably by the law . . .[1,2] Regardless of their discipline, forensic scientists play a critical role in the integrity of criminal justice systems across the world. Historical examples (e.g., the Salem Witch Trials) of accused persons receiving baseless or unjust legal outcomes that result in imprisonment and death are limitless.[3-5] Likewise, the number of victims who have been unbelieved, blamed, and have been ignored or denied justice is countless.[6] Rumors, unchecked political authority, flawed forensic science, and hysteria are some examples of historical reasons for miscarriages of justice.[3,4,7] For millennia, mankind has faced challenges with understanding forensic sciences' role in safeguarding justice regardless of culture, race, age, and form of the justice system.

Today, some of these underlying threats to justice continue to exist. Organizations focused on wrongful convictions and exonerations, including the Innocence Project, cite eyewitness misidentification, misapplication of forensic science, false confessions, official misconduct, and coerced pleas as threats to the accused.[8] Threats to a victim's ability to seek and obtain just outcomes in the world often include disbelief, policies that restrict access to help, and criminal justice systems that are not designed to account for the nuances of interpersonal crimes, with Gender-Based Violence and Femicide (GBVF) as one example of non-optimal systems globally.[9,10] Forensic scientists should understand how science applies to understanding truth and ensuring *Justice for All*.

This Interdisciplinary Symposium will explore the challenges, limitations, advances, and successes of forensic science in supporting justice today. The foundational principles of forensic science will be discussed by experts on the Sydney Declaration and describe why every forensic practitioner and researcher must be familiar with how its principles impact their work.[11] The topic of unjust outcomes and wrongful convictions in historical and modern times will be focused on by discussing innocence work and forensic science policy. This discussion will also explore the impact delays in justice and wrongful convictions have on victims who already face many challenges in receiving justice.

The final section of the symposium will consist of a panel of forensic experts from four countries and an expert in ethics. This interview-style panel will provide a forum for these experts to discuss the similarities and differences of forensic science application to justice systems across the world. The future of forensic science's role in ensuring impartial, fair, and proper application of justice will round out the discussion, followed by time for questions from the audience.

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Justice; Ethics; Forensic Science

CB1   The Boundaries of Judicial Discretion in Admitting Forensic Scientific and Technical Evidence

Stephanie Domitrovich, JD, PhD*, Administrative Office of PA Courts, Erie, PA; Milton Nuzum III, JD*, Vincent, OH

Program Description: In this presentation, Judge Stephanie Domitrovich and former Judge Milt Nuzum will facilitate an interactive dialog with the session attendees as they examine a series of cases and scenarios that test the limits of a trial judge's judicial discretion in admitting scientific and technical forensic evidence. Attendees will analyze a series of scenarios based on actual cases and place themselves in the role of a trial judge in making decisions on admissibility of the evidence in each scenario.

Educational Objectives: The goals of this presentation are: (1) to examine a series of cases and scenarios that test the limits of a trial judge's judicial discretion in admitting scientific and technical forensic evidence; and (2) to help attendees understand the Rules of Evidence and relevant case law that establish the guidelines a trial judge must follow in making critical decisions on the admissibility of scientific and technical forensic evidence.

Impact Statement: Attendees will analyze a series of scenarios based on actual cases and place themselves in the role of a trial judge in making decisions on the admissibility of the evidence in each scenario. This dynamic presentation will impact the forensic science community by enabling prosecutors, defense attorneys, judges, and expert witnesses to be prepared to successfully face the scrutiny of the court when seeking admissibility of scientific and technical forensic evidence in cases where the outcome likely hinges on the testimony of an expert witness.

The presenters will review the Rules of Evidence and relevant case law that establishes the guidelines a trial judge must follow in making critical decisions on the admissibility of scientific and technical evidence. Attendees will analyze a series of scenarios based on actual cases and place themselves in the role of the judge in making decisions on admissibility of the evidence in each scenario. This dynamic presentation will enable prosecutors, defense attorneys, civil litigators, and expert witnesses to be better prepared to successfully face the scrutiny of the court when seeking admissibility of forensic scientific and technical evidence in cases where the outcome likely hinges on the reliability of the testimony of an expert witness. Trial judges have wide discretion in *Daubert* jurisdictions versus how trial judges admit scientific and technical evidence in *Frye* jurisdictions. The differences between the admissibility of scientific and technical evidence in both types of jurisdictions will be discussed. Trial judges are considered to have more active roles as gatekeepers of expert testimony rather than trial judges in *Frye* jurisdictions. Under Federal Rule of Evidence 702, the trial judge must determine that the expert's opinion, as well as the basis and methods used by the expert to reach that opinion, are both relevant and reliable.[1] Federal Rule of Evidence 702 was recently amended to clarify the application of the preponderance of the evidence standard for admissibility of evidence as required by Federal Rule of Evidence 104(a). Moreover, the new language in Federal Rule of Evidence 702 clarifies the trial judge's role in determining the conclusions reached by the expert and the methodology applied to reach those conclusions. Previously, a significant number of courts had taken a liberal view of the admissibility standard and determined expert testimony was presumed to be admissible and hence improperly had the jury make these determinations. Indeed, the trial judge's gatekeeping role in *Daubert* jurisdictions means the trial judge must more closely scrutinize an expert's proffered testimony before said expert's testimony is presented to the jury. Court-appointed experts under Federal Rule of Evidence 706 can be very helpful to the court for their gatekeeping role.

Reference:

[1]   Federal of Evidence 702, Federal Rule of Evidence 706, Federal Rule of Evidence 104(a), *Daubert and Frye* cases.

Discretion; Judicial; Boundaries

CB2 Xylazine—An Emerging Cause of Death in Correctional Institutions

Marie E. Lasater, MD*, Texas County Coroner's Office, Licking, MO

Program Description: The unregulated veterinary drug xylazine is emerging as a cause of death in state correctional facilities. Unlike the drug fentanyl, there is no antidote. The origins and toxicity of the drug are discussed and illustrated with a case study of an offender death due to xylazine mixed with fentanyl.

Educational Objectives: The goals of this presentation are to: (1) identify physical symptoms of xylazine use, (2) identify treatment for xylazine overdose, and (3) obtain proper toxicology samples in case of fatal overdose.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the importance of recognizing signs/symptoms of xylazine use cannot be overstated. As an emerging drug, xylazine is not always included in basic forensic panels, so expanded testing is recommended. Xylazine has no approved antidote for humans. It is NOT a federally controlled substance, so has the propensity to be easily obtained.

Xylazine, known on the street as "tranq," is making its mark on a rural Missouri county, showing up in the local maximum security prison resulting in two offender deaths within six months. Unlike fentanyl, which is identified and treated emergently with narcan, xylazine has no approved antidote for humans. It is NOT a federally controlled substance.

Xylazine is an analog of the common drug clonidine, used to treat hypertension, but is only authorized for veterinary use due to its potentially lethal side effects that include bradycardia and profound hypotension. It is an α2-adrenergic agonist that acts via stimulation of central α2- receptors. Xylazine was discovered in 1962 by Farbenfabriken Bayer after Bayer and Farben reconsolidated in 1951 after the earlier IG Farber was dissolved by the allies following World War II. Early clinical studies confirmed its effects on the central nervous system, and today it is only approved in the United States for use as a sedative, analgesic, and muscle relaxer in dogs, cats, horses, and various deer species.

Human use of xylazine first became apparent in Puerto Rico in the year 2000. Philadelphia has been extremely hard hit by the drug, with the first detection in that city in 2006. The number of fatal overdoses in Philly involving xylazine have increased every year since its introduction. Despite being a medication approved only for use in certain animals, xylazine is relatively easy to purchase and is available online on several veterinary medicine sites (current price: $26.95 for 100mg), requiring a prescription from a veterinarian. In most cases, xylazine is mixed with another drug, frequently fentanyl. While narcan reverses the effects of fentanyl, death can still occur with fatal overdose cases seen with xylazine levels from trace to 16ml/L. There is no "safe" dose of xylazine in humans.

Effects are usually seen within 15 minutes after administration, with sedative effect lasting 1–4 hours. The drug diffuses readily throughout the body, penetrating the blood-brain barrier; 70% of the dose is eliminated in the urine, making it useful in detecting xylazine intoxication. Due to rapid metabolism, xylazine decreases fairly rapidly to undetectable levels, so procurement of toxicological specimens as soon as possible is recommended. Although there is no approved antidote to xylazine in humans, the drug has been effectively counteracted in veterinary practice. The natural herb yohimbine, a α2 adrenergic antagonist, has been shown to reverse the antihypertensive effects of xylazine in dogs and elephants.

The drug atipamezole is another α2-antagonist used to reverse the effects of xylazine, and while tested in humans in Phase I trials, it is not an approved medical treatment for xylazine overdose. As xylazine is commonly injected and mixed with other drugs, particularly fentanyl, the symptoms of pinpoint pupils, physical deterioration, dependence, and track marks will be evident. In addition, chronic xylazine users develop serious non-healing infected skin ulcers due to skin oxygen deficit following hypotension, bradycardia, and respiratory depression secondary to use of the drug. The ulcers may ooze pus and have a characteristic odor. In severe cases, amputations must be performed on the affected extremities.

As an emerging drug contributing to fatalities in correctional institutions with no antidote approved for human use, personnel in correctional medical facilities should be aware of the presence of xylazine. Markers of xylazine use include very distinct odorous pustular lesions that can be found anywhere on the body due to the ability of the drug to rob the skin of oxygen. Currently, there is no field test for xylazine, but it is readily detected in blood or urine in expanded testing completed by forensic laboratories.[1-9]

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Xylazine; In-Custody Death; Overdose

CB3   Vacuous Accreditation: The Illusion of Quality in Forensic Science

Tiffany A. Roy, MFSF*, ForensicAid, LLC, West Palm Beach, FL; Kimberly S. Kunkler, PhD, Marshall University, Huntington, WV

Program Description: This presentation will call attention to gaps in the quality oversight structure by questioning the current perceptions of forensic laboratory accreditation, highlighting its strengths and limitations, providing specific examples of where failures have occurred, and discussing potential opportunities for improvement.

Educational Objectives: This presentation is intended to highlight common misconceptions regarding accreditation and quality assurance in United States forensic laboratories. Accreditation is often misdescribed by practitioners and misunderstood by judges and juries. As a result of participation in this presentation, attendees will gain a clearer understanding of the role of forensic laboratory accreditation in the United States, including its strengths and limitations.

Impact Statement: This presentation will impact the forensic science community by fostering discussion about what meaningful and effective forensic science oversight would look like in the United States and why current accreditation frameworks are insufficient to ensure production of accurate and reliable forensic results. This presentation will draw the attention of justice partners to the need for statutory oversight of forensic science on the state and federal level in the image of the Texas Commission on Forensic Science.

Following nearly 15 years of calls for standardization and meaningful oversight in the forensic sciences by numerous independent bodies, significant efforts (including the investment of millions of dollars and thousands of volunteer hours) have been dedicated to the development of consensus standards to aid forensic science service providers on the path toward strengthening forensics in the United States. However, the United States still does not have a regulating body or other mechanism for meaningful oversight in place at the national level, or in most states. Forensic laboratory accreditation is often perceived by the community and stakeholders as a means of oversight through its role in ensuring that service providers are properly adopting and implementing the new and improved standards, and that service providers are producing accurate and reliable results. Unfortunately, such perceptions of forensic laboratory accreditation often far exceed its realistic abilities, making accreditation a poor substitute for oversight by a regulating body.

It is true that forensic laboratory accreditation can be effective at checking that a management system addressing concepts related to quality and reliability is in place, as well as checking that the laboratory is following the rules it sets for itself within its management system. However, forensic laboratory accreditation is currently not effective at ensuring that best practices are being utilized or that the reported results are complete and technically accurate. Furthermore, accrediting bodies often lack transparency in their operations, do not respond to stakeholder concerns or formal complaints in a timely manner, and actively perpetuate false claims of their function that are misleading to customers, stakeholders, and the public.

Accreditation; Accuracy; Quality Assurance

CB4 2023 State Legislative Encroachments Against Forensic Pathology Professionalism and Independence: Harbingers of Future Threats and the Opportunity for Strategic Planning and Action

M.J. Menendez, JD*, Willow Grove, PA; Joyce L. deJong, DO, Department of Pathology, Western Michigan University, Homer Stryker M.D. School of Medicine, Kalamazoo, MI

Program Description: In 2023, the independence of forensic pathology in the context of Medicolegal Death Investigations (MDIs) was directly attacked by the introduction of various state legislative proposals and bills passed into law. These legislative proposals and laws erode the ability of forensic pathologists to maintain impartiality and objectivity, mandate particular findings on death certificates, and allow non-physicians to perform autopsies. The cause for alarm is heightened by the apparent lack of scientific and medical underpinnings in the bills and laws. This program will address threats to the independence and neutrality of MDI and the medical practice of forensic pathology.

Educational Objectives: After attending this presentation, attendees will understand the importance of monitoring and evaluating state legislative proposals through a prioritized, anticipatory process. Vigilance in monitoring is warranted due to the recent uptick in legislative proposals and passed bills that dictate processes and outcomes in MDIs. The information obtained by attendees will enable proactive and informed responses to proposed legislation that encroaches on the independence of forensic pathology and MDIs.

Impact Statement: This presentation impacts the forensic science community by exposing threats to the independence and neutrality of MDIs and the medical practice of forensic pathology and by encouraging forward-leaning responses to ward off such threats.

MDIs are performed by coroner or medical examiner offices to explain the occurrence of unexpected, suspicious, and violent deaths, and to diagnose threats and offer preventative guidance for avoidance of premature death in the living. Forensic pathologists are essential to MDIs, as they employ specialized medical training to reach medical diagnosis relating to cause and manner of death. Forensic pathologists use external examination, medical records, pathology, histology, toxicology, and a surgical procedure known as the autopsy to determine presence or absence of diseases, poisons, or external factors that caused death. The Centers for Disease Control and Prevention Vital Statistics System, which provides foundational, baseline data for innumerable federal, state, and local entities, relies universally on accurate and objective autopsy, toxicology, and related findings as to cause and manner of death.

In 2023, the independence of forensic pathology in the context of MDIs was directly attacked by the introduction of various state legislative proposals and bills passed into law. These legislative proposals and laws erode the ability of forensic pathologists to maintain impartiality and objectivity, mandate particular findings on death certificates, and allow non-physicians to perform autopsies. The cause for alarm is heightened by the apparent lack of scientific and medical underpinnings in the bills and laws.

In Texas, HB 6 and companion SB 645 mandated that death certificates include the term "Fentanyl Poisoning" if toxicology examination reveals fentanyl or fentanyl-related substances in previously identified lethal amounts and autopsy results are consistent with opioid overdose as the cause of death. Illinois SB 1086 mandated the inclusion of "Fentanyl Poisoning" on death certificates under similar circumstances. These bills undermine the independence of forensic pathologists to consider all the facts and circumstances in a case, and additionally assume there is a universally agreed upon lethal concentration of fentanyl, which there is not. On June 14, 2023, Texas HB 6 was signed into law.

Indiana's HB 1286, another bill relating to the fentanyl crisis, mandated testing body fluids for any amount of xylazine, including trace amounts, if the coroner reasonably believes that the cause of death was due to overdose. Legislative proposals such as HB 1286 would increase the workload and costs for state health departments and toxicology laboratories, as well as compromising forensic pathologist independence. HB 1286 was signed into law on April 20, 2023.

In Oregon, SB 953 was engrossed by the Senate on April 17, 2023, after a March introduction, with a heading that reads, in pertinent part: "Provides that a physician assistant or nurse practitioner may be appointed as county medical examiner or assistant county medical examiner and may perform autopsies in deaths requiring investigation. Directs Chief Medical Examiner to provide training and supervision to physician assistants and nurse practitioners in performance of their duties." While the language of the bill was softened slightly in the amendment process, the bill was recommended for passage in committee and went to fiscal review and public comment before dying at adjournment of legislature.

Vigilant monitoring of state-specific legislation is essential to remaining in front of changes so that temporally effective, scientifically informed responses can be formulated. Attendees will be provided with governmental and open-source electronic resources that can be utilized to track bills and legislative movement in their respective states.

Conclusion: Laws and regulations that intrude into the independence of the medical diagnosis of death threatens the independence of medicine writ large. Heightened vigilance is called for among all those working in forensic science, and particularly in MDI systems, to monitor, challenge, and resist evolving legislative activity that threatens to encroach and erode the independence of forensic pathology diagnosis and conclusions.

Legislation; Fentanyl Toxicity; Xylazine

CB5   Protecting the Innocent: The Urgent Need to Research When, and Under What Circumstances, DNA Evidence Can Lead to Wrongful Convictions

J.D. Schmid, JD, Sixth District Public Defender's Office, Duluth, MN*

Program Description: This presentation will address how two recent trends in forensic DNA analysis require stakeholders to re-evaluate the power of DNA evidence to accurately distinguish the guilty from the innocent. First, courts must learn to account for the increased sensitivity of current DNA testing methods and the ensuing risk that DNA recovered from a crime-scene is unrelated to the crime. Second, courts must learn to account for the increased use of probabilistic genotyping software programs and common misunderstandings about the meaning and limitations of the subsource likelihood ratios generated by such programs. Research exploring how each of these trends impacts stakeholders' abilities to distinguish the guilty from the innocent is urgently needed to prevent wrongful convictions.

Educational Objectives: The goal of this presentation is to explore the urgent need to further research how recent technological advancements in forensic DNA analysis are impacting the administration of justice in the American criminal court system.

Impact Statement: Several members of the forensic science community have either: (1) identified common misunderstandings about likelihood ratios among lay people, or (2) recognized the impact of highly sensitive testing methods on the detection of irrelevant DNA at a crime scene. The materials discussed in this presentation will impact the forensic science community by hopefully providing empirical guidance to practitioners and decision makers in cases where the presentation of subsource likelihood ratio could lead to a wrongful conviction. This presentation will also hopefully encourage researchers with technical expertise and resources to study whether existing procedural safeguards are sufficient to protect innocent defendants faced with incriminating DNA evidence.

The primary goal of criminal courts is to distinguish the guilty from the innocent. Forensic DNA analysis has historically provided the court system with a powerful tool to make this distinction. DNA evidence has allowed courts to hold perpetrators of previously unsolvable crimes accountable for their conduct. Hundreds of exonerees also owe their freedom to the power of DNA evidence.

Two recent trends, however, require careful consideration of the potential for DNA evidence to mistake the innocent for the guilty. First, current DNA testing methods have become incredibly sensitive. The risk that an innocent person's DNA will be detected in crime-scene stain is greater now than at any other time. This risk will continue to grow as technological advances allow laboratories to generate full profiles from increasingly small amounts of DNA. Courts must therefore learn how to account for the irrelevance of some DNA associations. Knowing when, and under what circumstances, irrelevant DNA poses an intolerable risk of wrongful conviction must be understood.

Second, courts must learn how to account for common misunderstandings about the meaning and limitations of subsource likelihood ratios. Most laboratories have implemented, or are in the process of implementing, probabilistic genotyping software programs. The laboratory reports and analyst testimony derived from these programs generally express weight of evidence in the form of a subsource likelihood ratio. Subsource likelihood ratios are often misunderstood and/or misrepresented by stakeholders in the criminal justice community. Research has identified a tendency among laypersons to misinterpret likelihood ratios by "transposing the conditional" or by using a subsource likelihood ratio to draw inferences about source or activity level propositions. Little is known about whether such misunderstandings persist in the face of properly limited testimony and, more importantly, how misunderstanding the meaning and limitations of a subsource likelihood ratio impacts a stakeholder's ability to distinguish a guilty defendant from one who is innocent.

Several procedural safeguards exist that may allow courts to account for the risks outlined above. Tools such as cross-examination and evidentiary rules are frequently used to mitigate the impact of potentially misleading evidence. Confidence in the ability of such safeguards to prevent wrongful convictions should not, however, be assumed. Empirical data is necessary to determine when, and under what circumstances, DNA evidence may lead to wrongful convictions, and whether any of the procedural mechanisms available to courts are sufficient to prevent such injustices.

Upon completion of this program, attendees should be able to recognize how recent trends in forensic DNA analyses could lead to wrongful convictions if not properly addressed. Attendees should also be able to recognize the urgent need to research whether, and to what extent, existing procedural safeguards are sufficient to prevent wrongful convictions.

Activity Level; Score Likelihood Ratios; Wrongful Conviction

CB6 New Jersey's Top 10 Most Wanted: Anthony Mota Apprehended

Anna W. Delaney, MA*, New Jersey State Police, Hamilton, NJ

Program Description: This presentation will cover how the New Jersey State Police Fugitive Unit, New Jersey State Police Office of Forensic Sciences Forensic Anthropology Unit, and the United States Marshal's Office were able to locate and close one of New Jersey's Top 10 Most Wanted cases—the case of Anthony Mota. Even in death, we strive to resolve and provide closure and justice to our victims and their families.

Educational Objectives: After attending this presentation, attendees will understand how to execute an international exhumation, gain knowledge of the valuable resources that the United States Marshal's Office can provide for assistance in cases, learn what samples provide the best DNA results, and the importance of documentation throughout a complex case.

Impact Statement: This presentation will impact the forensic science community by bridging the gap between international agencies working to achieve the same goals, demonstrating how to work collaboratively with multiple agencies across domestic and international jurisdictions, and informing attendees of the resources available from federal agencies.

Anthony Mota committed several heinous crimes, including a brutal murder in 1997 that occurred in New Jersey. Mr. Mota was federally indicted in 1998 for charges including murder, kidnapping, racketeering, arson, possession of a weapon for unlawful purposes and theft by extortion; however, he evaded apprehension by fleeing to his native Dominican Republic after the murder was committed.

This presentation will discuss how the New Jersey State Police Fugitive Unit and United States Marshals were able to locate Mr. Mota in the Dominican Republic, where he lived a double life under an alias until his death in June 2021. Due to his death, authorities were unable to extradite him alive back to the United States. Through investigative interviews in the United States and Dominican Republic, the true identity of his alias became apparent, showing that Mr. Mota had finally been found after all these years. The New Jersey State Police Office of Forensic Sciences Forensic Anthropology Unit helped develop and implement an international exhumation and retrieved his remains for anthropological examinations and DNA analysis in the United States. Through the assistance of multiple state and federal agencies, the identity of one of New Jersey's most wanted was confirmed.

This presentation will demonstrate how, after 25 years on the run, the collaboration of multiple agencies led to justice for all through the identification of one of New Jersey's Top 10 Most Wanted Individuals and the closure provided for the family of the victim. A plethora of technologies were employed to not only locate Mr. Mota, but also to scientifically confirm his identity. No matter the length of time or how a cold case seems impossible to resolve, the dedication of the men and women in law enforcement continue to provide justice for the citizens of New Jersey.

Anthropology; Exhumation; Homicide

CB7   Operation Death Harvester: How the FBI Is Dismembering the Illegal Trade in Human Body Parts

Paul Micah Johnson, PhD*, Federal Bureau of Investigation, Detroit, MI

Program Description: This presentation is a case review of the largest human body parts industry investigation in the Federal Bureau of Investigation's (FBI's) history. Death Harvester was opened by the author as an organized crime case in the Detroit Field Office in 2011. Its purpose was to investigate the illegal sale of human remains for research and education that was taking place through a network of anatomical supply companies. What started with the finding of severed heads in a rusty van ultimately resulted in the seizing of thousands of human remains across the country, the closure of four body donation programs, the arrest and conviction of seven individuals, and the identification, cremation, and return of human remains to hundreds of victim families.

Educational Objectives: This presentation will help the attendee to better understand the global trade in human remains for research and education, the common crimes associated with the trade, what has been, and can still be, done to stop the illegal activity, and the roles that forensic science professionals can play.

Impact Statement: The expertise of forensic pathologists and forensic anthropologists is regularly needed in criminal investigations of the human body parts trade, as are the skills of fingerprint examiners and a range of DNA specialists. Having a broad understanding of this industry and its common crimes will better prepare these forensic science professionals to assist with ongoing efforts to stem some of the rampant illegality. Additionally, this presentation will impact the forensic science community by helping all attendees better understand the risks associated with donating one's body to science.

On the night of October 23, 2011, the driver of a rusty navy-blue Ford® Econoline® van attempted to cross the international border between Canada and the United States. In the back of the van were several picnic coolers and in them, something the United States Customs and Border Protection agent did not expect. Each cooler contained five standard kitchen trash bags and each trash bag contained a wet object about the size of a large bowling ball. What the agent saw when he opened the bags was "the most cruel and inhumane thing that I have ever encountered on the job." He followed up with "I want to see everyone associated with this brought to justice."

Death Harvester is the name of the FBI case that was initially opened to investigate the illegal activity of Arthur Rathburn, former University of Michigan Willed-Body Program Coordinator, owner of International Biological, Inc. (IBI) and the driver of that navy-blue van. Death Harvester was the first criminal enterprise case of its kind in the FBI, investigating the international market in human body parts. The case centered on a network of so-called "body brokers" and willed-body-to-science businesses with extensive interstate and international connections. These entities fraudulently obtained, then dismembered, packaged, and sold or leased human body parts to a vast network of medical researchers, medical equipment developers, pharmaceutical companies, and individual domestic and foreign buyers. Many of the body part donors had tested positive for serious infectious diseases—a fact that sellers went to great pains to hide from buyers. This presentation will discuss the details of the Death Harvester case and will include the roles played by forensic professionals in its successful outcome.

Death Harvester was worked with direct assistance from the Centers for Disease Control and Prevention (CDC), the Arizona Office of the Attorney General, and the United States Department of Transportation, Office of Inspector General. Additionally, the Office of the Wayne County Medical Examiner, the FBI Technical Hazards Response Unit, the FBI Evidence Response Team Unit, and the FBI Laboratory were critical to the safe handling and analysis of thousands of seized human remains. Much of the case was prosecuted by the United States Attorney's Office for the Eastern District of Michigan.

Cadaver; Human Remains; Unidentified Persons

CB8   Online Child Exploitation: Proactive Law Enforcement Strategies

Paul C. Graf, MS*, Independent Consultant of Child Abuse Legal Matters, Sr. Special Agent, NCIS (Retired), Castle Rock, CO

Program Description: Under any other circumstances, a child sex offender would have to approach a child and converse, which is a much more difficult hurdle in terms of circumstance, intellectual capability, and social functioning. Online communication has dispensed with much of the social skills and verbal communication required to seduce a child, which may have deterred some offenders, and given them direct access to children, many of whom are online with little or no adult/parental supervision. In response to this crisis, law enforcement has adopted a number of proactive strategies to enforce child-protective criminal statutes. Those statutes, the communication elements of the internet, and the development of law enforcement efforts in this field will be discussed.

Educational Objectives: After attending this presentation, attendees will: (1) learn what conduct on the part of individuals expressing a sexual interest in children is illegal, and what conduct, though possibly objectionable, is protected by United States Constitutionally guaranteed freedoms; (2) learn to identify the various communication elements of the internet; (3) be able to understand how persons expressing a sexual interest in children make use of the various communication components of the internet to contact both persons who share similar interests and potential child victims; and (4) be introduced to several proactive techniques used by law enforcement, and the technology that makes them possible, to target and investigate criminal child sex offenders online.

Impact Statement: Federal statutes prohibit the possession of graphic Child Sexual Abuse Materials (CSAM), the distribution of CSAM images by any means, including computer, the buying and selling of children for the purpose of producing CSAM images, and interstate or foreign travel with the intent to engage in a sexual act with a child. Law enforcement's proactive investigations often involve undercover communication with individuals who display a sexual interest in children; they document exchanges of illegal CSAM and rely heavily on face-to-face undercover meetings. This presentation will impact the forensic science community by delineating what conduct is and is not prohibited by federal and various state laws. A discussion of proactive law enforcement methods will be presented, as well as an explanation of the internet and its communication media components, such as the World Wide Web (WWW), the Dark Web, online gaming platforms, Internet Relay Chat (IRC), chat rooms, newsgroups, and file servers, all with case examples.

Due to the incredible popularity and utility of the global linking of computer systems known as the internet, the incidence of child pornography possession and distribution has exploded. The internet is the ideal medium for such criminal activity, as it affords individuals with a sexual interest in children a degree of anonymity and ease of communication—both with others who share their interest in children and thus, help validate their behavior, and with actual (potential) child victims. Under any other circumstance, a child sex offender would have to approach a child and converse, a much more difficult hurdle in circumstance, intellectual capability, and social functioning. Online communication has dispensed with much of the social skills and verbal communication required to seduce a child, which may have deterred some offenders, and given them direct access to children, many of whom are online with little or no adult/parent supervision. In response to this crisis, law enforcement has adopted a number of proactive strategies to enforce child-protective criminal statutes. Those statutes, the communication elements of the internet, and the development of law enforcement efforts in this field will be discussed. Federal statutes prohibit the *possession* of graphic Child Sexual Abuse Materials (CSAM) images, the *distribution* of CSAM images by any means including computer, the *buying and selling* of children for the purpose of producing CSAM images, and *interstate or foreign travel* with the intent to engage in a sexual act with a child. Law enforcement's proactive investigations often involve undercover communication with individuals who display a sexual interest in children, they document exchanges of illegal CSAM and rely heavily on face-to-face undercover meetings. This training seminar will delineate what conduct is and is not prohibited by federal and various state laws. A discussion of proactive law enforcement methods will be presented, as well as an explanation of the internet and its communication media components, such as the World Wide Web (WWW), the Dark Web, online gaming platforms, Internet Relay Chat (IRC), Chat Rooms, Newsgroups, and File Servers, all with case examples.

Child Abuse; Law Enforcement; Sexual Crimes

LS1 W.W.G.D. (What Would Gibbs Do?): The Real Work of NCIS Special Agents

Paul C. Graf, MS*, Independent Consultant of Child Abuse Legal Matters, Sr. Special Agent, NCIS (Retired), Castle Rock, CO

Program Description: The duties and responsibilities of a Naval Criminal Investigative Service (NCIS) Special Agent, along with real case examples, will be discussed through the lens of the real-life Jethro Gibbs.

Educational Objectives: After attending this presentation, attendees will be familiar with the duties and responsibilities of NCIS Special Agents and understand the similarities, and the many differences, between the *NCIS* television shows and "real life" NCIS work.

Impact Statement: This presentation will impact the forensic science community by developing awareness of the critical missions of NCIS Special Agents and their roles in federal law enforcement.

When the presenter, Paul C. Graf, began his career with the United States Naval Investigative Service (NIS), he often said it took well over 20 minutes to tell someone who he worked for and why he was asking so many questions. Now, and since the advent of the wildly popular CBS Television series *NCIS* and its offshoot programs (*NCIS Los Angeles, NCIS New Orleans, NCIS Hawaii*) the re-named Naval Criminal Investigative Service (NCIS) has become a household word. That said, many viewers still believe it to be a fictional agency and are surprised to hear the presenter spent 33 years conducting criminal, counter-intelligence, counter-terrorism, and espionage as a Senior Special Agent of NCIS (a.k.a.: The Real Gibbs). Mr. Graf was assigned to offices in Long Beach, CA, Naples, Italy, Newport, RI, Brunswick, ME, and Guantanamo Bay, Cuba. His duties included narcotics investigation, grand larceny, sabotage, espionage, Double Agent Operations, homicide, arson, rape, procurement fraud, cold case homicide, and child abuse investigations, his specialty. In his role as an Executive Protection Supervisor, he led teams of armed Agents who protected military and civilian VIP's and world leaders, worldwide. Mr. Graf conducted NCIS investigations and operations in 26 countries of the world. Therefore, the duties and responsibilities of an NCIS Special Agent, as seen through the lens of his extensive career, along with real case examples, will be discussed. The forensic science community will, thus, develop an awareness of the critical missions of NCIS Special Agents and their role in federal law enforcement.

Crime Scene Investigation; Death Investigation; Law Enforcement

LS2   Getting Things Started With a Bang: Interpreting Gunshot Sounds in Audio Forensic Analysis

Robert C. Maher, PhD, PEng*, Montana State University, Bozeman, MT

Program Description: This presentation begins with the historical background of gunshot audio interpretation, then provides several fascinating case studies of the current state-of-the art, demonstrating how gunfire sounds can provide essential forensic information. Attendees will learn the essential physical principles of how firearms make sounds and how those sounds are affected by the recording location and the recording system. At the conclusion of the seminar, attendees will understand how to interpret audio waveforms and spectrograms from gunshot audio recordings and how to apply their new knowledge to examples and case studies.

Educational Objectives: After attending this presentation, attendees will: (1) understand the basic physical principles of how firearms make sound and how gunshot sounds propagate through the air to the microphone; (2) have viewed waveforms and spectrograms of gunshot sounds, identifying the key features and important details; (3) understand how to interpret and knowledgeably discuss the strengths and weaknesses of various sources of gunshot audio recordings; (4) understand the basic features of contemporary gunshot detection and location systems, such as ShotSpotter™ from SoundThinking, Inc.; and (5) recognize the proper steps in handling user-generated audio evidence in forensic investigations.

Impact Statement: This presentation will impact the forensic science community by introducing the attendees to gunshot audio forensic examination.

More and more criminal forensic cases involve audio forensic evidence in general, and gunshot audio recordings in particular, due to the millions of newly installed residential security and doorbell camera systems, the growing use of body-worn cameras by law enforcement officers, and the significant number of communities with gunshot detection systems such as ShotSpotter™. The sounds of gunfire—although generally familiar to most people—actually may contain many details about the circumstances of the shooting incident and the type(s) of firearm(s) involved.[1] Audio recording devices have become omnipresent, so gunshot acoustic evidence is more common than ever before. Gunshot sounds can play a unique role in forensic investigations. Gunshots recorded near a crime scene help answer key questions about the timeline of a shooting event and the circumstances of the incident.[2] Forensic recordings nowadays can come from numerous possible sources, including law enforcement body-worn cameras, residential doorbell cameras and commercial surveillance systems, bystanders with mobile phones, and emergency call center recordings.

The presentation will cover the historical background of gunshot audio interpretation and provide fascinating case studies of the current state-of-the art, demonstrating how gunfire sounds can provide essential forensic information.[3] Audio forensic examiners are commonly asked to determine the location of gunfire, the type of firearm(s) involved, and the time sequence of successive shots ("who shot first?"). Recent research studies have revealed how the relative orientation between the firearm and the microphone affects the recorded sound. Examiners also must understand the role of acoustic reflection, diffraction, and reverberation when interpreting gunshot recordings.[4]

Time will be provided during the session for attendees to ask questions and interact with other participants interested in expanding their knowledge of audio forensic analysis of gunshot acoustics.

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Audio; Forensic Analysis; Crime Scene Investigation

LS3   Human Spaceflight Medicolegal Death Investigations

Edward Mazuchowski, MD, PhD*, Forensic Pathology Associates, Division of HNL Lab Medicine, Allentown, PA

Program Description: This presentation will give an overview of the phases of human spaceflight and the unique challenges each phase presents. Past fatal mishaps will be reviewed. The current applicable protocols in regard to human spaceflight medicolegal death investigations, current knowledge gaps, and future challenges to these investigations will be discussed.

Educational Objectives: Upon completion of this workshop, attendees should be able to describe the phases of spaceflight and the unique challenges these phases present during a spaceflight death investigation.

Impact Statement: This presentation will impact the forensic science community by providing a historical overview of human spaceflight medicolegal death investigations and an overview of current knowledge gaps and challenges for these investigations.

Since Cosmonaut Yuri Gagarin orbited the earth on April 12, 1961, over 600 individuals have traveled past the Karman line (altitude of 100km)—the boundary of Earth's atmosphere and space. As part of the National Aeronautics and Space Administration (NASA) Apollo program, 24 astronauts flew to the moon and 12 astronauts walked on the lunar surface. Over the past two decades, astronauts have continuously occupied and operated the International Space Station in low Earth orbit. Historically, human spaceflight has been conducted by government-sponsored space agencies. Recently, commercial spaceflight companies have been successful in achieving human spaceflight, including travel to the International Space Station and low Earth Orbit. Future programs include travel to the lunar surface, Mars, and deep space.

The phases of spaceflight include ground/launch, ascent, earth orbit, entry, and landing phase for missions orbiting Earth. Outbound, lunar orbit, lunar surface, and earth return are additional phases of spaceflight for lunar missions. Each phase of flight poses unique hazards to the astronaut, and those supporting them, that may result in death. Understanding these hazardous and the changes the human body undergoes during various phases of flight is critical for comprehensive human spaceflight death investigations.

Although there have been human spaceflight mishaps, there have been relatively few incidents resulting in loss of crew. Loss of crew incidents have occurred during the launch/ground, ascent, entry, or landing phases of the flight. There have been no fatalities during the earth orbit, outbound, lunar orbit, lunar surface, or earth return phases of flight. Deaths during these phases present unique challenges for declaration of death, preservation of evidence, examination of the remains, and containment of the remains to prevent injury to the remaining crew.

This presentation will review the various phases of human spaceflight and highlight the loss of crew incidents that have occurred over the past six decades. The current applicable protocols and documents regarding human spaceflight death investigations will be presented. The challenges and knowledge gaps of human spaceflight, including return to the lunar surface, mission to Mars, and future deep spaceflight, as they relate to death investigations will be discussed.

Human Spaceflight; Medicolegal Death Investigation; Phases of Spaceflight

LS4   There Is No I in TEAM: The Importance of Leadership, Personal Accountability, and Personality Type in High Stress Work Environments

Charla Skinner Perdue, MFS*, Florida State University, Panama City Campus, Panama City, FL

Program Description: This presentation's goal is to show the importance of the role each person plays in high stress, collaborative work groups that make up many of the various positions in the fields of forensic science. High stress work environments are where the substance of the work is difficult, there is increased pressure to be right, and heavy caseloads and rapid pace take a toll on both mental and physical health. Reducing the stress on the team improves the work culture for everyone. There is no I in TEAM, but the argument can be made that there should be. The only person whose decisions and actions we can control is ourselves. We will take a quick 11-question multiple choice quiz to determine personality color and then explore how understanding ourselves and just as importantly, how others perceive us, can lead to better workplace interactions, reduce errors, and improve the overall quality of our work and our agency culture.

Educational Objectives: At the completion of this luncheon presentation, attendees will be able to: (1) identify their personality color by completing a short 11-question multiple choice quiz; (2) recognize the characteristics of their personality color and how they may be perceived by others; (3) discuss the importance of using personality color to improve communication; (4) examine studies related to secondary trauma in high stress work environments; (5) relate the concept of stress in the workplace to noise and errors; (6) describe the physical and mental effects of the high stress workplace, both primary and secondary; (7) discern the role one plays in challenging work situations; (8) evaluate the importance of leadership; and (9) explore ways to leverage the team's talents.

Impact Statement: This presentation will impact the forensic science community by highlighting the benefits of knowing the individual's personality color and the color of those they work with. It will include a brief 11-question multiple choice quiz to identify their personality color, to better understand how others perceive them, and consider studies and techniques from the field of sociology to improve how they interact with others. We will examine the importance of leadership as a tool to enhance the workplace culture and how a positive work environment may even reduce errors. By understanding that the only person whose decisions and actions we can control is ourselves, we can endeavor to improve our high stress work environment for ourselves and everyone on the team.

Sociologists approach personality in terms of an individual's status in a group.[1] Since crimes are not solved by any one person but by everyone working together, it is imperative that the group members can cooperate. Personality determines how we interact with colleagues, management, and those we are serving in the community. Even more critical is how we are perceived by our peers. Using the research of sociologists to find one's personality type and that of those we work with can improve communication and reduce error.

The work of solving crime by its nature creates trauma. Studies have shown vicarious trauma in those working in these roles can compound the harm caused.[2] High stress work environments where the substance of the work is difficult, the increased pressure to be right, heavy caseloads and rapid pace take a toll on both mental and physical health. Reducing the stress on the team can decrease the secondary traumas of working in this type of environment.

Recent studies on the concept of noise have increased awareness of how error rates can be affected by these types of distractions. Examples of noise include emotional reactions, mood, fatigue, and group dynamics and are areas where the workplace can be a factor.[3] A positive work environment can reduce noise and in turn may reduce errors in casework.

One of the best indicators of ethics in any agency is the culture. Leadership perpetuates the culture of an agency, and the culture sustains behavior.[4] In difficult workplace situations, each of us plays one of four roles: victim, bystander, perpetrator, or leader.[5] Frequently, those in forensics tend to be more introverted and the role of leader can be self-contradictory.[6] Understanding the importance of leading even when difficult gives one confidence to speak up when ethical issues arise. As individuals working in the criminal justice system, we promote public trust and must be able to make sound decisions based on ethical principles.

Understanding one's personality type and how we are regarded by others leads to better interactions at work. It allows self-reflection of one's potential blind spots, weaknesses, and strengths.[7] Knowing how to find one's voice can allow us to be part of workplace solutions, preserving peace and allowing for better team dynamics.

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Ethics; Mental Health; Workplace

W1　Recommendations From the NIST/NIJ Expert Working Group on Human Factors in Forensic DNA Interpretation

Jarrah Kennedy, MSFS, Kansas City Police Crime Lab, Kansas City, MO; Angela Spessard, Maryland State Police, Pikesville, MD; Tiffany A. Roy, ForensicAid, LLC, West Palm Beach, FL; Melissa Taylor, National Institute of Standards and Technology, Gaithersburg, MD; Mikalaa M. Martin, RTI International, Greenville, SC; Britton Morin, Union County Prosecutor's Office Forensic Laboratory, Westfield, NJ; Nikola Osborne, National Institute of Standards and Technology, Auckland, New Zealand; Hope Zagaria, RTI International, Alexandria, VA

Learning Overview: Upon completion of this workshop, participants will be familiar with the recommendations in the National Institute of Standards and Technology (NIST)/National Institute of Justice (NIJ) Expert Working Group on Human Factors in Forensic DNA Interpretation report. They will know the implications of these recommendations for their own practice and how the recommendations serve to improve the practice of DNA examination. Participants will be invited to discuss how relevant and feasible the recommendations are to their own practice and to the wider forensic DNA community.

Impact Statement: The study of human factors in forensic science is an essential element to inform our understanding of the interaction between humans and the systems they use. In understanding human factor issues, we can identify and address the potential for error and bias. Furthermore, we can develop ways to shape positive laboratory culture, improve staff morale, and increase work productivity. This workshop presents three years' worth of effort to understand human factors as they apply to forensic DNA interpretation. This effort provides guidance to DNA analysts, laboratory management, and legal practitioners on how to improve practice for safer justice outcomes.

Studying human factors is essential to inform our understanding of humans' interactions with the systems they use. This is especially important in forensic science, where the outcomes of these interactions can have a direct impact on an individual's life or liberty. Human factors are often associated with cognitive bias, an issue that continues to receive significant attention in forensic science. Human factors extend far beyond bias, however, and include any activity in which a human is involved. For example, human factors study can also assist in developing effective ways to shape positive laboratory culture, improve staff morale, increase work productivity and quality, inform training standards, enhance communication of results, inform research gaps, and much more.

In recognizing the importance of human factor issues in forensic science, the NIST and NIJ collaborated to develop an Expert Working Group Series on Human Factors in Forensic Sciences. To date, the series has produced two successful reports: one in latent print examination and another in handwriting examination.

In February 2020, the NIST/NIJ Expert Working Group (EWG) on Human Factors in Forensic DNA Interpretation first convened and was charged with conducting a scientific assessment on the effects of human factors in forensic DNA analysis and interpretation with the goal of recommending approaches to improve its practice and reduce the likelihood of errors and bias. The EWG has since evaluated relevant scientific literature and technical knowledge to develop a report and recommendations. This evaluation serves to educate members of forensic DNA laboratories and allied criminal justice system partners (e.g., attorneys, investigators, parent organization leadership).

In this workshop, we will walk participants through the EWG's process to develop the report, highlight key findings, discuss some of the more controversial topics, and provide steps to implement the resulting recommendations. Topics and recommendations will relate to DNA interpretation and:
- Education, Training, and Professional Credentialing
- Quality Assurance/Quality Control
- Cognitive Bias and Error Reduction
- Reporting and Testimony
- Management
- Work Environment
- Expressing Evidence Strength (e.g., likelihood ratios)
- Research
- Emerging Technology

This full-day workshop will comprise a mixture of lectures, discussions, and interactive activities. Workshop participants will be expected to engage with the material and provide feedback on the relevance and feasibility of implementing the recommendations in their own practice (i.e., on the individual-, team-, and organization-level). This workshop is relevant to forensic DNA professionals and other forensic science professionals, laboratory leadership, forensic science and human factors researchers, educators, legal practitioners, and anyone interested in understanding and improving decision-making in forensic science.

Forensic DNA; Bias; Reliability

W2   Get Fired Up for Improvements to Fire Debris Standards! (Overview, Use, and Implementation of the Major Revisions to ASTM E1618)

Brenda B. Christy, Virginia Department of Forensic Science, Norfolk, VA; Kimberly S. Kunkler, Marshall University, Huntington, WV; Laura J. Hernandez, Verity Labs Inc., Las Cruces, NM

Leaning Overview: Attendees of this workshop will receive information and training on major revisions to the American Society for Testing and Materials (ASTM) E1618.[1] Presenters will discuss why the revisions are needed, explain the new specifications for the ignitable liquid classification system, provide an overview of the performance-driven instrumental analysis requirements, and facilitate implementation of the new interpretation and reporting requirements through practical exercises. Intended as an introductory workshop, attendees will gain experience using these new specifications for classification of ignitable liquids and a practical understanding of how the updated requirements can reduce misattribution or misidentification of ignitable liquids in complex matrix samples.

After completing this workshop, attendees will: (1) be familiar with the major revisions currently being made to ASTM E1618-19 Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry; (2) understand the potential impact of the revisions on their laboratory's standard operating procedures; and (3) have basic experience in utilization of some of the revisions through completion of practical, hands-on exercises.[1-4]

Impact Statement: The major revisions to ASTM E1618-19 provide additional information that is designed to clarify its requirements and improve consistency of its use in fire debris analysis. This workshop will impact the forensic science community's practice by providing orientation and training related to implementation of the major revisions to ASTM E1618.

The Organization of Scientific Area Committees for Forensic Science (OSAC) Subcommittee on Fire Debris, Explosives, and Gunshot Residue (SC), responsible for reviewing fire debris related standards, initially assessed ASTM E1618 to determine if it provided sufficient information to be considered a best practice and if it was suitable for placement on the OSAC Registry. The SC members identified several key areas where additional information was needed to strengthen the standard, clarify intended requirements, and improve the consistency of its use.

Since then, the SC has compiled and carefully reviewed existing literature and data resources to provide this additional information through major revisions of ASTM E1618 with the intent of guiding labs to current best practices in validation, instrumental analysis, classification, interpretation, and reporting of results. These revisions resulted in the creation of two new, separate standards (one detailing the classification system, one providing requirements for reporting of results) and an intensive revision to E1618 detailing the interpretation process. These three documents are currently progressing through the ASTM balloting process as a prerequisite to placement on the OSAC Registry. In addition, guidance for validation and verification of methods used in fire debris analysis is slated to become an annex to an overarching standard focused on validation in the various forensic chemistry subdisciplines.

References:
1. ASTM E1618-19 Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry (under revision as OSAC 2022-S-0004 & OSAC 2022-S-0005).
2. ASTM WK73482 Standard Practice for Reporting Results and Opinions of Ignitable Liquids Analysis.
3. ASTM WK81720 E1618 Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry.
4. ASTM WK81724 Standard Classification for Ignitable Liquids Encountered in Fire Debris Analysis.

Fire Investigation; Standards; Interpretation

W3   **Machine Learning-Powered SpectrApp: An Open-Source Tool for Analyzing Forensic Spectroscopic Data**

Eugenio Alladio, Department of Chemistry, University of Torino, Torino, Piemonte, Italy; Paolo Garofano, Centro Regionale Antidoping "A. Bertinaria," Roma, Lazio, Italy

Learning Overview: Upon completion of this workshop, attendees will gain a comprehensive understanding of SpectrApp, an open-source tool developed by the Department of Chemistry at the University of Turin, its capabilities in forensic spectroscopic data analysis, and utilize multivariate data analysis. This user-friendly tool combines advanced machine learning techniques with a seamless interface, catering to both experts and non-experts in the field. Participants will delve into the power of SpectrApp's multivariate data analysis and chemometric strategies, facilitated by the R Shiny environment, explore a vast array of graphical approaches, including univariate, bivariate, and multivariate visualizations, and uncover intricate patterns within complex datasets.They will learn about SpectrApp's support for cluster analysis, Principal Component Analysis (PCA), and other advanced methodologies, such as PLS-Discriminant Analysis (PLS-DA) and PLS-Regression (PLS-R), and witness how SpectrApp enables the prediction of new samples, empowering forensic analysis like never before. Don't miss this enlightening workshop that showcases the potential of SpectrApp to revolutionize the future of forensic science through machine learning and data analysis.

Impact Statement: This session on SpectrApp will have a profound impact on the forensic science community by introducing a transformative tool that leverages machine learning for spectroscopic data analysis. Participants will gain invaluable insights and practical skills to navigate the complexities of forensic data, propelling them towards more accurate and efficient analyses. The workshop's educational objectives emphasize the integration of cutting-edge techniques, such as multivariate data analysis and chemometric strategies, enabling forensic experts to extract meaningful information from intricate datasets.

By empowering both experts and non-experts with the user-friendly interface of SpectrApp, this workshop aims to democratize advanced data analysis in forensic science. This accessibility will promote broader adoption and foster a culture of collaboration and knowledge-sharing within the community. The practical application of PLS-DA and PLS-R will enable participants to make informed decisions and enhance the efficacy of forensic investigations. The ability to predict new samples using SpectrApp further strengthens the community's capacity for real-world applications, fostering more accurate and reliable results. Furthermore, SpectrApp's role as an open-source tool encourages transparency and reproducibility in forensic data analysis. Researchers can verify and build upon existing work, ensuring the reliability and credibility of scientific findings.

By showcasing SpectrApp's potential to revolutionize forensic spectroscopic data analysis, this workshop encourages innovation and drives advancements in the field. This newfound integration of machine learning methodologies in forensic science will undoubtedly influence research directions and encourage further exploration of data-driven approaches.

A hallmark of SpectrApp lies in its exceptional user-friendliness, making it accessible to both seasoned experts and non-experts in the fields of machine learning and forensic science. Its intuitive interface empowers users to seamlessly evaluate spectroscopic data through sophisticated multivariate data analysis and chemometric strategies. Powered by the versatile R Shiny environment, SpectrApp elevates the data analysis experience, streamlining visualization, preprocessing, and analysis tasks.

During this immersive session, participants will embark on a captivating journey into SpectrApp's expansive repertoire of graphical approaches, granting them unprecedented insights into the complex relationships and patterns within the datasets. Through an array of univariate, bivariate, and multivariate visualizations, attendees will glean valuable understanding from the data, revealing crucial information that transcends traditional data representations.

The workshop will delve deep into SpectrApp's impressive support for Cluster Analysis and PCA, showcasing its efficacy in uncovering hidden structures and dependencies within the data. Additionally, participants will have the privilege of exploring a diverse selection of advanced multivariate modeling methodologies, including the powerful PLS-DA for classification and PLS-R for quantification.

The true power of SpectrApp lies in its capacity to enable the prediction of new samples, an essential feature that has transformative implications for forensic analysis. Armed with SpectrApp's predictive capabilities, attendees will be able to assess the origin, classification, and quantity of new samples with precision and accuracy, bolstering the efficacy of forensic investigations.

Machine Learning; Spectroscopy; Forensic Analysis

W4   Inside the Black Box: Forensic Psychiatry for Lawyers 2.0

Corina Freitas, Freitas and Associates LLC, Alexandria, VA; Vivian Shnaidman, Jersey Forensic Consulting, Princeton, NJ; George D. Annas, Forensic Psychiatry Consulting, LLC, Syracuse, NY

CANCELED

W5   Forensic Postmortem Radiology and Medicolegal Death Investigations

Summer J. Decker, Department of Radiology, University of South Florida, Tampa, FL; Edward Mazuchowski, Forensic Pathology Associates, Division of HNL Lab Medicine, Allentown, PA; Natalie L. Adolphi, New Mexico Office of the Medical Investigator, Albuquerque, NM; Fabrice Dedouit, Hopital Rangueil, Toulouse, Midi-Pyrenees, France; Lars Ebert, 3D Center, Institute for Forensic Medicine, University of Zürich, Zürich, Switzerland; Jamie Elifritz, Office of the Medical Investigator, Affiliate, Albuquerque, NM; Michael J. Thali, Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland; Jonathan M. Ford, Department of Radiology, University of South Florida, Tampa, FL

Learning Overview: This workshop is an update on current practices in forensic postmortem radiology and medicolegal death investigations. National and international forensic pathology and radiology practitioners will provide an introduction to and update on best practices in the field. After attending this workshop, attendees will: (1) be informed of the different modalities for postmortem radiology, (2) understand the strengths and weaknesses of the different current modalities, (3) learn about the interpretation of the postmortem radiology scans and levels of forensic evidence, and (4) be aware of current advances, collaboration, and potential resources available to interested practitioners.

Impact Statement: This presentation will impact the forensic science community by providing attendees with expertise on best practices, interpretation of radiology imaging, and incorporation of forensic postmortem radiology into medicolegal death investigations.

It has been over a decade since the publication of the National Academy of Sciences Report calling for the strengthening of the forensic sciences. In this seminal report, the critical role that imaging, specifically radiologic imaging, plays in the documentation of findings sufficient for courts, for providing the opportunity for review by outside experts, as well as for allowing for reevaluation of evidence as medical knowledge advances was noted. Since then, forensic radiology has grown significantly as a field to incorporate advanced radiology techniques into medicolegal death investigations. However, the type of modality and incorporation into the daily medicolegal death investigation varies widely across medicolegal jurisdictions.

Currently, there are four main radiologic imaging modalities that are used in medicolegal death investigations: fluoroscopy, digital radiography (X-ray), including whole-body radiography, Computed Tomography (CT), and Magnetic Resonance Imaging (MRI). There are strengths and weaknesses of each modality. Location/infrastructure, equipment cost, personal cost, time to scan, interpretation and incorporation of findings, presentation at medicolegal proceedings, and storage of results must all be evaluated.

This workshop will provide an overview of how the different radiologic imaging modalities have been incorporated into specific medicolegal death investigations. Through directed lectures and case presentations, the advantages and limitations of the modalities will be presented. How postmortem radiology enhances the medicolegal death investigation will be discussed. Topics will include natural disease, trauma, overdose, postmortem changes, and artifacts. Representative digital radiographs, CT images and Three-Dimensional (3D) reconstructions will be presented. Techniques that overcome some of the limitations of different modalities, such as postmortem angiography for the evaluation of vascular/soft tissue injury, will be presented. The incorporation of these findings into legal proceedings will also be discussed. Participants will have the opportunity to discuss with the presenters through a panel discussion implementation of forensic radiology imaging into their medicolegal system.

Similar workshops have been presented at AAFS by the same team over the past few years, and they have been highly attended by members from different sections. This workshop represents new and updated content to present new advances in the field.

Forensic Pathology; Forensic Radiology; Postmortem Imaging

W6   Don't Know Much About Standards: Incorporating Forensic Science Standards Into University Curricula

Heidi Eldridge, The George Washington University, Washington, DC; Julie A. Howe, Saint Louis University, Saint Louis, MO; Teresa L. Ambrosius, AAFS Standards Board, Colorado Springs, CO; Sarah Seashols Williams, Virginia Commonwealth University, Richmond, VA; Jennifer Limoges, New York State Police Forensic Investigation Center, Albany, NY; A. Karl Larsen, Jr., University of Illinois at Chicago, Chicago, IL; McKenzie Weyh, Michigan State Police, Lansing, MI; Arely J. Parra López, The George Washington University, Eaton, CO; Britni Skillman, Sam Houston State University, Huntsville, TX; Peter Stout, President and CEO, Houston Forensic Science Center, Houston, TX

Learning Overview: During this presentation, representatives from universities and the Forensic Science Education Programs Commission (FEPAC) will present on ways they have incorporated standards into their university programs, examples of specific courses where students' knowledge has been enhanced by such standards, and how to overcome obstacles of changing curriculum to include private sector standards. Representatives from forensic laboratories will present on how hiring practitioners with a knowledge of standards affects their practices, as well as how they are implementing standards into lab training and standard operating procedures. The latter portion of the workshop will be comprised of a panel, including educators, lab managers, young forensic professionals, and recent university graduates, to discuss how forensic standards are changing, and will continue to change, the landscape of forensic education and training.

After attending this presentation, attendees will have a better understanding of how incorporating standards in an educational curriculum affects students' understanding of the discipline and forensic sciences when they enter post-graduate employment.

Impact Statement: This presentation will impact the forensic science community by creating greater awareness of how standards are currently being implemented in university level educational programs, and how lab managers have both incorporated standards into their training and how they use standards for minimum qualifications and education of practitioners.[1]

Multiple forensic and standards organizations are working with members, partners, and constituents across the standards community to develop education initiatives that highlight the economic, societal, and environmental benefits of standards to students, emerging professionals, and the general public. These responsive programs and activities are designed to engage students of all ages in the world of standards and conformity assessment and ensure the strength and efficacy of the next generation of United States standardization leaders.

Avenues exist for professional development and training of practitioners at workshops, conferences, and webinars, but little opportunity currently exists for students in higher education to learn about forensic science standards. Expanding training at this level presents an opportunity to fill a gap by introducing private sector standards and better prepares students for entry into the workforce, while also aligning with recent national strategies that support fostering development in the forensic sciences through "enriching undergraduate experiences."[2]

Implementation begins with awareness. Some agencies are not aware of the standards that are available, why they are needed, how they are developed, or how they can be implemented. Still others recognize the value of standards but fight an uphill battle in garnering the enthusiasm and cooperation of their employees. An incoming workforce of forensic science students who have already learned the ins and outs of forensic science standards and expect their use in the workplace could go a long way toward closing this gap. However, some universities also struggle with the best way to incorporate standards into a curriculum that is already packed with university general education requirements, accreditation science, and discipline-specific requirements, while leaving some space for electives.

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Education; Standards; Forensic Science

W7   Emerging Semi-Synthetic Cannabinoids: THC Isomers and Analogs

Ayako Chan-Hosokawa, NMS Labs, Horsham, PA; Sarah A. Shuda, Center for Forensic Science Research and Education, Willow Grove, PA; Barry K. Logan, Center for Forensic Science Research and Education, Horsham, PA; Nicole Lattanzio, NMS Labs, Willow Grove, PA; Joanna De Morais, European Monitoring Centre for Drugs and Drug Addiction, Lisbon, Portugal; Erin Karschner, Armed Forces Medical Examiner System, Dover Air Force Base, DE

Learning Overview: This workshop will address the growing semi-synthetic cannabinoid market from both a toxicological and a seized drug chemistry perspective. It will provide an overview of the legislation and scheduling of Tetrahydrocannabinol (THC) -related compounds in the United States, discuss drug trends in semi-synthetic cannabinoids in both Europe and the United States, and review analytical methods for the separation of THC isomers in seized drug material. This workshop will also focus on the known pharmacological activity and potency of currently available semi-synthetic cannabinoids, provide reviews of cross-reactivity of the cannabinoid isomers and analogs on the commercially available immunoassay platform, and describe analytical methods for isomer separation in toxicological samples. Finally, delta-8-THC blood concentrations and trends in the Driving Under the Influence of Drugs (DUID) cohort will be presented.[1,2]

After attending this presentation, attendees will be familiar with semi-synthetic cannabinoids such as delta-8-THC, THC-P, HHC, etc., their appearance in various cannabis-containing products, their potency, and their legality. Additionally, attendees will understand analytical challenges associated with these newly emerging THC isomers and analogs and how to resolve chromatographic interferences.

Impact Statement: This presentation will impact the forensic science community by providing a legal, pharmacological, and analytical overview of emerging semi-synthetic cannabinoid products.

The 2018 Farm Bill legalized industrial hemp production in the United States, making cannabis and cannabis-derived products containing less than 0.3% delta-9-THC more commercially accessible. This also led to overproduction of Cannabidiol (CBD), which can serve as a precursor for synthesis of psychoactive isomers and analogs of THC. Cannabis manufacturers have exploited legislative loopholes since the Farm Bill's introduction and successfully promoted products containing THC isomers and analogs whose legal status is unclear, or variable, state to state, as an attractive alternative to the clearly federal Schedule I drug, THC. Products are often advertised to contain THC isomers or analog such as delta-8-THC, delta-10-THC, delta-6a,10a-THC, exo-THC, THC acetate (THC-O), THC-P, and HHC, among many others; however, upon analysis, these products could contain not only delta-9-THC but also a combination of compounds. This leads to analytical challenges as well as interpretation challenges.

Edibles, candies, baked goods, personal care products, beverages, dietary supplements, e-cigarette liquids, and other cannabis-derived products containing <0.3% THC and other semi-synthetic THC derivatives manufactured from CBD have proliferated and are sold not only at smoke shops, but also at gas stations, in malls, and in boardwalk storefronts, providing consumers with ample options for purchasing products containing psychoactive THC isomers.

References:
1. Chan-Hosokawa A., Nguyen L., Lattanzio N., Adams W.R. Emergence of Delta-8 Tetrahydrocannabinol in DUID Investigation Casework: Method Development, Validation and Application. *J Anal Toxicol*. 2022 Feb 14; 46(1):1-9. doi: 10.1093/jat/bkab029. PMID: 33754645.
2. Chan-Hosokawa A., Nguyen L., Logan B.K. Estimation of Delta-8 Tetrahydrocannabinol (THC) Concentrations in DUID Investigation Casework. *J Anal Toxicol*. 2023 Feb 21; 47(1):e14-e16. doi: 10.1093/jat/bkac068. PMID: 36056934.

Cannabinoids; Isomer; Analogs

W8   Non-Fatal Strangulation: An Update on Practice and Current Literature

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Learning Overview: "He only choked me . . . " Anoxic brain injury can occur in minutes. Non-fatal strangulation and its associated effects are not well understood by responding providers. This potential lethal act is present in more cases than one thinks. The workshop presenters plan discussions around how the current evidence impacts the investigation, documentation, care, and prosecution of strangulation cases. Long-term effects such as traumatic brain injury are now being studied, with current information to be discussed. Key concepts will be illustrated through the use of case studies and group discussion. We will: (1) discuss the prevalence of non-fatal strangulation; (2) appraise current literature related to addressing non-fatal strangulation in the clinical arena; (3) associate linkages between non-fatal strangulation and traumatic brain injury; and (4) evaluate assessment and documentation of non-fatal strangulation through the use of case studies.

Impact Statement: Many disciplines that investigate, provide care, or prosecute cases where non-fatal strangulation is a component will benefit from understanding this topic. Investigators and crime scene personnel will be able to better recognize and investigate this potentially lethal act. Health care providers must properly assess and treat those who have experienced strangulation. Finally, prosecutors must develop knowledge about how to charge and prosecute for this action and to effectively use the expert witness to convey the significance that strangulation has on the victim.

Strangulation is a lethal form of violence and occurs in intimate partner violence, sexual assault, and other types of physical assault. Since the early 2000s, non-fatal strangulation has been recognized as a method of control, harm, and even death. Victims often do not disclose that strangulation has occurred unless asked by the health care provider or first responder. It is critical that first responders, law enforcement, and crime scene analysts understand injury mechanisms and the health outcomes that may affect a criminal investigation. Health care must recognize the signs and symptoms to determine an appropriate plan of care for both the short- and long-term effects.

Non-fatal strangulation has two primary mechanisms of action. One prevents inflow and the other prevents outflow of either blood or oxygen. The presentation and symptoms are different. Some symptoms of non-fatal strangulation are memory loss, confusion, difficulty breathing, and trouble talking. Non-fatal strangulation may result in anoxic brain injury where consequences include seizures and loss of bowel and bladder function, to name a few. Understanding the strangulation event is the first step in the response. This workshop plans to take participants step by step through what occurs in a non-fatal strangulation case and the response necessary to discover what actually happened.

"Audrey" reports her boyfriend pushes her against the wall and puts his arm around her throat. "Sandra" says her husband puts both of his hands around her throat, shaking her when they argue. She reports she has passed out. Marcus" says that the neighbor bully knocks him to the ground and puts him in a choke hold where he cannot breathe. These are all forms of non-fatal strangulation.

In developing the skills to assess and document strangulation events, health care delivery and investigations will become more robust. As prosecutors understand the magnitude of the long-term effects of non-fatal strangulation and associated traumatic brain injury, charges may be better determined. Expert witnesses are better utilized to describe the physical and psychological effects. Workshop presenters will provide information that will assist professionals who encounter cases of non-fatal strangulation to better be prepared to respond to the unique needs of this population.[1-9]

References:
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2. Donaldson, A. E., Hurren, E., Harvey, C., Baldwin, A., & Solomon, B. (2023). Front-line health professionals' recognition and responses to nonfatal strangulation events: An integrative review. *Journal of Advanced Nursing* (John Wiley & Sons, Inc.), 79(4), 1290–1302. https://doi.org/10.1111/jan.15601.
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9. Wilkes, N. (2023). The Pursuit of Medical Care for Female Victims of Nonfatal Strangulation at the Time of Police Response. *Violence Against Women*, 29(2), 388–405. https://doi.org/10.1177/10778012221140133.

Non-Fatal Strangulation; Traumatic Brain Injury; Violent Behavior

W9 **The Requirements and Implementation of ANSI/ASB Standard 123—Standard for Routine Internal Evaluation of a Laboratory's DNA Interpretation and Comparison Protocol: Laboratory and Legal Perspectives**

Charlotte Word, Richmond, VA; Virginia Barron, Law Office, Westbrook, MN; Terri Rosenblatt, Post Conviction Justice Unit, New York County District Attorney's Office, New York, NY; Brian Higgins, USACIL, Forest Park, GA; Ashley Luther, Indiana State Police Laboratory, Indianapolis, Indianapolis, IN

Learning Overview: After attending this workshop, attendees will have a heightened understanding of: (1) the need to monitor the efficacy and consistency of use of the laboratory DNA interpretation and comparison protocols; (2) the specific requirements of Standard 123 and how to implement the standard in a DNA testing laboratory effectively in conjunction with other relevant DNA standards; (3) how to assess the results of the internal evaluation process with appropriate corrective actions; and (4) the legal perspective regarding the consistent use of high-quality interpretation and comparison protocols prior to and post-conviction and how that may help to provide "Justice for All." The attendees will have the opportunity to network with the speakers and other attendees while discussing implementation options.

Impact Statement: This workshop will impact the forensic science community and the "Justice for All" initiative by providing information to laboratory staff, attorneys, and judges regarding the value of performing an annual review of the forensic science service provider's interpretation and comparison protocols. Guidance for monitoring and evaluating the consistency of use and suitability of the protocol within a DNA laboratory via implementation of the American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 123, Standard for Routine Internal Evaluation of the Laboratory's DNA Interpretation and Comparison Protocol, and its possible effect on the judicial system will be the main focus of this workshop.

Several studies conducted over the past 20 years have demonstrated the inconsistent use of the interpretation and comparison protocols within some DNA testing laboratories as well as variability in the reporting of results and conclusions, especially when complex DNA profiles were evaluated that fell outside of the types of profiles routinely evaluated during mandatory proficiency testing. A standard, drafted by the Organization of Scientific Area Committees (OSAC) and developed by the ASB, provides for the continued monitoring of the efficacy and consistent use of the laboratory's protocol. This workshop will focus on the specific requirements of Standard 123 and provide effective mechanisms for implementation of the standard, including the evaluation of the resulting data to ensure quality interpretation and comparison of DNA data. DNA analysts and attorneys will provide insights regarding the importance of and value added through the implementation of this standard.

DNA; Interpretation; Quality Assurance

W10   Evolving Approaches and Technologies to Address Existing Challenges in Seized Drug Analysis

J. Tyler Davidson, Sam Houston State University, Conroe, TX; Edward Sisco, National Institute of Standards and Technology, Gaithersburg, MD; Ira Lurie, The George Washington University, Washington, DC; Frances Scott, National Institute of Justice, Washington, DC; Amber K. Burns, Maryland State Police Forensic Sciences Division, Pikesville, MD; Sherri L. Tupik, Drug Enforcement Administration, Dulles, VA

Learning Overview: After attending this workshop, attendees will have learned about readily implementable evolving approaches and technologies to address existing challenges in seized drug analysis. The attendees will learn about emerging technologies, such as Direct Analysis in Real Time-Mass Spectrometry (DART®-MS), Gas Chromatography-Vapor-Phase Infrared spectroscopy (GC-VIR), Gas Chromatography-Vacuum Ultraviolet spectroscopy (GC-VUV), and Ultra-High Performance Liquid Chromatography-Photo Diode Array Ultraviolet single quadrupole Mass Spectrometry (UHPLC-PDA UV-MS). In addition, attendees will also learn about evolving approaches, such as microcrystal tests and the analysis of marijuana and marijuana products.

Impact Statement: This workshop will impact the forensic science community by providing chemists, section leaders, and laboratory managers with the necessary information to determine which evolving approach or technology may be most appropriate to address their specific laboratory needs. Aspects such as the approximate cost, analysis time, capabilities, limitations, and specific challenges that each approach or technology can help address will be provided. Informing the seized drug community about the strengths and limitations of the identified evolving approaches and technologies will better prepare forensic laboratories to make informed decisions about how to combat their specific challenges in seized drug analysis.

Challenges with the analysis of seized drugs have necessitated research into alternative approaches and technologies to assist forensic laboratories with the identification and quantification of unknown seized drugs. These challenges include the rapidly evolving nature of the seized drug landscape, with the continued emergence of Novel Psychoactive Substances (NPS), the identification of closely related chemical substances, the detection of minor components in mixtures, the differentiation of hemp and marijuana, screening of multiple subunits, and growing backlogs. However, there is a considerable divide between the research community developing these alternative approaches and technologies and actual implementation by practicing forensic laboratories. The Evolving Approaches and Technologies for Seized Drug Analysis subcommittee was established to specifically address this divide by the Forensic Laboratory Needs Technology Working Group (FLN-TWG), which is housed at the National Institute of Justice (NIJ) and supported by the Forensic Technology Center of Excellence (FTCOE) through a cooperative agreement with RTI International.

This workshop provides a summary of the subcommittee's developed technical notes and overarching white paper designed to help address the divide between the research community and practicing forensic laboratories. During this workshop, attendees will learn about readily implementable evolving approaches and technologies that are either not widely known or are currently underutilized. These approaches and technologies have been identified by the subcommittee as potential solutions to address existing challenges with the analysis of seized drugs. Each approach and technology will be introduced, including the principles of operation or analysis, approximate cost and analysis time, and specific capabilities and limitations as they pertain to seized drug analysis. In addition, this workshop will help address implementation needs, including a discussion about installation requirements, user training, and the availability of validated methods and searchable libraries.

Drug Analysis; Novel Psychoactive Substances; Education

W11 Police Officers Injured in the Line of Duty: The Role of Peer Support in Recovery

Gregory I. Mack, Psychological Evaluation Section, Medical Division New York City Police Department, Shrub Oak, NY; Daniel A. Martell, UCLA School of Medicne, Irvine, CA; Learie C. Johnston, Westchester B.L.U.E. Foundation/Mount Vernon Police Department, Mount Vernon, NY; Matthew Frank, Westchester B.L.U.E. Foundation, Port Chester, NY; Paul Puccini, Westchester B.L.U.E. Foundation, New Rochelle, NY; Jamie M. Elifritz, Forensic Radiology Group/Department of Radiology, University of New Mexico, Albuquerque, NM

Learning Overview: After attending this workshop, attendees will: (1) better understand the experiences of police officers who have been severely injured in the line of duty; (2) build skills to identify the many challenges one can face while recovering from a Traumatic Brain Injury (TBI) caused by a gunshot wound to the head; (3) be made aware of the physical damaged caused by a Gunshot Wound (GSW) to the head; (4) appreciate the effects of TBI on cognitive and psychological assessment protocols; (5) be informed about the current empirical research and literature on peer-assistance programs, peer support, and peer-facilitated interventions for police officers; and (6) have a better understanding of the role social support, gratitude, resilience, and satisfaction with life have on recovery.[1-4]

Impact Statement: Presentations in this workshop will impact the forensic science community through detailed accounts of police officers who were injured in the line of duty. Through their first-hand accounts, these officers will discuss their road to recovery and associated psychological sequela and its impact on their families. The forensic science community was previously introduced to one of the officers (LCJ) through a presentation at the 75th Annual AAFS conference. This workshop will also impact the forensic science community by providing the most up-to-date information on the role, responsibilities, and best practices associated with a Peer Support Program.

This presentation will provide an overview of the of circumstances that led up to the wounding of New York police officers and the challenges that they faced and still endure. We will engage in an interactive discussion with these officers and learn how they faced aspects of their physical and psychological recovery and the role other factors played in their seeking help such as individual characteristics, the availability of peer support, stigma, family, and social support, gratitude, resilience, and satisfaction with life. Special focus will be given to a traumatic brain injury caused by firearm. This workshop will discuss topics requiring basic knowledge pertaining to brain anatomy, brain function, and cognitive and psychological effects of TBI.

In their July 2023 "Law Enforcement Officers Shot in the Line of Duty" report, the National Fraternal Order of Police reported that there have been 226 officers shot in the line of duty, of which 31 were killed. Sixty-seven of these officers were shot and wounded and 14 were killed in 69 separate ambush-style attacks.[5] Officers who survive these devastating injuries face many long hours of rehabilitation and recovery. If an officer survives a deadly encounter such as a gunshot wound to the head, the road to recovery is unimaginable.

Ninety percent of victims of a gunshot wound to the head die. Many die before they make it to the hospital. Fifty percent of those victims who do survive the initial trauma end up dying in the emergency room. Gunshot wounds to the head account for 12% of all TBI and are the cause of death in an estimated 35% of all deaths attributed to TBI.[6]

On October 30, 1993, 23-year-old Mt. Vernon, NY, police officer LCJ was shot with a .380 caliber handgun. He was struck by a single round that entered the left frontal side of his head without exiting. The officer was in a coma for several weeks. LCJ sustained severe neurological damage. During his initial recovery and rehabilitation, he had to relearn how to speak and ambulate and was left with partial paralysis of the right side of his body. He was subsequently retired at the rank of detective.

On April 20, 2006, a Mount Vernon, NY, detective was walking out of the police interview room after questioning a 33-year-old Bronx man in connection with the April 14 killing of a local businessman when the man jumped out of his chair and grabbed the detective's gun. A life-and-death struggled ensued, and the man was able to get his hands on the trigger and fired a shot that penetrated the detective's left foot.

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Traumatic Brain Injury; Police Peer-Support Programs; Resilience

W12   Reading the Blueprint to Construct the Framework: Demystifying Forensic Accreditation

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Learning Overview: After attending this workshop, attendees will understand some principles of International Organization for Standardization (ISO) accreditation programs and quality management, necessary elements of the requirements in each standard (ISO/IEC 17020 and ISO/IEC 17025), and guidance on how to meet the requirements. Attendees will be provided with resources to help them develop an effective quality management system and become an accredited Forensic Service Provider (FSP).

Impact Statement: This presentation will impact those in forensic science who are considering accreditation, or who are beginning the process of obtaining accreditation, by explaining the pieces needed to build a framework of accreditation. It has been over a decade since the National Academy of Sciences (NAS) published recommendations to the forensic science community about how forensic science can be strengthened in the United States, which includes accreditation of FSPs.[1] Since then, accreditation of Forensic Science Service Providers (FSSPs) (e.g., Crime Scene Investigator [CSI] units, crime labs, latent print units, etc.) has been endorsed by the National Commission on Forensic Science (Recommendation to the Attorney General Universal Accreditation 2015), forensic science professional organizations (e.g., the American Academy of Forensic Sciences [AAFS], the American Society of Crime Laboratory Directors [ASCLD], the Association of Forensic Quality Assurance Managers [AFQAM], the International Association for Identification [IAI]), and even mandated by organizations such as the United States Department of Justice and state forensics oversight boards (e.g., New York, Texas). Many conference presentations and webinars have been given and reports written that advocate for accreditation in FSPs, giving explanations for why it is beneficial to an organization, but what comes next? Where do they start? What do these standards mean? How are they supposed to meet them? This workshop will provide attendees with answers to these questions as well as practical guidance and considerations when building the quality assurance framework to obtain accreditation.

This workshop will provide an overview of the Framework for Accreditation of Forensic Units developed as part of cooperative agreement 70NANB21H098 with the National Institute of Standards and Technology (NIST) that can be used by FSPs through the accreditation process.

The workshop will review the benefits, and the drawbacks, to accreditation for an organization, particularly with respect to CSI and latent print units. It will also discuss why accreditation of FSSPs is important to the criminal justice system, using practical examples in forensics as well as other industries. Attendees will learn what a forensic accreditation program consists of and the structure of the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17020 and ISO/IEC 17025 standards used to build a framework, along with the similarities and differences between the two. Attendees will learn how to approach building a quality management system and the overall accreditation process as well as tips for reading and interpreting standards.

Case studies about CSI units and small labs, some with only one or two employees, obtaining accreditation will be presented and discussed. Resources and ideas to help the attendee design and build their quality management systems to become accredited will be shared. Chances are their organization currently meets many of the requirements, and they may just need to document them. Participants should leave with a better understanding of quality management and accreditation in forensic science and their importance and feel more confident in the possibility of incorporating both into operations in their units and labs.

Accreditation of CSI units is important because the scene investigation is the main gateway for the recognizing, recording, collecting, transporting, and storage of forensic evidence, which can have lasting impact on the analysis, interpretation, reporting of results, and ultimately the outcome of the investigation. Accreditation also gives the criminal justice system and the public confidence in the agency's results and ensures that the agency maintains impartiality. Accreditation is a formal recognition that an agency has implemented internationally recognized standards and best practices and demonstrates and maintains competency with a commitment to continuous process improvement. The 2009 National Academy of Sciences (NAS) Report documented the need for mandatory accreditation for forensic disciplines, including many that police agencies and identification units engage in.[1] This is particularly important in the practice of CSI and forensic science as, unlike other industries, there is no mandatory regulatory approach to ensuring quality. Therefore, a voluntary standardization approach is important for maintaining public trust and transparency.

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Standards; Forensic Accreditation; Forensic Service Provider (FSP)

W13   Beyond the "Who Done It . . . ." DNA Interpretation Given Activity Level Propositions

Tim Kalafut, Sam Houston State University, Department of Forensic Science, Huntsville, TX; Simone Gittelson, District of Columbia Department of Forensic Sciences, Washington, DC

Learning Overview: After attending this workshop, attendees will be able to communicate expert knowledge on the evaluation of DNA evidence given activity level propositions using a scientifically sound framework and published practices. Participants will understand how to apply the Case Assessment and Interpretation (CAI) framework to perform case pre-assessments, formulate activity level propositions, and assign likelihood ratios for biological evidence given activity level propositions. This knowledge provides attendees with tools to handle hypothetical questions commonly asked in court, how to recognize poorly worded questions, and provide answers to these hypotheticals in a scientifically correct manner.

Impact Statement: This presentation will impact the forensic science community by providing guidance for real-world testimony on how to handle DNA testimony when the court's interest is beyond that of an "inclusion" and its associated sub-source likelihood ratio.

This workshop will demonstrate peer reviewed, published methods for evaluating DNA evidence given activity level propositions as well as related testimony at court. DNA testimony has moved past the "Who?" questions related to the source of the DNA and it is now common for questions to be asked about "How?" the DNA ended up on the evidence item. While this is often done using hypothetical questions designed to elicit vague answers about "possible" or "could have" or "consistent with", there are published frameworks and reporting and testimony guidelines available to assist both experts and the court. This workshop will cover the theory and application of Case Assessment and Interpretation (CAI) and Bayesian networks (BN) to evaluate DNA evidence profiles given activity level propositions and the communication of these results via report writing and testimony. A combination of lectures, demonstrations, and hands-on group activities will be used in this workshop. Challenges in both the evaluation of the evidence and testimony in an adversarial court system will be addressed as well.

There are numerous misconceptions and misunderstandings regarding the interpretation of DNA evidence given activity level propositions. Questions in court often focus on hypothetical DNA transfers (e.g., "Is it possible that DNA transferred during such-and-such activity?"), though many people don't realize that by answering, they are testifying about activity level propositions. This workshop will demonstrate how to interpret DNA evidence and share expert knowledge using a scientifically sound framework and published practices when questions about the evidence relate to what may have happened.

In the morning session, participants will learn (1) how to formulate activity level propositions in accordance with the 2020 recommendations of the DNA Commission of the ISFG [1], (2) conduct a case pre-assessment using the Case Assessment and Interpretation (CAI) framework [2,3], and (3) assign likelihood ratios for DNA evidence given activity level propositions. After these lectures, the participants will understand the difference between Transfer-Persistence-Prevalence-Recovery (TPPR) and an activity level proposition. In addition, an introduction to Bayesian networks will provide the participants with an understanding of what Bayesian networks are, and how this approach may be used to assign the probabilities in the numerator and denominator of a likelihood ratio.

In the afternoon sessions, participants will first explore a hands-on exercise to apply this newly acquired knowledge and skill set on a mock case. Strategies for how to read forensic DNA literature to inform probabilities will be discussed. In the second half of the afternoon, the workshop will cover court testimony of biological evidence. In this part of the workshop, the participants will learn to handle the hypothetical questions that are commonly asked in court, and importantly, learn how to model responses to hypothetical questions.

After attending this workshop, participants will be able to recognize poorly worded questions and how to answer them in a scientifically correct manner by testifying on the evidence rather than on what happened (i.e., avoid the transposed conditional). The workshop will conclude with a discussion and a question and answer session. This workshop will provide attendees with examples of the scientific framework, knowledge, and skills to effectively evaluate DNA evidence given activity level propositions and to correctly present this information in court testimony.

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Forensic DNA; Evaluation; Activity Level

W14   Applications of Digital Forensic Audio/Video for Real Life Challenges With DeepFake Video, Audio, and Artificial Intelligence

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Learning Overview: After attending this workshop, attendees will: (1) be familiar with the latest developments in forensic audio analysis and authentication; (2) understand criteria used for media authentication; and (3) understand how to conduct analysis within a forensic framework, and (4) multiple frameworks to use for DeepFake video detection.

Impact Statement: This workshop will impact the forensic community by: (1) explaining the scientific approach in forensic audio authentication; (2) demonstrating an authentication investigation framework; (3) discussing tools used to combat multimedia forgery; (4) showing multiple frameworks to use for DeepFake video detection.

Digital multimedia authentication seeks to determine the validity of digital multimedia containers and contents by investigating their format, structure, time, frequency, pixel, and/or sample level features. This workshop will discuss the digital audio and video analysis process providing the user with methods of detecting and authenticating audio, including DeepVoice and DeepFake videos. It will also demonstrate the incorporation of multiple tools and techniques into unified frameworks appropriate in forensic examinations where reducing examiner bias and error is crucial.

Section 1—Audio: We propose a one-hour workshop covering audio analyses and authentication. The goal of this workshop is to provide an overall view of conducting comprehensive examinations that rely on the results of multiple analyses to inform an ultimate finding or opinion.

The workshop will focus on real-life audio challenges and solutions. The proposed audio authentication frameworks combine both container and content analysis to determine authenticity of the recording as well as the purported source.[1,2] Audio container analysis will exploit characteristics of the multimedia file format and structure while content analysis will cover time and frequency domain techniques including quantization level, power, direct current offset, butt splice, spectral, and Multidetector Computed Tomography (MDCT) analysis.

Section 2—Video: We will discuss the use of Artificial Intelligence (AI) to detect deepfakes with explainable methods. We will also cover other methods for analyzing deepfakes, including:

- Blood flow analysis can be used to detect deepfakes by looking for inconsistencies in the blood flow of the face. Deepfakes often have unnatural blood flow patterns, which can be identified by AI-powered software.

- Lip sync analysis can be used to detect deepfakes by looking for inconsistencies between the movement of the lips and the audio track. Deepfakes often have lip movements that are not synchronized with the audio, which can be identified by AI-powered software.

- Electric network frequency analysis can be used to detect deepfakes by looking for inconsistencies in the electric network frequency of the video. Deepfakes often have a different electric network frequency than the original video, which can be identified by AI-powered software.

- Photo Response Non-Uniformity (PRNU) analysis can be used to detect deepfakes by looking for inconsistencies in the PRNU pattern of the image. PRNU is a unique pattern of noise present in all cameras. Deepfakes often have a different PRNU pattern than the original image.

- Visual analysis can be used to detect deepfakes by looking for inconsistencies in the appearance of the face. Deepfakes often have unnatural skin texture, lighting, or shadows. Visual analysis can be done by humans or by AI-powered software.

We will also discuss the limitations of each of these methods and how they can be used together to improve the detection of deepfakes. We hope this workshop will provide you with a comprehensive overview of the methods available for detecting deepfakes.

References:
1. *SWGDE Best Practices for Digital Audio Authentication*. Version: 1.3 (September 20, 2018).
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DeepFake; Digital Forensics; Audio Video

W15   To Tell the Truth: Perspectives on the Practice of Courtroom Testimony

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Learning Overview: Upon completion of this workshop, attendees will be better able to understand and perform one of the most challenging parts of any forensic practice—courtroom testimony in its many forms, be it civil or criminal; federal, military, or state; and deposition or trail.

Impact Statement: This workshop will impact the forensic science community by providing a multidisciplinary look at the why and how of the practice of courtroom testimony, including recognizing potential challenges and pitfalls.

This session is a multidisciplinary workshop focused on courtroom testimony. The sessions are geared for all levels of experience from novice to seasoned professionals. While the speakers will focus on subjects that include training, preparation, cognitive bias, courtroom technology, and "high-profile" cases, the sessions will explore criminal versus civil, deposition versus trial, state versus federal versus military, etc. Various perspectives from the different stakeholders involved are addressed—judge, prosecutor, defense, professional, and consultant.

Regardless of the particular section, all the forensic sciences converge in the courtroom with testimony regarding the findings. While there are expected customs and duties assigned to the various parties involved, there is not—nor can there actually be—any single "standard" for a witness and how they tell their story. While the basics are generally well-known, the court officials have various interests in their individual takes of "the truth, the whole truth, and nothing but the truth." Certain topics can affect that sworn duty: training, preparation, cognitive bias, courtroom technology, and "high-profile" cases.

In our adversarial justice system, the judge – as the gatekeeper – is the arbiter of what is allowed. In the criminal courts, the jurisdiction sets the rules, with an overall movement toward the Federal Rules of Evidence. The prosecution has specific duties to provide information to the defense. Generally, the government has as its witnesses those government personnel already assigned to the task as day-to-day forensic scientists employed by the venue's forensic laboratory. On occasion, consulting experts are called in to assist with the case, but this can prove expensive and be a "difficult sell" to those in control of finances, since the work has already been done and paid for by a regular employee. The defense generally can seek out an expert, but there are various constraints they potentially face, two significant ones being funding and availability. In civil proceedings, attorneys for the plaintiff and respondent are both reliant on those same government employees, but both sides are free to and do routinely engage consulting forensics practitioners.

Regardless of the venue, information is elicited from witnesses by means of a series of questions intended to provide the desired information *in a specific way* and *in a specific order. How* information is elicited varies widely, depending on the attorney's strategy. The witness is limited in how they are allowed to respond, with the judge serving as final authority as to what is responsive to the question asked. A witness cannot volunteer information but should only respond to the question asked. Attorneys for both sides should be fully cognizant of the facts and should follow up as needed to fully provide the information sought from the witness. Prosecutors additionally have a duty to provide any information they believe to be potentially helpful to the defense. The witness, unlike counsel, is not an advocate but should be an objective—a neutral analyst who looks at the data in a specific case and then provides a reasoned interpretation of the information with their ultimate conclusion. Although the mechanics are similar across all jurisdictions and in all courts, human personalities come into place to make the whole process even more interesting.

This workshop will focus on the many factors that make up court testimony. Various perspectives from the different stakeholders involved are addressed, with special focus on training, preparation, cognitive bias, courtroom technology, and "high profile" cases. The purpose is to better understand and perform one of the most challenging parts of any forensic practice—courtroom testimony.

Evidence; Lawyer; Court

W16   Statistics and Forensic Science: From Probability to Black Box Studies to Likelihood Ratios

Linton Mohammed, Forensic Science Consultants, Inc., Poway, CA; James Green, Eugene, OR; Michael J. Salyards, Compass Scientific Consulting LLC, Tucson, AZ; Hal S. Stern, University of California, Irvine, Irvine, CA

Learning Overview: After attending this workshop, attendees will: (1) be familiar with the language and key concepts of probability, including the role of populations and sample, laws of probability, dependence/independence of events, conditional probability and Bayes' rule, and the Likelihood Ratio (LR); (2) be familiar with the language and key concepts of statistical inference, including the role of data collection, measurement, error rates, reliability and validity; (3) understand issues associated with the design, execution, and interpretation of black box studies; (4) understand statistical hypothesis testing and its relevance for the two-stage approach for analyzing forensic evidence; and (5) understand the likelihood ratio/Bayes factor approach to assessing forensic evidence along with its strengths and weaknesses.

Impact Statement: Understanding the applications of statistics by the forensic science community will assist practitioners, attorneys, and jurists in evaluating evidence and opinions. This workshop will impact the forensic science community by leaving attendees in a position to better understand and engage in ongoing discussions (in the Organization of Scientific Area Committees [OSAC] and other places) about appropriate approaches to assessing forensic evidence and reporting forensic conclusions.

This workshop introduces key concepts in probability and statistics for forensic practitioners by linking them to key topics that are currently impacting the forensic community. The target audience is anyone from the forensic science community (practitioners, lawyers, judges, advocates) with a desire to better understand ongoing conversations about error rates, black box studies, inconclusive findings, and LRs.

The analysis of many types of forensic evidence, including handwriting, firearms (bullets and cartridge cases), latent prints, and glass fragments, requires the comparison of items, usually one of questioned origin and one or more from a known source, to assess their similarity and probative value. Tools from probability and statistics play a critical role in the logical assessment of forensic evidence.

The workshop is organized into four sections. The first section reviews the definition of probability and key concepts, including conditional probability, independence, and Bayes Rule that are relevant to forensic scientists. The concepts are illustrated through court cases where they arise naturally.

The second session addresses data collection (statistical sampling), design of experiments and studies, and statistical measurement. Important measurement concepts like bias, variance, reliability, and validity are discussed. This leads naturally to a discussion of the strengths and weaknesses of black box studies. Results from some of the published black box studies are reviewed.

The third section reviews more formal statistical concepts like estimation and hypothesis testing. These are discussed primarily in the context of a two-stage approach to the analysis of forensic evidence; the two-stage approach first makes a determination as to whether a questioned and known sample can be distinguished, then attempts to assess the probative value of the resulting conclusion.

The final part of the workshop addresses the LR framework for assessing forensic evidence. The LR has been used in the analysis of DNA evidence for many years. This section of the workshop reviews the motivation for the LR, the way it works in DNA, the challenges associated with using the LR for pattern evidence, and the relationship of the LR with other approaches. Newer approaches that rely on scores (e.g., FRSTATS) are also discussed.

The workshop is intended to leave participants in a position to better understand and engage in ongoing discussions (in Academy Standards Board [ASB], OSAC, and other places) about appropriate approaches to assessing forensic evidence and reporting conclusions.

Statistics; Forensic Science; Evaluation

W17 Crime Scene Reconstruction: Using Critical Thinking, Logic, and Reflection

David Pauly, Methodist University, New London, NC; Steve Downs, Methodist University, Fayetteville, NC; Bryan W. Brendley, Methodist University, Greensboro, NC; Douglas Young, Thornton Police Department, Broomfield, CO

Learning Overview: The goal of this workshop is to assist attendees in understanding and implementing the scientific method of inquiry to criminal investigations and forensic science problems by the use of physical evidence, objective data/information, artifacts of affect, and methods/processes of reasoning, logic, and cognitive learning within the contextual bounds of where this data/information is located, to derive knowledge regarding a particular incident and, when possible, to provide a chronological sequence to the incident.

Impact Statement: This workshop is critical to all forensic scientists, crime scene investigative personnel, detectives, pathologists, and attorneys charged with investigating violations of law. By understanding logic, critical thinking, and reasoning, the law enforcement and forensic professional will better understand how their own biases and/or lack of knowledge and understanding can impact this forensic science community's decision-making process that could lead to a flawed outcome or conclusion.

This workshop is applicable to all forensic science disciplines and will begin with an overview of forensic reconstruction in a broad sense, followed by detailed information on how people process information to come to a conclusion, and culminating with a hands-on practical exercise involving all attendees who will implement the concepts studied and presented. During this presentation, attendees will be instructed on the background of reconstruction and how, by using a multidisciplinary systematic method of inquiry to problem-solving, they will be more likely to come to a logical and factual outcome.

Since the mid-1950s, the United States population has nearly doubled to an estimated 330 million citizens. Crime of all types remains a significant problem, with most metropolitan centers having a higher concentration. Estimates place law enforcement solve rates of most crimes below 20%, with homicide hovering around 60% nationally.

Technology has exploded in law enforcement with the use of aerial drones, 2D panoramic and 3D scanners, along with portable alternate light sources being more available and utilized than ever before. Despite all the advances in technology, there remains a need for law enforcement and forensic science professionals to understand *Reconstruction*. Reconstruction is most thought of as the physical reconstruction of a motor vehicle collision, bloodstain pattern, fire scene investigation, aviation disaster, or bullet trajectory.

This workshop will begin by introducing attendees to the fundamentals of how a person thinks, how they process information, and some of the pitfalls of drawing a conclusion without sufficient knowledge. Evaluating a crime scene, damage to a vehicle, or injury to a body remains a crucial part of crime solving, including the process of refuting that a crime occurred as alleged, or at all. This workshop will provide attendees with a better approach to all investigations by providing a better foundation to problem solving that directly impacts the physical reconstruction process.

Attendees will receive instruction on the implementation of the scientific method of inquiry to criminal investigations and forensic science problems by use of physical evidence, objective data/information, artifacts of affect, and methods/processes of reasoning, logic, and cognitive learning within the contextual bounds of where this data/information is located, to derive knowledge regarding a particular incident and, when possible, to provide a chronological sequence to the incident.[1-11]

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Decision Making; Shootings; Bloodstains

W18   Campus Shootings in America: Is There More We Can Do?

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Learning Overview: The goal of this presentation is to provide an overview of campus shootings in America and continue with legislative and legal updates through case analyses. This workshop will explore gender, mental illness, behavior, victimology, and weaponry. Attendees will be able to assess their role in making positive and effective changes to policy, practice, and research within and among multiple disciplines. Attendees will have the opportunity to discuss their existing practices and brainstorm alternates throughout the presentation.

Impact Statement: This presentation will impact the forensic science community by reminding all attendees of their role in making positive and effective changes to eliminate campus shootings by focusing on our historical, at the time of, and post-incident responses and by using case analyses to encourage accountability and promote real-time collaborative research and practices within multiple disciplines.

The goal of this presentation is to create an open and honest dialog about shootings on campuses across the United States. "Violence . . . crosses racial and gender boundaries, as these horrific acts affect every racial group, social class, ethnicity, gender, and any time other category one can image."[1] The forensic science community and the criminal justice system as a whole has the responsibility of ensuring public safety through meaningful and structured analyses, collaboration, and behavior-based changes to prevention and responding to incidents.

The United States has seen staggering numbers of mass shootings since the 1960s and increasing incidents at educational institutions.[1] Mass shootings are statistically rare, accounting for less than 1% of all firearm homicides in the United States.[2] However, mass shootings on campuses are growing and leaving behind a trail of gross destruction that emotionally outweighs the number of incidents.

According to The Violence Project, most mass shooters experienced childhood trauma; were exposed to violence at a young age; had an identifiable grievance or crisis point; have studied the actions of past shooters and sought validation for their methods and motives; and had the means to carry out an attack.[2] A mass shooter profile is unique to their targeted location and often includes some level of mental illness. A one-size-fits-all profile does not exist.

Gender does play a minor role in the type of shooter, although most are male. According to a November 2011 United States Department of Justice Report, women committed just 6% of all murders with multiple victims, four studied under the Violence Project, and two of those cases involved a male partnership.[3]

There are signs of shooter crises. There are specific changes in behavior that are noticeable to others. There are frequent incidents where concerns about a shooter's behavior was identified and reported but not taken seriously or connected until after an incident.

Weaponry and access to firearms brings up much concern and debate. Handguns were present in over three-fourths of all mass shootings. However, at least one semiautomatic assault weapon, rifle, and/or shotgun were included.[2] Background checks are required, but sharing background check information has demonstrated inconsistency and, in some cases, failed completely.

This presentation will include an overview with case analyses. We will discuss law enforcement responses and what improvements can be made at all phases of an incident. Further, we will discuss the type of weaponry and how mental illness plays a role in the incidents and what practical implications can be derived from our case analyses through open dialog with the audience, hoping to reduce violence on campuses and improve related policy and practice through inclusive and diverse research and analyses.

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School Shootings; Weapon Analysis; Mental Health

W19   Forensic Scientists on the Front Lines

Agnes D. Winokur, Laboratory Director, DEA, Miami, FL; Barry K. Logan, PhD, Executive Director, CFSRE, Horshan, PA; Alex J. Krotulski, Associate Director, CFSRE, Abington, PA; Larry D. Fluty, Assistant Commissioner, U.S Customs & Border Protection, Montgomery, TX; Scott R. Oulton, Deputy Assistant Administrator, Drug Enforcement Administration, Springfield, VA; Jonathan McGrath, Principal Technical Advisor, U.S. Customs and Border Protection, Houston, TX; Sarah A. Shuda, Senior Scientist, CFSRE, Willow Grove, PA;

Learning Overview: After attending this workshop, attendees will be able to discuss examples of teamwork approaches that place forensic scientists on the front lines, addressing the challenges associated with the growing number of emerging drug substances and precursors, the complexity of the analysis, and the impact on enforcement and intelligence investigations. Attendees will be able to: (1) describe how forensic techniques can be used beyond simply reporting the presence of controlled substances to provide insights into changes in drug manufacturing and distribution practices; (2) evaluate the differences in analytical capabilities and workflow strategies provided in central laboratories versus front line laboratories; and (3) explain how forensic analysis impacts intelligence gathering to support investigations, health, and safety.

Impact Statement This workshop will benefit the forensic science community by highlighting the impacts of forensic science collaborations on investigations, epidemiology, drug intelligence, public health, and canine training and operations. It will also highlight how deployment of laboratories on the front lines strengthens the efficiency of these operations, utility of the results, and timeliness of testing.

Forensic science is typically practiced in the controlled environment of a laboratory. The laboratory setting can house high-tech instrumentation, offer a more stable environment for standardization and contamination control, and provide optimal conditions for peer review and quality systems implementation.

The needs, role, and impact of forensic science have been transformative, increasing its use by the investigative sciences, law enforcement, medical examiners and coroners, crime and death scene response, border agencies, and national security agencies, and even extending to other professional domains like canine detection teams, public health agencies, drug intelligence agencies, and others.

These initiatives require scientists to leave the laboratory setting and employ best practices to support these investigative efforts, explore new field-friendly technologies, develop creative ways to expand laboratory services to the field environment, rely on reach-back approaches to support what is feasible in the field, and make the best of difficult environments without sacrificing the quality and standards expected by the public and the courts for both investigative and forensic work.

This workshop will provide insights into various examples of forensic scientists working on the front lines supporting investigations or collection of intelligence that supports public health and public safety agencies. Examples include satellite laboratories from the Canada Border Services Agency, known as Designated Safe Sampling Areas, within Ports of Entry with high interdictions of illicit drugs, equipped with advanced real-time drug identification technology. Similarly, the United States Customs and Border Protection (CBP) established Forward Operating Laboratories, which utilize Fourier-transform infrared spectroscopy, Gas Chromatography/Mass Spectrometry (GC/MS), and colorimetric technologies to perform preliminary drug screening at United States border inspection sites, including international mail facilities. These compact, chemist-led labs are designed to provide border and law enforcement officers along with crime and intelligence analysts the advanced real-time drug identification needed to improve their ability to seize narcotics, collect intelligence information, decrease response-time of informing law enforcement partners, and support border security.

A key component of border security and drug interdiction is the use of specially trained canines to aid with screening and searches. CBP will present on their laboratory program to test, develop, and maintain narcotics and pseudo-narcotics training aids. The CBP program is developing science-based strategies to strengthen canine training and operations, using solid phase microextraction GC/MS to characterize the chemical odor profiles of target materials. This CBP canine program provides a model for how forensic laboratories can strengthen drug interdiction strategies.

Working in collaboration with customs officers to obtain authentic current drug seizures and test them for potency, purity, the presence of adulterants, and evidence of unreacted precursors and reaction byproducts, in addition to supporting the development of drug canine training aids, helps investigators identify changes in the methods of drug synthesis, packaging, and tableting. This workshop will feature data from a CBP collaboration with the Center for Forensic Science Research and Education (CFSRE) on fentanyl profiling to determine insights into changing drug manufacturing and smuggling practices. This will include a review of current synthetic methods for fentanyl and related compounds and the identification of analytical markers for different synthetic routes as well as data analytics on fentanyl signatures from various points of seizure. The CFSRE will also present data from collaboration with public health agencies, which provides a real-time look at what drugs and drug combinations are circulating at the street level. Scientists from the Drug Enforcement Administration (DEA) will also discuss their collaboration with CBP and Food and Drug Administration (FDA), bringing scientists to the front lines and expanding scientific expertise to supplement intelligence and investigative information, providing a more comprehensive and holistic picture of the illicit fentanyl supply.

Fentanyl; Canines; Forensic Analysis

W20   Moving Toward High Reliability in Forensic Science

Brian J. Gestring, 4n6services, Guilderland, NY; Laura C. Fulginiti, Maricopa County Office of the Medical Examiner, Phoenix, AZ; K. Scott Griffith, SG Collaborative Solutions, Roanoke, TX

Learning Overview: After attending this workshop, attendees will have learned to see, understand, and manage risk within their operations.

Impact Statement: The work product of forensic providers is critical to the criminal justice system. While there have been advances in overall quality over the years, forensic providers still find themselves reacting to adverse incidents as they become front-page headlines. This presentation will impact the forensic science community by informing attendees of proactive approaches that will prevent adverse events from occurring and mechanisms that can be put in place to quickly recover from these incidents if they do occur.

Socio-technical systems are made up of humans interacting with technology within the confines of an organizational culture. Forensic practice is socio-technical because it involves all these elements. Attendees will learn to see, understand, and manage risk in each of these elements to increase their overall organizational reliability and will be informed as to how to take steps to make their operations resilient.

In recent years, there have been significant advances in the quality associated with forensic practice. Systems are being put in place to manage quality assurance and quality control, but forensic practitioners are still inexperienced in managing risk.

In layman's terms, risk is the potential of something going wrong. As individuals, we manage risk every day, and our judgments differ based on the severity of the potential negative outcome. Our risk tolerance is higher when we rummage through the refrigerator evaluating expiration dates than when we evaluate getting an elective surgery.

For organizations to become reliable, they must learn how to evaluate the risk in each element of the socio-technical system. The first step in this process is seeing the risk. While there are many cognitive biases that can interfere with seeing risk, the most pernicious is outcome bias. If a specific behavior is "risky," it must be changed, regardless of the outcome. Unfortunately, the outcome of an adverse event will dramatically change an organization's response. We even see this in our society. The penalty for being caught driving under the influence is significantly different for drivers that are apprehended after a routine traffic stop than for those involved in fatal accidents.

Even if we see a risk, we don't always fully understand it. After the outbreak of the novel coronavirus (COVID-19), authorities were aware of the disease, but they did not fully understand how COVID-19 was different from a previous outbreak of respiratory disease that occurred in 2003.

Once risk is seen and understood, it then has to be managed. Risk avoidance is not always possible, so high-risk processes must have recoveries in place should an adverse event occur. In the medical field, administering certain medications can be a high-risk process and it can't be eliminated. Both technological and human processes can be put in place to try and prevent the wrong medication from being administered. Technologically, a system can be put in place to only dispense the medication and doses prescribed. Humans can also be used to double-check that the proper medication is being dispensed.

While these barriers and redundancies will make the system reliable, if the improper medicine or dose is inadvertently administered, having an antidote on hand will make the system resilient because it will allow the reliable system to quickly recover from an adverse event.

There is a pattern to how bad things happen and a science for preventing them. Moving toward high reliability and resiliency in forensic practice should include learning how to see, understand, and manage the risks associated with our work.

High Reliability; Risk; Resiliency

W21   Investigation and Certification of Intoxication Deaths for the Non-Pathologist

James R. Gill, Office of the Chief Medical Examiner, Farmington, CT; Laura Labay, NMS Labs, Langhorne, PA; Kelly Keyes, RTI, International, Yorba Linda, CA; Candace H. Schoppe, San Diego County Medical Examiner's Office, San Diego, CA

Learning Overview: The goal of this presentation is to introduce attendees to the essential components necessary for competent medicolegal death investigation and proper certification of suspected substance-related deaths. Experts from their respective fields will discuss death investigation, autopsy, and forensic toxicology. This program focuses on practical information that can be used by the non-pathologist and the pathologist-in-training to understand the necessary task of investigating and certifying these deaths. The importance of proper death certification for public health and safety purposes will be stressed. Upon completion of this educational activity, attendees should be able to: (1) recognize relevant investigative information and where to obtain it; (2) understand the role of the autopsy in these investigations; (3) critically interpret toxicology testing in the context of the entire case investigation; (4) understand the role of the forensic toxicologist; and (5) understand how drug intoxication deaths are certified and how they affect the public health and criminal justice systems.

Impact Statement: This presentation will impact the forensic science community by providing non-pathologists with practical information to help them understand the medicolegal investigative process, toxicological testing, and result interpretations to facilitate the recognition, investigation, and certification of drug intoxication deaths. Attendees will learn what investigative findings raise suspicion for an intoxication death, the importance of interpreting postmortem toxicology results in light of the investigative and autopsy findings, and the critical roles of the forensic toxicologist and forensic pathologist. Ultimately, this will lead to an appreciation of how accurate death certification impacts public health and the criminal justice system.

This workshop brings together experts in medicolegal death investigation, forensic pathology, and forensic toxicology to discuss the investigation and toxicological analysis when substance use is suspected, focusing on identifying and certifying these deaths. Common problems faced during the investigation, confounding or competing findings at autopsy, and pitfalls in postmortem toxicological testing and interpretation will be explored. To properly certify these deaths, experts will discuss the impact of information gained from the investigation of the circumstances, autopsy, and appropriate postmortem toxicological testing.

Over the past ten years, the number of accidental, substance-related deaths has increased to epidemic levels and constitutes a public health crisis. Annually, over 100,000 people have died from accidental drug intoxications in the United States in 2021 and 2022. This, combined with a workforce shortage of forensic pathologists, has further strained medicolegal death investigation systems and forensic toxicology laboratories. Despite these hindrances, the joint efforts of the medicolegal death investigator, forensic toxicologist, and forensic pathologist are still needed to investigate and certify deaths due to drug intoxications.[1]

Attendees will be introduced to the essential components necessary for competent medicolegal death investigation and proper certification of suspected substance-related deaths. This program focuses on practical information that can be used by the non-pathologist and the pathologist-in-training to understand the necessary task of investigating and certifying these deaths. This workshop brings together experts to illustrate their specialized roles in investigating accidental substance use deaths.

The medicolegal death investigator considers the circumstances and looks for clues at the scene and on the body to assess the likelihood that a particular death requires further investigation (e.g., a postmortem examination). The initial triage of the report of any death is a critical step. Once jurisdiction is declined, it is rare to get a second opportunity to reconsider. Numerous investigative signs and autopsy findings associated with drug use, some subtle, are presented.

The forensic pathologist is at the center of the investigation of the cause of death and, in addition to performing the autopsy, correlates the death investigator's and toxicologist's findings.[2] Not performing an autopsy may lead to missing other possible causes of death, such as disease or trauma, that supersede the drug intoxication as the cause of death. The benefits of the autopsy in excluding other or contributing causes of death are highlighted.

The forensic toxicology laboratory analyzes biological samples using the context of the case history to help direct testing so that drugs of forensic relevance are detected and quantified. Analytical scopes and methodologies will be discussed, particularly in the context of novel psychoactive substances.[3]

Common pitfalls encountered during each stage of the investigation and death certification process also will be demonstrated through practical case examples, as they can affect proper scene investigation, triage, toxicology testing, result interpretation, and certification.[4] The important professional relationships and interactions among the medicolegal death investigator, toxicologist, and pathologist are elucidated.

Overall, this presentation will impact the forensic science community by providing non-pathologists and pathologists-in-training with an understanding of how toxicology testing factors into death investigations and how the pathologist determines if a person died *of* drug intoxication or simply died *with* a drug intoxication so that the death is correctly certified. The importance of proper death certification for public health and safety purposes will be stressed.

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Certification; Intoxication Deaths; Death Investigation

W22   How Should Forensic Genetic Genealogy (FGG) Navigate the Court System? A Mock Cross Examination

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Learning Overview: By attending this workshop, attendees will gain a perspective on important issues related to the introduction of FGG in court, from both the prosecution and defense points of view. The mock trial and subsequent discussion will also provide FGG practitioners with insight into how to present expert testimony.

Impact Statement: This presentation will impact the forensic science community through a demonstration of the cross examination of a qualified, competent FGG expert witness, to show in real time various technical and legal challenges that may arise in court as FGG moves further into the judicial system.

This workshop provides insight into the technical and legal challenges that may arise during cross examination, in the context of challenges the community faces if FGG should move further into the court system.

FGG was recently introduced into court as an investigative lead in the trial of Patrick Leon Nicholas for the 1991 murder of high school senior Sarah Yarborough in Federal Way, WA, as a way to address privacy concerns that the defense raised related to the state constitution. This landmark case has brought to the forefront important issues concerning the relevance of introducing FGG in court. FGG can only produce investigative leads; ultimately, legal identification depends on a Short Tandem Repeat (STR) match to crime scene DNA.

As FGG has helped solve many hundreds of cold cases, it is inevitable that it will move further into the criminal justice system. The Yarborough case is only one of a very few examples to date of expert witness testimony on the subject; as more occur, it is critical to develop an understanding of the role that FGG plays in solving a case in relation to issues that may arise with its introduction at trial. At present, the FGG community offers few courses in professional development in a larger sense and very few, if any, covering expert witness testimony.

An expert witness must meet ANSI National Accreditation Board (ANAB) and Organization of American Studies (OAS) standards, which brings up concerns over the lack of training and professional background of FGG practitioners. An expert is viewed not as a subject matter expert in a narrow sense, but as a person qualified by knowledge, skill, experience, training, or education; that is, a practitioner broadly defined as an expert by his education and relevant work experience. Without further training, practitioners called to testify in the near future will have little experience preparing for trial testimony, with insufficient knowledge of what to expect.

By observing a mock FGG cross examination in real time involving a case where FGG provided investigative leads resulting in an arrest, attendees will learn how a qualified, competent expert witness can respond to issues that may arise at trial, such as credentialling of FGG practitioners, ongoing communication with an agency, apparent biases for or against law enforcement, and the use of unaccredited laboratories for producing FGG Single Nucleotide Polymorphism (SNP) data.

Cold Case; Genealogy; Court

W23   The Impact of Burning on Skeletal and DNA Evidence

Jane E. Buikstra, Arizona State University, Tempe, AZ; Katelyn L. Bolhofner, Forensic Anthropology, Arizona State University, Glendale, AZ; Joanne Devlin, University of Tennessee, Knoxville, TN; Giovanna Vidoli, Forensic Anthropology Center, University of Tennessee, Knoxville, Knoxville, TN; Anne Stone, Arizona State University, Tempe, AZ; Laura C. Fulginiti, Maricopa County Office of the Medical Examiner, Phoenix, AZ; Cody E. Parker, School of Human Evolution and Social Change, Arizona State University, Gilbert, AZ; Erin M. Rawls, Arizona State University, Tempe, AZ

Learning Overview: After attending this workshop, attendees will have learned how fire affects bodies and the ability of researchers and forensic practitioners to obtain skeletal and DNA evidence. Attendees should be able to: (1) describe methods used to characterize the extent of burning in bone; (2) understand the effects of burning on bodies, (3) appreciate the different extraction methods applied to burned and highly degraded skeletal tissues, and (4) evaluate which downstream analyses are likely to produce useful data for case identification.

Impact Statement: The recovery and analysis of skeletal evidence and DNA from burned human remains represent some of the most challenging cases in medicolegal investigations. This workshop will impact the forensic science community by increasing attendees' understanding of how burning affects the body and DNA preservation. Specifically, attendees will understand the characteristics used to score the level of burning in bones and the protocols used for optimal recovery of DNA from burned bone and tissue.

The presenters will provide an overview of how bodies burn from case examples as well as experimental burning of complete human bodies and will discuss the classifications used to describe the extent of burning (i.e., the temperatures reached) in bone by previous researchers. Attendees will also learn about DNA extraction methods that have been applied to burned tissue and bone. The presenters will discuss the success of these methods based on data from research projects examining the effects of burning on skeletal remains and on the recovery of DNA from those remains. Attendees will learn how the DNA recovered from these burned remains performs in subsequent Combined DNA Index System (CODIS) Short Tandem Repeat (STR) and Next Generation Sequencing (NGS) analyses that aim to test the limits of DNA recovery from fire-death victims. This workshop probes the methods (and challenges) for obtaining DNA using novel ancient genomic technologies optimized for maximum ultra-short molecular recovery as well as for obtaining skeletal evidence, including evidence for trauma, from burned human remains.

Thermal alteration of the human skeleton reduces the amount of DNA available for STR genotyping and downstream NGS analyses. As a consequence, the identity of many who died as the result of fire, such as some victims recovered from wildfires, house fires, or other medicolegal contexts, remain unknown. In this workshop, the presenters will discuss how fire affects bodies and the ability of researchers and forensic practitioners to obtain skeletal and DNA evidence. Attendees will first hear an overview of how bodies burn from case examples as well as experimental burning of complete human bodies. This discussion will include how burning affects evidence of trauma as well as the classifications that have been used by previous researchers to describe the extent of burning (i.e., the temperatures reached) in bone based on coloration and other tissue characteristics. The presenters will also provide details about DNA extraction methods that have been applied to burned tissue and bone, and we will discuss the success of these methods based on data from research projects conducted by researchers from Arizona State University with the Maricopa County Office of the Medical Examiner (MCOME) and the Forensic Anthropology Center (FAC) at the University of Tennessee, Knoxville. Attendees will learn how the DNA recovered from these burned remains performs in subsequent CODIS STR and NGS analyses. The NGS data enable comparisons of the success rates of DNA libraries generated using both single- and double-stranded methods for mitochondrial genome and genome-wide single nucleotide polymorphism (SNP) capture. These analyses aim to test the limits of DNA recovery from fire-death victims using a suite of these cutting-edge genomic technologies.

Skeletal Remains; DNA; Burn

A1   Increasing Access and Engagement at Academic Conferences: Comparing Modalities and Facilitating Interactions

Lisa Monetti*, Drew University, Madison, NJ

Learning Overview: After attending this presentation, attendees will understand barriers to access to academic conferences in the fields of forensic and biological anthropology. Attendees will also understand possible new directions for conference and session structure that will be more accessible and increase diversity of presenters.

Impact Statement: This presentation will impact the forensic science community as it will address barriers to access to academic conferences. It will highlight the value of diversity of presenters at academic conferences and show ways to make conferences within the forensic sciences more accessible. Developing more accessible spaces for sharing knowledge within forensic science propels the field toward greater inclusivity and diversity of scientific thought.

Changes implemented within higher education in response to the COVID-19 pandemic are innumerable. For one, international academic conferences shifted from in-person to virtual and hybrid modalities. By now, many have returned to in-person formats while others retain hybrid options.

Prior to the COVID-19 pandemic, hybrid conference modalities were rare, but it had been suggested that their implementation could increase accessibility, particularly for early career researchers and folks with other barriers to access, including geographic location. The pandemic posed an opportunity to interrogate whether this was, in fact, the case. Of course, geographic location of the author and the conference itself are not the only barriers to access, though many of the challenges are related to location, including travel costs, visa considerations, as well as perceived safety during travel and when sharing accommodation or staying alone. The author analyzed conference proceedings from the fields of forensic anthropology and biological anthropology to identify the proportion of first-author presenters who were from countries and continents other than those of the host institution, following the methodology of Hirst et al.[1] Findings suggest that these proportions remained largely unchanged regardless of the modality of the conference. Twenty-two percent of first authors were from out of the host country for in-person years, and 24% for hybrid and virtual modalities when considering the American Academy of Forensic Sciences (AAFS) and American Association of Biological Anthropologists (AABA) conferences from 2016–2022. Because we understand that diversity and accessibility are essential for progressing scientific thought, what can be done to increase access and international engagement at academic conferences?

An often-mentioned challenge within the discourse on virtual and hybrid conference modality is that of networking, which is seen as more difficult when attendees are not physically in the same space as one another. The author developed and employed an adapted conference session format during a virtual conference in 2021 that aimed to increase engagement, networking, and international collaboration in the virtual space. This included organizer engagement with authors ahead of the conference, leading to the authors writing open-ended questions focused on topics adjacent to their research but which they could not answer without increasing their collaboration with others. An increased number of discussion blocks were added to the session and these questions were discussed in groups to identify areas of future collaboration and increase interaction between speakers and attendees.

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Conference; Anthropology; Diversity

A2   **Assessing Opportunities for Forensic Casework Experience for Students Through Educational Programs and Internships**

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Learning Overview: This presentation utilizes survey data from forensic anthropology students to explore their opportunities and experiences with casework through academic programs and/or internships. After attending this presentation, attendees will have a better understanding of the opportunities available for forensic anthropology students and the gaps in accessibility for students to attain this crucial training.

Impact Statement: This presentation will impact the forensic science community by demonstrating that forensic anthropology students regard applied casework experience as essential to their professional growth in the field. Adequate forensic casework experience is crucial to prepare them to become forensic anthropology practitioners.

The American Board of Forensic Anthropology (ABFA) provides certifications for forensic anthropology practitioners who have demonstrated expertise in their field. However, currently no qualifications and competencies have been defined for forensic anthropology students, who are poised to become the next generation of practitioners. In order for students to become qualified forensic anthropology practitioners, there needs to be access to applied training in forensic anthropology while attaining undergraduate/graduate education. While other core competencies are required of forensic anthropology students, perhaps the most crucial is that of obtaining sufficient knowledge for practice and receiving practical experience. Therefore, students interested in pursuing forensic anthropology, and especially ABFA certification, must primarily master these skills and have the casework background necessary for practice while completing their degree(s).

This study investigates the opportunities for and experiences with forensic anthropology casework through degree programs and internships from the perspective of students. An Institutional Review Board (IRB) -approved electronic survey was distributed on July 12, 2023, and remained open through October 31, 2023. The survey is advertised to both student and practitioner participants, and the survey questions are generated based on which category defines a respondent. Both undergraduate and graduate students are able eligible to participate, and students are asked questions regarding basic demographics and relating to their personal experiences with forensic casework.

Descriptive statistics were generated for participant responses. Currently, 32 current forensic anthropology students (or recent graduates) have participated in the survey. Most respondents (56%) have completed a master's degree, followed by a bachelor's (36%). Of the 32 initial respondents, only 23 participants answered, "Have you assisted with/conducted forensic casework?"; of these, approximately 82% of respondents had casework experience. Students reported contributing to anywhere from 1 to 150 forensic cases, though only four respondents indicated analyzing more than 30 cases. Many students attained casework experience through degree programs (undergraduate [9%], master's [30%], and doctoral [21%]), while others (26%) received casework experience through an internship and/or visiting scientist program; 7% had not assisted with forensic casework. Of those students who had casework experience, they were able to contribute roughly equally to search and recovery, maceration, photography, skeletal analysis, and case report writing. Of those who assisted with writing case reports, the majority (36%) did not sign as author/co-author while 28% did. Through free-form questions, students shared similar sentiments about the importance of gaining casework experience while in school. For example, respondents noted that assisting with casework provides them with the opportunity to determine if forensic anthropology is the appropriate career path for them, apply the methods they have learned through coursework, learn how to write case reports, learn how to navigate the larger death investigation system in which forensic anthropologists operate, and become qualified to be a practicing forensic anthropologist.

These preliminary results indicate that, of the surveyed students, many forensic anthropology students have had experience with at least one forensic case through academic programs and/or internships. These findings demonstrate most forensic anthropology students are receiving some applied training, either in their educational programs or through internships. These results will be useful for students who do not receive hands-on learning to advocate for themselves or to provide emphasis for searching for external learning opportunities, such as internships. This study aims to stress the importance of obtaining practical forensic anthropology experience as a student.

Casework; Survey; Anthropology

A3   How Contextual Information and Consideration of the Type of Bones Present Influence Interpretations of Identifiability

Andrea Palmiotto*, Indiana University of Pennsylvania, Indiana, PA; Allysha P. Winburn, University of West Florida, Pensacola, Florida; Christine M. Pink, Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, MI

Learning Overview: After attending this presentation, attendees will better appreciate the impacts of human decision-making when assessing the identification potential of human skeletal remains.

Impact Statement: This presentation will impact the forensic science community by demonstrating that interpretations of the potential for skeletal remains to lead to individual identifications are minimally influenced by biasing contextual information. However, interpretations of identifiability may be swayed by the type of bones present in an assemblage.

Previous studies have demonstrated that forensic scientists are susceptible to the biasing effects of contextual information. In forensic anthropology, for example, indicators of cognitive bias have been identified in interpretations of both metric and morphological estimates of sex.[1,2] However, forensic anthropologists have also emphasized the capacity for quality-assurance protocols like blind analysis, peer review, and linear sequential unmasking to mitigate such contextual effects.[1-3]

The potential relationship between biasing contextual information and perceptions of identifiability is as-yet uninvestigated and formed the focus of this study. An online, anonymous, Institutional Review Board (IRB) -approved survey was sent to forensic anthropologists (n=155) and other individuals (including other forensic practitioners [n=121] and members of the public [n=48]). Participants examined a series of photographs of incomplete human skeletal remains and were asked to interpret the potential for the remains to be identified. Possible responses were limited to "Unlikely," "Possible," "Probable," and "Very Likely." One-third of participants viewed these photos with no accompanying contextual information, one-third were exposed to limited biasing context (e.g., "The following set of remains was recovered from a forensic context with several personal artifacts and other material evidence."), and the remainder were exposed to extensive biasing context (e.g., "The following set of remains was recovered in association with panties, a bra, and breast implants."). Two hypotheses were examined: (1) contextual information and (2) the type of bones present will impact perceptions of identifiability among other forensic practitioners and the public—but forensic anthropologists will not be biased by either context or type of bones present.

These hypotheses were based on the assumptions that forensic anthropologists, with their expertise in skeletal analyses and their familiarity with methods of skeletal identification based on multiple bodily regions, would recognize that biological inferences can be made from nearly any bone in the human skeleton. We assumed that other individuals, who may be less familiar with anthropological analytical capabilities, would be influenced by the contextual information and type of bones present. Therefore, we anticipated that forensic anthropologists would indicate that each set of remains had a "Very Likely" chance of being identified, while other respondents would vary in their responses.

Chi-square contingency tables were used to examine differences in responses based on type of participant ("forensic anthropologists" and "others") and exposure to contextual bias ("none," "limited," or "extensive"). Responses indicated that participants generally were not swayed by biasing information. Regardless of contextual details or anthropological experience, participants reported similar interpretations of identifiability for two of the three photographs (p >0.05). However, all participants, including forensic anthropologists, were influenced by the type of bones present in the photographs. No matter the type of contextual details provided, each group ("forensic anthropologists," "others") reported "Very Likely" identifiability statistically significantly more frequently when the skull was present in an image compared to images with no skull present. Moreover, for the image of predominantly long bones (no skull, ribs, vertebrae, or bones of the hands and feet), interpretations varied significantly among all groups.

These outcomes indicate that estimates of identifiability are relatively resistant to the biasing effects of contextual information, regardless of who is making the estimate. This also suggests a greater awareness of forensic science analytical capabilities among the public. However, additional underlying assumptions—specifically about the analytical value of the human skull and other elements—may be affecting interpretations of skeletal assemblages and their identification potential. This study highlights an additional area that forensic anthropologists should consider when assessing human skeletal assemblages.

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Anthropology; Identify; Bias

A4 Mapping Barriers to Entry of Spanish-Speaking Students of Forensic Anthropology: Diversity in Academic Opportunities at United States Hispanic-Serving Institutions

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Learning Overview: After attending this presentation, attendees will be informed about barriers to entry in forensic anthropology for Spanish speakers within the United States and understand the need for increased availability and resources of forensic anthropological programs/degrees and opportunities available in Hispanic-Serving Institutions (HSI).

Impact Statement: This presentation will impact the forensic science community by demonstrating the need for recruitment and retention strategies for Spanish-speaking students in forensic anthropology by analyzing the availability and resources of forensic anthropology programs within United States HSI. This presentation further benefits the forensic science community by offering new strategies for improving educational opportunities for Spanish-speaking and other diverse students in forensic anthropology.

Diverse research groups have been shown to produce more novel findings, introduce more technological innovations, and research cited more often and by a wider audience.[1] Yet, the lack of diversity within forensic anthropology is well documented, with 87% reporting as non-Hispanic, White, forensic anthropology lacks diversity among its practitioners.[1,2] This lack of diversity of practitioners likely produces blind spots in regard to the needs, challenges, and culturally specific solutions of those the field serves. Even though 84.3% of forensic anthropologists believe that it is fundamental to recruit and retain diverse practitioners in the field, no equitable outreach strategies have been put into place. In fact, the homogeneity of the field increases from undergraduate to graduate to professional ranks, sifting-out diversity at each level.[2] While scholars have begun to examine the history of inequity in the field (e.g., race and gender, primarily) and call for strategies necessary to increase and support diversity (e.g., Goliath et al. 2023), language barriers have not been explored.[3] Numerous factors affect the enrollment and retention of minority students, but without interrogating the data, the forensic sciences cannot self-examine the barriers to diversity within the field or enact changes to attract and retain minority practitioners.

The lack of Spanish-language scholarship in forensic anthropology appears to reflect the lack of the field's diversity.[4] As part of a larger study exploring the experiences of Spanish-speaking forensic anthropology students and practitioners and the gaps in Spanish-language scholarship in the field, this study will present a Global Information System (GIS) map using the HSI database of the: (1) locations,(2) availability of biological and forensic anthropology courses, (3) degrees and certificates, (4) faculty mentors, and (5) enrollment costs, and other resources of United States HSI. As defined by the United States Department of Education, an HSI is an institution that has an enrollment of at least 25% full-time undergraduate students making them eligible for funding to provide expanded opportunities and resources for these students.[5] As of Fall 2020, 451 universities/colleges were classified as HSIs with 2,044,533 enrolled Hispanic students within 25 states and Puerto Rico. This data is compared to results from a random selection of non-HSI institutions within the United States that are listed as having forensic anthropology programs on the bioanth.org and AAFS.org websites. The map is intended for use as a resource for prospective students, committees on diversity, and demonstrates one aspect of gatekeeping for minorities in our discipline.

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Spanish Speakers; Diversity; Hispanic-Serving Institutions

A5   Using Cause of Death and Skeletal Indicators of Health and Stress to Understand Structural Inequality

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Learning Overview: This presentation will demonstrate how Cause Of Death (COD) is related to different skeletal markers of physiological stress and health, namely linear enamel hypoplasia, stature, and antemortem tooth loss. After attending this presentation, attendees will understand how COD and skeletal markers of stress and health can provide useful information in medicolegal investigations when considered together. Both measures contribute contextual information about the deceased's lived experiences.

Impact Statement: This presentation will impact the forensic science community by showing that COD and skeletal markers of physiological stress and health are tools to uncover context about people's lived experiences. Combining information from COD and skeletal markers of stress and health can advance forensic investigations by reducing the number of possible identities and identifying events or factors that contributed to an individual's death.

The cumulative burden of physiological stress during an individual's life is reflected in a complex interplay of disease vulnerability, morbidity, and mortality. Stress responses are often related to COD, both of which can be used as a measure of population health.[1] Here, we investigate the association between COD and markers of physiological stress in a sample of 297 individuals from the Hamann-Todd Osteological Collection (HTOC), comprised of individuals from predominantly low socioeconomic statuses from the late 19th- to early 20th-century Cleveland. Those in the HTOC were largely employed as laborers or in unskilled trades and would have lived in areas of overcrowded housing causing high disease spread. Thus, the background of the HTOC is comparable to forensic samples that often include victims of multifaceted structural inequality.[2] Linear Enamel Hypoplasia (LEH) incidence and stature were used as indicators of physiological stress during growth and development, while Antemortem Tooth Loss (AMTL) was used as an indicator of health during adulthood. LEH were scored as present/absent for each individual. Stature was available through medical and autopsy records. AMTL was calculated as the proportion of teeth missing antemortem.

Documented CODs were available for most individuals in the sample from death certificates. CODs varied by socially ascribed race and sex within the sample. Black Americans were twice as likely to die from tuberculosis than White Americans (χ^2=4.793, p=0.029). Individuals that died from degenerative diseases, such as cardiovascular disease, had a significantly more AMTL (H=18.53, p=0.002). Differences in AMTL among COD categories were still significant when controlling for age at death (F=2.388, p=0.038). Females that died from degenerative diseases had significantly shorter statures compared to those that died of infectious disease (t=2.903, p=0.004). There were no significant differences in LEH prevalence among the different CODs represented (χ^2=4.449, p=0.487), although a previous study found an association between stature and LEH presence for females in the HTOC.[3]

These trends show that several skeletal markers of stress are related to degenerative disease in this sample. These results demonstrate how combining information from COD and skeletal markers of physiological stress and health may provide additional context about an individual's lived experiences, which can aid forensic investigations by narrowing the pool of possible identities and understanding the events that led to an individual's death.

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Cause of Death; Anthropology; Mortality

A6   The Development of a Migrant Profile for Unidentified Human Remains Found Across Three Medicolegal Offices in Greece

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Learning Overview: This presentation will utilize demographic data from unidentified human remains cases originating from three offices within Greece to demonstrate documented demographic trends between individuals considered migrants and individuals considered non-migrants. The goal of this presentation is to contribute to ongoing research that is working toward building a migrant profile for remains discovered in border regions globally.

Impact Statement: This presentation will impact the forensic community by highlighting the potential for anthropological analysis to assist in the determination and identification of migrant remains. This presentation will also inform the forensic community about the diversity of migrant remains within the Greek medicolegal system and the similarities between the Greek and United States migrant humanitarian crises to foster collaboration in identification efforts.

For this study, the demographic and Global Positioning System (GPS) data from 770 cases between the years 1995 and 2022 were gathered from three offices in Greece in which unidentified human remains were recovered. The offices were located across a diversity of landscapes, including the Forensic Medicine Unit of the University of Crete in Heraklion on the island of Crete (n=99), the Forensic Anthropology Unit of the Department of Forensic Medicine and Toxicology at National and Kapodistrian University of Athens located in the city of Athens (n=317), and the Department of Forensic Medicine of the Democritus University of Thrace located in the city of Alexandroupoli (n=354). From the combined dataset, a total of 367 individuals were considered migrants, while 403 individuals were considered non-migrants. For the individuals considered migrants with a known country of origin, the majority of the individuals originated from Pakistan (n=35), followed by Syria (n=33) and Afghanistan (n=19), but in total 18 countries were represented in the data from both the western and eastern hemispheres. The migrant cases consisted of primarily males (n=322, 87%) with an average age of 27.1 years. The female migrant cases had an average age of 28.9 years. The primary cause of death for the migrant cases was drowning, followed by traffic/train accidents. This migrant demographic profile differs from the non-migrant profile of unidentified individuals from among these offices in that the non-migrant cases consisted of 49% males (n=201) with an average age of 59.4 years, while the females in this group had an average age of 66.4 years. The primary cause of death for the non-migrant cases was undetermined.

The identification rate for the migrant group was 47%, while the identification rate for the non-migrant group was 34%. The identified non-migrant cases included individuals of both Greek and non-Greek ancestry. For both groups, the means of identification included combinations of visual identifications, personal effects, fingerprints, and genetic methods. The GPS data for each case was used in hotspot analysis to qualitatively examine any differences in concentrations across the Greek landscape between the migrant and non-migrant groups. The hotspot analysis shows a higher concentration of migrant remains in the northeastern/Thracian region of Greece. While the data presented in this study indicate a specific demographic and geographic trend of migrant remains, the study is limited by the inclusion of only three offices, an absence of data from major shipwrecks containing migrants which are handled by other jurisdictions, and the data mining from a combination of pathology and anthropology reports. This presentation will also discuss these limitations and directions for future research to build stronger migrant profiles and increase identification rates for both migrant and non-migrant unidentified human remains in Greece.

Anthropology; Human Remains; Mass Fatality

A7   The Development of a Multidisciplinary, Minimally Invasive Approach for the Forensic Examination of Skulls

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Learning Overview: After attending this presentation, attendees will have a greater understanding of the accuracy and consistency of the age at death, sex, and ancestry estimations through anthropological, odontological, radiological, isotope analysis, proteomic, and genetic methods. Through the discussion of the findings, attendees will ultimately appreciate the recommended multidisciplinary approach to forensic human identification for skeletal remains.

Impact Statement: This presentation will impact the forensic science community by assessing various forensic profiling methods for human remains identification to showcase the strengths and limitations of each when applied to skull-only remains. The presented findings will help guide investigators to choose optimal methods and samples for age at death, sex, and ancestry estimations to aid missing persons investigations and disaster victim identification.

Forensic examination of human skeletal remains is important to establish the unknown deceased person's identity using an accepted scientific method such as dental or DNA analysis or to estimate the individual's age at death, sex, ancestry, lineage, externally visible characteristics, and other information such as geographical region of origin.[1-3] Such approaches to human remains identification typically involve one or more anthropological, radiological, isotopic, and genetic methods.[4]

This research focuses on cranial remains to evaluate a multidisciplinary, minimally invasive approach for the forensic examination of skulls. The absence of a post-cranial skeleton often presents challenges for identification by limiting the skeletal elements available to generate investigative leads when traditional dental and/or DNA matches fail. Therefore, this research aims to recommend an accurate, comprehensive, and least-destructive sampling and examination workflow for skull-only cases to maximize identifying information.

Eight human cadavers donated to the Australian Facility for Taphonomic Experimental Research (AFTER) and subjected to different environmental conditions were examined by various forensic experts applying different forensic examination approaches, starting with an anthropological assessment, using both non-metric and metric approaches. The non-metric approach utilized a visual assessment of the skull for age, sex, and ancestry estimation, while the metric approach included 30 cranial measurements analyzed through CRANID, FORDISC®, and AncesTrees programs for sex and ancestry estimation. Different skeletal and dental elements were then sampled and submitted for DNA-based sex, phenotype, ancestry, and paternal and maternal lineage estimations. These samples were also submitted for expert radiocarbon dating and stable isotope analysis.

The examination results obtained from each forensic technique/expert were reviewed to determine the accuracy of the age at death, sex, and ancestry estimations by comparing them to documented and self-reported donor data.

The expert non-metric anthropological assessments provided accurate results for all age, sex, and ancestry estimation of the donors, showing a more conservative result for age and ancestry predictions (e.g., "adult Caucasian male" instead of a specific age range or ancestral group). The metric evaluation produced accurate sex estimation but showed only moderate success with ancestry prediction, showing a broad range of possible ancestries, and proving to be more useful for accurate elimination of ancestral groups not corresponding with the donors' measurements. Both nuclear and mitochondrial DNA were successfully recovered from the petrous bone with the phenotype, ancestry, maternal lineage, and paternal lineage results agreeing with the reported donor phenotype, ancestral, and sex information. The radiocarbon dating results for date of birth and death provide a comparable age estimation with higher precision than the non-metric anthropological age assessment. The oxygen isotope results for region of origin information are currently under progress.

The findings of this research highlight the need for forensic experts from different disciplines to work together for a holistic investigation to ensure accurate and comprehensive human remains identification in skull only cases.

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Human Remains; Identification; Multidisciplinary

A8 An Investigation Into the Facial Dimensions of a North Indian Population: Implications in Facial Identification

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Learning Overview: After attending this presentation, attendees will understand the importance of facial morphometrics, the dominant and rare facial features present in a north Indian population, and the usefulness of facial dimensions and their prediction in forensic examinations.

Impact Statement: This presentation will impact the forensic science community by providing in-depth knowledge of facial features and the classification of faces for personal identification. The knowledge will be helpful for anatomists, plastic surgeons, and forensic experts to identify and isolate the common and rare characteristics in the population.

Personal identification is a distinctive approach that distinguishes people from each other and makes them unique. The human face is the most common approach that represents the uniqueness of a person.[1] Facial features are the results of environment and genetics that represent the specific characteristics of the population in specific regions. Moreover, facial identification from video surveillance systems is becoming a common approach in the current era.

The present study was conducted in the Mandi district of Himachal Pradesh, India, after obtaining ethical approval from the institution and written informed consent of the participants. This study incorporated 228 participants (158 males and 70 females) who were healthy and young with an age range of 18–35 years. A total of 19 facial measurements were taken in a Frankfurt horizontal plane using a sliding and spreading caliper following the standard procedure. Statistical analysis was done using the IBM® SPSS Statistics Version 20. Sexual dimorphism in the mean values of the measured parameters was analyzed by using an independent sample t-test.

The present study evaluated three facial indices (i.e., facial index, nose index, and lip index) among the participants following the classification by Williams et al., Martin and Saller, and Sarkodie et al.[2-4] In males, the mean facial index, nasal index, and lip index were found to be 81.139, 71.18, and 39.73, respectively, whereas mean facial index, nasal index, and lip index of 78.78, 69.83, and 36.72 were observed in females, respectively.

By calculating the facial index, the most common and rare facial types present in the target population were studied. The most dominant face was found to be hypereuryprosopic (very broad face) and the least was hyperleptoprosopic (very long face). The most common nose type was the mesorrhine, whereas platyrrhine and hyperplatyrrhine types of noses were the least commonly present. In most of the target population, intermediate types of lips were observed, whereas the least commonly found lips were of a thick type. Sexual dimorphism was also observed in the facial dimensions. Furthermore, a low to strong correlation was observed among the various facial dimensions considered in the study. Afterward, the regression models were formulated, which will be helpful for forensic anthropologists and law enforcement agencies for facial reconstruction and identification. This study provided data for facial phenotype among the North Indian population, which will be helpful for plastic and oral surgery, biometric purposes as well as facial identification in forensic science, etc.

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Facial Identification; Forensic Science; Facial Shapes

A9 The Identification of Fragmented Cranial Remains Exhumed From a Site Adjoining the Ajnala Site: A Forensic Anthropological Case Report

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Learning Overview: After attending this presentation, attendees will be sensitized about the importance of the application of advanced forensic anthropological techniques for forensic identification of badly damaged skeletal remains.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the multidisciplinary approach of the anthropological, molecular, and chemical examinations of the isolated and badly damaged or commingled human remains can be very helpful in the identification of such remains retrieved from forensic or bioarcheological contexts.

Mankind have suffered several unexpected disasters, heinous crimes, and brutalities, resulting in a large number of unknown skeletal remains to be identified and repatriated to their relatives. Forensic anthropological identification of badly damaged and commingled human remains is a very difficult task owing to poor preservation, severe fragmentation, missing elements, and the inadequate records about the conflicts or disasters that produced such human remains. Establishing identity to such remains is the ultimate purpose of almost all forensic anthropological examination.[1] Integrated inputs from archaeology, modern genetics, ancient DNA, and proteomics and stable isotope analysis has made such identifications possible.[2]

In one such incident of mass killings, 282 Indian-origin soldiers were killed by the colonial rulers, and the remains were dumped in an abandoned well at Ajnala, India in in 1857; forensic anthropological examinations of these remains found them belonging to different Indian states as mentioned in the written accounts. However, the damaged cranial fragments belonging to two individuals were retrieved from the area adjoining to Ajnala mass-burial site during a construction activity carried out later in 2014.

The present study was conducted to ascertain whether the recovered cranial fragments were synchronous to Ajnala skeletal remains or if they belonged to a different traumatic situation, with the objective of estimating the identity and population affinity of these cranial fragments. The morphological examination of jaw fragments and teeth was used for their forensic anthropological examination. The ancient DNA from the second molar and temporal auditory meatus of both the specimens was extracted and analyzed using standardized protocol, and library preparation was performed.[4-6]

The morphoscopical, osteological, molecular, and stable isotope results supported the versions that cranial fragments of two individuals excavated from an adjoining site to Ajnala well belong to the same group of individuals who were captured and killed at Ajnala and whose cadavers were dumped into an ancient abandoned well. It may be possible that cadavers of these individuals may have been buried after the main mass burial site (the Ajnala well) was filled with sand. The general robusticity of the skull, and the size and shape of the gonial angle, mental eminence, and the mastoid process, showed that fragments supposedly belonged to a male. The eruption of third molars with relatively minimal wear of the teeth, and the apparent degree of cranial suture fusion (although this may be affected through taphonomic damage/distortion), indicated they were young-mature adults, though other skeletal indicators may put these specimens in an older age category. The occlusal surface wear analysis shows that the teeth belong to a pretty youthful individual, but the possibility of using a soft diet by these individuals cannot be ruled out. The pubic symphysis or auricular surfaces were not available among the Ajnala skeletal assemblage for ascertaining the age of the specimen. The ancient DNA results were in accordance with the observations reported in the previously published article where the geographical affiliation of the remains have been found to be non-local to the site, potentially belonging to Eastern Uttar Pradesh, Bihar, and some Northeastern Indian.[3,6]

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Forensic Archaeology; Fragments; Exhumation

A10 Forensic Investigations on a Skull Found Inside a Roman Villa in the City of Diamante, Italy

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Learning Overview: After attending this presentation, attendees will be able to describe the management protocol in the case of human remains discovery.

Impact Statement: This presentation will impact the forensic science community by showing the usefulness of protocol-based procedures in the context of human remains discovery.

In this case report, we describe a case of a skull discovery in Diamante, Italy, near a Roman archeological site. We carried out: a skull external examination with photographic surveys before and after washing of internal surface of the skull; a radiological evaluation by performing a computer tomography scan before and after washing of the internal surface; after skull sampling, a chronological evaluation was performed by determining the radiocarbon concentration and comparing it with values of current 12C and 13C, and the calculation of 14 C with C6 Sucrose standard samples. An anthropological analysis was performed to estimate age at death by an examination of the degree of suture closure (the "Mindle Lovejoy" method) and an ancestry evaluation was conducted by application of the Hefner method. For taphonomy evaluation, the soil was sampled.

From anthropological and forensic examinations that were conducted, the skull was estimated to be a male subject. Age-at-death estimation was performed both with a mean age of 51.5 (± 12.6) years and with the latero-anterior system with a mean age of 56.2 (± 8.5) years. Human ancestry was comparable at 60.63% with Europoid one (Caucasoid). Radiocarbon dating collocates the skull to between 1877 and 1916 with a 29.5% interval of confidence. The skull examination revealed multiple alterations on the cortical surface of the bone consisting of postmortem fractures, discolorations, cracking, exfoliations, and erosions. Postmortem fracture can happen due to the soil weight acting on the bone surface or due to other pressure/mechanical factors. Some areas of ethmoidal bone and zygomatic processes were presenting postmortem fractures. Discoloration phenomenon was observed mainly in the right side of the skull, with the presence of yellow/orange spots, most likely determined by the position of the skull in contact with the soil.

In the posterior-superior portion of the left parietal bone, an irregular oval area was present with a brownish mark in the center, referable to a combustion sign that occurred after death. At morphological and radiological examinations, the presence of suspicious perimortem cut-marks, traumatic cut lesions, caused by a blade with a thin section inflicted just before death were detected.

The investigations performed showed that the suspicious skull injuries described before were not compatible with perimortem lesions but to a postmortem disarticulation. Moreover, from the anthropological and forensic analyses, it was possible to determine that the skull belonged to a male subject who underwent a postmortem disarticulation, attributable to funeral rituals, which were conducted during prehistoric ages in Europe.[1,2] In order to reach those conclusions, we employed this protocol: external examination with photographic survey; radiological imaging with evaluation of the results; anthropological analysis; radiocarbon dating; cataloging of circumstantial data present inside the skull (in order to perform future analysis).

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Forensic Archaeology; Human Remains; Forensic Analysis

A11   Skeletal Indicators of Down Syndrome: A Case Study in Forensic Anthropology

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Learning Overview: After attending this presentation, attendees will understand the importance of studying the skeletal manifestations of Down syndrome and how the condition was represented in the remains included in this study.

Impact Statement: It is estimated that over 200,000 people are living with Down syndrome today.[1] However, current forensic anthropological literature does not comprehensively address recognizing this condition in skeletal remains. The impact of this research will hopefully be the beginning of establishing methods for recognizing Down syndrome in remains.

Estimated to affect 1 in 800 births, Down syndrome is a chromosomal condition caused by a mutation referred to as trisomy 21.[1] There are between 200 and 300 genes on chromosome 21; the main characteristics of this condition include hypotonia, respiratory issues, delayed development, intellectual impairments, and a higher risk for infections.[2]

According to clinical and dental literature, skeletal phenotypes with varying rates of presence include: short stature, shortening of hand and feet bones, unstable atlanto-axial joint, 12th rib absence or poor formation, shortened long bones, low nasal bridge, a narrow and short and deep and high palate, changes in dental eruption timing, brachycephaly, malocclusion underdevelopment of osseous tissues, and mid-facial hypoplasia.[13]

In addition to the listed skeletal abnormalities, delayed skeletal and dental growth could affect established age estimation methods in forensic anthropology. Earlier onset of osteoporosis and increased rates of obesity could skew degradation-based age methods higher. The overall small size of people with Down syndrome and hypotonia likely results in more gracile muscle attachment sites and therefore sex estimations could skew toward female. Abnormal long bone ratios could cause errors in stature estimates which are primarily based on long bone measurements. Population affinity is often assessed qualitatively by examining facial features, with cranial facial alterations such as a low nasal bridge and palate shape changes; therefore, these methods could be affected.

In this case study, an individual with Down syndrome from the Texas State Donated Skeletal Collection was examined for non-metric variation, and the biological profile was assessed utilizing common current methodologies. A complete biological profile was assessed, age was determined utilizing ADBOU 2.1, sex was determined using MorphoPASSE, and FORDISC® 3.1; FORDISC® 3.1 was also used for stature and population affinity. Two authors assessed each aspect of the biological profile with strong agreement between observers. Sex estimation incorrectly classified the individual as female with high probabilities for all utilized methods. Population affinity was correctly estimated to be White with both cranial and post-cranial methods with high probabilities. Stature estimation was calculated utilizing FORDISC® 3.1 and the estimated stature with a 90% confidence interval; most resultant estimations contained the living stature; however, it was often the low point of the estimates. The individual's age was contained in the age estimate. The individual was also examined for any skeletal phenotypes listed in clinical literature, and the majority of traits listed above were observed with the exception of a low nasal bridge, absence of the 12th rib, and brachycephaly. Due to edentulism, palate and dental differences could not be observed.

This research demonstrates the importance of recognizing Down syndrome is skeletal remains, as doing so can aid in identification of individuals and prevent the use of methodologies that are not appropriate for estimating the biological profiles of people with Down syndrome. Further research is needed with an expanded sample size to better determine a set of skeletal characteristics that forensic anthropologists can utilize to potentially recognize Down syndrome.

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Anthropology; Inclusion; Casework

A12   The Characteristics of a Vertebroplasty in Human Skeletal Remains

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Learning Overview: The goal of this presentation is to explain the surgical procedure of a vertebroplasty and demonstrate its dry bone characteristics.

Impact Statement: This presentation will impact the forensic community by providing explanations of the physical modifications associated with a vertebroplasty procedure, which may be confounding to forensic anthropologists and other medicolegal death investigation experts.

Vertebroplasties are safe and effective medical procedures where bone cement is injected into a vertebral body in order to reduce pain and increase stability of the area. Vertebroplasties are typically performed to correct or stabilize a Vertebral body Compression Fracture (VCF), usually resulting from osteoporosis. However, vertebroplasties may also be used to reinforce vertebral bodies in cases of Schmorl nodes or other pathological conditions that weaken a vertebral body.[1]

Riggs and Melton report that there are over 700,000 VCFs diagnosed every year in the United States, resulting in ~115,000 hospital admissions.[2] VCFs are three times more likely to occur in females compared to males and may result in chronic pain, immobilization, kyphosis, pulmonary deterioration, and depression.[1,2] Clinically, VCFs are often treated with analgesics, bed rest, and external bracing; however, approximately one-third of patients require the invasive treatment of a vertebroplasty.[1] Vertebroplasties are typically performed using fluoroscopy; a needle is inserted through the pedicle of vertebra into the vertebral body, where the bone cement is injected. The volume of bone cement for a vertebroplasty will vary but is typically between 3.5–5.0mL depending on the size of the vertebra.[1] The most widely used bone cement for VCFs is Polymethyl Methacrylate (PMMA), which has a curing time of approximately nine minutes.[1]

Here we present the dry bone characteristics of an individual with a vertebroplasty from Western Carolina University's (WCU) willed body donation program. WCU's willed body donation program operates in support of its human decomposition facility, the Forensic Osteology Research Station (FOREST). In this case, the individual had consented to taphonomic research and was used in a controlled burn as part of a continuing education course. After the thermal alterations, the individual decomposed in the FOREST and was subsequently cleaned and curated in WCU's John A. Williams Donated Skeletal Collection. During processing for curation, abnormal characteristics were observed on the first lumbar vertebra. This vertebra presented as abnormally heavy with a compressed vertebral body, where portions of the superior vertebral body's cortical bone were absent and a hard, off-white substance was protruding in some areas. Upon donation, this individual did not record previous medical procedures in their optional donation paperwork, so the cause of the abnormality was unclear with multiple possible origins initially hypothesized (e.g., antemortem neoplasm, antemortem surgical intervention, postmortem fungal growth).

Laboratory analyses were performed to investigate the abnormal vertebra. Gross observation of the vertebra did not locate any obvious areas of antemortem needle insertion; however, the cortical bone of the vertebra was porous, which may have obscured surgical defects. The cortical bone on the superior vertebral body was thin and fragile, suggesting that the absent portions of bone in this area likely occurred postmortem. Radiography of the vertebra demonstrated the unknown substance was present throughout the inside of the vertebral body. The unknown substance was analyzed using Fourier Transform Infrared Spectrometry (FTIR). The resulting spectrum showed the presence of PMMA and barium sulphate, consistent with bone cement. Based on these results, the compressed vertebral body and presence of bone cement are indicative of a vertebroplasty. Due to a lack of medical history for this donor, the age of the vertebroplasty and cause of the VCF is unknown.

With almost 20,000 vertebroplasties performed in the United States every year, forensic anthropologists are likely to encounter them occasionally in their casework.[3] Understanding this medical procedure as well as its characteristics in dry bone will assist forensic anthropologists in correctly identifying these vertebral abnormalities and potentially assist in the identification process of unknown individuals expressing these characteristics.

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Vertebroplasty; Pathology; Trauma

A13   An Unusual Case of Human Remains Found in an Urban Aqueduct

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Learning Overview: This presentation will outline the unique taphonomic effects observed on remains discovered in an urban aqueduct. After attending the presentation, attendees will better understand taphonomic changes that can occur in a man-made water system and how it could impede trauma identification.

Impact Statement: This presentation will impact the forensic science community by sharing how taphonomic changes in an urban water system differs from a naturally occurring water system. Temperature, currents, fauna, flora, and water chemistry are among factors that can affect human remains during decomposition. All these variables are drastically different in a man-made water system where the water is chemically treated and filtered to remove contaminants, microorganisms, and debris. Distinguishing perimortem trauma from postmortem changes in forensic anthropology relies on whether the bone was wet or dry when the damage occurred. In the case presented, the chemically treated water likely prolonged decomposition and the highly pressurized water pipe created ample opportunity for postmortem damage on bone that retained its moisture content.

The skeletal remains of a single 30-year-old decedent were discovered in an urban aqueduct and water feeder pipe. Based on the timeline from investigators, the decedent was likely in the pipe from 2015 to 2022. The remains were mostly skeletonized with remnant wet soft tissue surrounding the vertebrae. No evidence of animal or insect activity was observed. The water was treated, clean water, which would reduce the presence of biological agents normally found in natural bodies of water such as invertebrates and microorganisms.

The water pressure of the pipe caused significant damage to the skeleton, which occurred in the perimortem period, as defined by the period in which the bone retains enough collagen and moisture to resist fracturing.[1] Several ribs and the scapula exhibited significant plastic deformation, with some ribs bent as much as 90 degrees with little or no external or internal fracturing. Longitudinal fractures were observed on the superior and inferior margins of the bent ribs. Rib fractures can be particularly challenging to assess given the shape of the bone, dynamic responses to loading specific to ribs, and the scarcity of forensic anthropological literature on the subject.[2-5] Abrasion consistent with the remains being in a turbulent aquatic environment for some time was observed but it was much more localized than would be expected in a natural water context, taking on the appearance of incised defects and even saw marks to the untrained eye.

This case emphasizes the need for decomposition equations for different types of aquatic environments as well as more trauma and taphonomy studies in urban aquatic environments. By sharing the details of this unique case, anthropologists can better understand the taphonomic changes caused by water movement in the context of the specific biological and chemical environment of an urban aqueduct.

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Aquatic; Taphonomy; Plastic Deformation

A14   Understanding the Potential of Chemical Anthropology in Burned Human Remains

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Learning Overview: This presentation will provide a deep understanding of the challenges forensic anthropologists face when analyzing burned human remains due to heat-induced changes. After attending this presentation, attendees will understand how vibrational spectroscopy techniques like Fourier Transform Infrared FTIR and MicroRaman can help assess the structural and chemical degradation of burned skeletons, focusing on key spectral markers and their correlation with exposure temperature. In fact, it demonstrates the potential for using vibrational spectra to distinguish burned remains, especially in complex scenarios, shedding light on the role of exposure duration in degradation. This knowledge equips attendees to apply vibrational spectroscopy effectively in forensic anthropology, advancing the field's capabilities.

Impact Statement: This presentation will impact the forensic science community by introducing innovative methods using vibrational spectroscopy for assessing the structural and chemical degradation of burned human remains, significantly advancing the field of forensic anthropology. By providing forensic anthropologists with precise tools to determine bone exposure temperatures and identify key spectral markers, this study enhances the accuracy of forensic analysis, particularly in complex scenarios involving commingled or scattered remains, while also contributing to broader interdisciplinary research.

Burned human remains pose challenging analytical problems to forensic anthropologists due to heat-induced changes. The determination of the maximum temperature of exposure through bone has been frequently addressed in the past but still requires additional investigation. This research explores the potential of vibrational spectroscopy in determining the structural and chemical degradation based on spectra from the organic and inorganic composition of burned human skeletons.

To explore this, an assemblage of 560 samples from 40 skeletons of the 21st-Century Identified Skeletal Collection (University of Coimbra) experimentally burned at different maximum temperatures (from 500°C to 1,050°C) and durations (from 45 to 240 minutes) were analyzed through vibrational spectroscopy, namely FTIR and MicroRaman.[1] The relationship between the spectra of individual bones (long bones and hip bones) and the variation of the intensity of the bands assigned to either organic or inorganic components such as amide I and II vibrational modes of proteins, CH_2 bending and stretching vibrational modes of lipids, carbonate, hydroxyapatite, phosphate, hydroxylation patterns were investigated. The correlation of the spectra of the organic and inorganic components with the maximum temperature of exposure is clear. Transition from polymorphic hexagonal to monoclinic hydroxyapatite occurs at a temperature higher than 800°C. The band (Ca-OH) (*ca.* 345 cm^{-1}) is detected only for burning temperatures equal to or above 700°C, being absent for lower temperatures.

However, the chemical degradation of the burning experiment is still a variable that needs to be further investigated since it could reveal discriminatory "fingerprinting" in cases of commingled or scattered contexts. Therefore, duration of the exposure plays an important role in the structural and chemical degradation of burned human remains. This study obtained promising results regarding the potential of analyzing vibrational spectra from burned human remains to assess the structural and chemical degradation correlated with the maximum temperature to which they have been exposed.

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Burned Human Remains; Forensic Anthropology; Vibrational Spectroscopy

A15 Is It Scientifically Impossible to Burn Human Bodies to Ashes? Revisiting the 2014 Case of the 43 Missing Students from the Ayotzinapa Rural Teachers College in Guerrero, Mexico

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Learning Overview: This presentation reexamines some of the older forensic and fire science that was used to investigate a 2014 case involving the disappearances and incineration of 43 students in an outdoor dumpsite in Guerro, Mexico. In 2015, an investigation by the Inter-American Commission of Human Rights (IACHR) had appointed the Interdisciplinary Group of Independent Experts (GIEI) to independently investigate the case. Portions of the GIEI's report were based on research findings from burning large wood cribs (pyre) with pig bodies to model the incineration of humans.[1] The GIEI final report concluded that the 43 students were not incinerated in the dumpsite. In 2015, the IACHR had rejected the claim that 43 victims were incinerated to "ash" at that site and concluded that the official account of events was scientifically impossible. Some of the decade-old forensic and fire science that were used to investigate this case were compared to current findings from experimental fatal fire research involving n=10 outdoor incendiary fire tests that demonstrated the multiple variables and processes associated with intentional cremation into fragmentary burned bones as ashes. After this presentation, the attendees will understand the process of how human cadaver bodies are cremated based on current fatal fire research.

Impact Statement: This presentation will impact the forensic science community by demonstrating the importance of reevaluating older forensic casework with updated scientific research findings, which can offer newer information into the case.

Over the past decade, there have been advances in the analysis of burned human remains from fatal fire research and the analysis of fire victims by forensic anthropologists. Current research from forensic anthropology and fire science were applied to a 2014 case in Guerrero, Mexico, involving the disappearances and incineration of 43 male victims. We revisited some of the original case findings and compared them with current research models to test the hypothesis that it is scientifically impossible to burn 43 bodies to ashes.

We analyzed the results from n=10 experimental outdoor incendiary fire experiments using non-embalmed human cadaver bodies (donated with family consent), different types and amounts of fuels, and burned for varying durations for forensic training and research during the Fatal Fire Death Investigation Course by the San Luis Obispo Fire Investigation Strike Team (SLO FIST) in California[2]. Data collection from these tests documented different fuel types and amounts, durations, passive and active involvement (refueling), the processes of cremation into burned tissues, bones, and teeth, and how much time, fuels, and efforts are needed to intentionally cremate a human body to ashes. These results were compared to the research tests and findings by fire scientist Dr. Torero in 2015 who burned pigs on wood cribs (pyre) to replicate human cremation.[3]

A comparative analysis of the research methods, materials, and findings from our current tests and those from the original investigation was conducted to examine how much time, fuels, and efforts are needed to intentionally cremate a body. Torero's research had estimated that to cremate one pig body would require ~700kg of wood to burn for 12 hours to ash. Our research results demonstrated that full cremation resulted from significantly less fuels of ~150kg–200+kg of wood for ~2 hours. We observed that the most extensive damage resulted from activities of refueling and intentionally stoking, crushing, and pulverizing fragile burned bodies and burned bones into smaller fragments, which significantly reduced the time and materials needed for cremation. In 2014, the site was excavated by the Equipo Argentino de Antropología Forense (EAAF) forensic anthropologists who recovered multiple darker charred fragments of burned human bones and teeth that had remained camouflaged within the black fire debris and overlooked until site excavation. White calcined bones would be easier to identify and remove from black fire debris, which could explain their absence and why so few bone fragments were recovered.

Our research results demonstrated that it is scientifically possible to intentionally cremate bodies to ashes as charred and calcined fragmented bones and teeth burned in a large outdoor incendiary fire event with the intent to destroy evidence and that these newer scientific findings should be considered to revisit the case. Three of the missing victims have been identified through DNA testing and newer technologies (i.e., Rapid DNA) that provide better results for thermally degraded bone, especially for the charred remains. For almost a decade, the families and communities of the 43 missing victims have been given false hope and are still searching for their loved ones after the Mexican government's investigation concluded that it was scientifically impossible that a mass cremation had occurred at the dump site and to keep looking elsewhere.

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Fire Investigation; Thermal Damage; Charred Body

A16 An Evaluation of Commingled Remains Using Computational Methods to Reconstruct Dental MNI Based on Dental Wear

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Learning Overview: This presentation will utilize computational methods to reconstruct dental Minimum Number of Individuals (MNI) based on dental wear. After attending this presentation, attendees will have a better understanding of this pilot study that shows a different approach to estimating the MNI and the importance of improving method efficiency and accuracy.

Impact Statement: This presentation will impact the forensic scientific community by highlighting the use of age-related dental wear to re-associate commingled teeth and reconstruct MNI using Python®. Attendees will grasp a better understanding of the use of Python® for purposes of re-associating commingled teeth and the potential use of this particular method to a wide range of commingled osteological datasets.

A high recovery of commingled osteological human remains at an archaeological site is quite difficult due to a wide range of factors.[1] Existing methods used in the estimation of MNI are time-consuming and present with accuracy challenges and embedded bias.[2] Osteo-sorting methods are limited to reconstructing MNI based on the most frequent recurrence of a diagnostic skeletal or dental element. Commingled and disarticulated dentition is exceptionally problematic in the absence of associating alveoli. An experimental approach using computational methods was developed to reconstruct MNI using age-related dental wear scores following Lovejoy (1985) in a commingled dental assemblage (1,278 adult teeth; 22 cist graves) from the Kourion Amathus Gate Cemetery (KAGC) in Cyprus.[3]

For the purposes of this study, a script was written in Python® that automatically calculated the MNI per tomb based on the amount of repetitive teeth and dental wear score in order to reconstruct individuals. The code looped over every tooth per tomb and created new individuals based on dental wear and tooth type. When the tooth did not fit any of the requirements to be placed within an already existing individual, a new individual was created. The dental wear scores per individual varied only +/- 1 phase – as described in the Lovejoy method – from the first tooth listed in the individual. These results were compared to the already determined MNI based on repetitiveness. It showed that a higher MNI was found in almost all the tombs when taking dental wear into account. The total MNI before the Python® method was used was 66. After applying the method, the MNI was calculated as 111. The Wilcoxon test found that this difference was statistically significant with a p-value of 0.000599.

It is, however, suggested that caution is applied in using this method because at this pilot stage, it is recognized that more research on this topic is required by testing the method on different datasets, preferably of known demographics and established dental MNI based on the level of wear present. This will allow us to exploit weaknesses in the method and prevent these by tackling them in advance. In addition, the possibility of uneven dental wear in an individual should be researched further to include that as a bias in the method to make it more reliable. The results of this pilot study show promising potential and should be explored further in the future.

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Dental Wear; Commingled Remains; Python®

A17 MNI, NIDS, Centroids: Additional Considerations for Individuating Commingled Remains Using Stable Isotope Ratios

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Learning Overview: After attending this presentation, attendees will have a better understanding of the intraperson variation seen in the stable isotope ratios of human bone collagen (carbon and nitrogen), and how this can and cannot be used in the segregation of commingled human remains.

Impact Statement: This presentation will impact the forensic science community by providing a more complete understanding of intraperson isotopic variation seen in human bone collagen, as well as a novel approach to the segregation of commingled human remains assemblages using stable isotope delta (δ) values.

Fragmentary and/or highly commingled assemblages can make the anthropological segregation of skeletal elements into discrete individuals more difficult. Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) measured for bone collagen have been shown to reliably sort modern commingled human remains by creating an isotopic Minimum Number of Individuals (MNI) for an assemblage.[1,2] Similar to morphological MNI in biological anthropology, the application of isotopic MNI has limitations for large-scale commingled assemblages from similar backgrounds (e.g., individuals consuming similar diets), often underestimating the number of individuals represented in an assemblage.

Isotope δ values have also been shown to reliably segregate faunal remains into a Number of Isotopically Distinct Specimens (NIDS). In the NIDS approach, Euclidean distances using a combination of $\delta^{13}C$ and $\delta^{15}N$ values in tandem rather than independently are applied to an assemblage for individuation.[3] Currently, there is no comprehensive understanding of skeletal turnover rates within the literature. We hypothesized that bone turnover could affect the isotopic profile of an individual, possibly leading to one element, or grouping of elements (e.g., lower body, upper body), to be consistently "average" compared to the individual's "overall" combined isotopic profile. In other words: Is there an element that consistently represents the average isotopic signature of the entire human body? Additionally, we hypothesized that a NIDS metric calculated for a human assemblage would be smaller than that previously calculated for faunal assemblages, based on the relative isotopic homogeneity of the modern human diet.

Collagen isotope ratios from 21 modern adult male individuals were examined. Each individual had a minimum of five long bones (at least two upper and two lower body elements); in total, 118 elements were included. The individuals originated from Northeast Asia, Southeast Asia, and the United States, and is a subset of the dataset previously used to determine human isotopic MNI.[1]

Chi-square tests were used to determine that there was no statistically significant difference between the mean or median elements when comparing all long bones (statistically assuming all elements had an equal likelihood of being the centroid). Chi-square tests were also used to determine that there were no statistically significant differences between various groupings (e.g., upper versus lower body, also assuming equal likelihood). Lastly, the mean intraindividual Euclidian distance was calculated as 0.29‰ ($\sigma = 0.16$), compared to the 0.52‰ ($\sigma = 0.45$) seen in faunal remains.[2] This supports the assumption that modern human diets are overall isotopically simple.

We found that there is no one element, nor grouping of elements within the long bones, that can be reliably used as a centroid for intraperson isotopic variation in the segregation of large-scale commingled assemblages. While bone turnover rates are still scarcely understood, our results suggest that intraskeletal variation in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values is not statistically affected by typical turnover processes. Additionally, the NIDS metric calculated in this study is smaller than what was seen previously in faunal remains, which suggests that a NIDS metric built from a human model could also be used for individuation, although further examination is required. This should be tested in real-world applications using other commingled remains.

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Stable Isotope Ratio Analysis; Bone Collagen; Commingling

A18   **The Accuracy and Reliability of Visual Pair Matching Lower Limb Bones in a Commingled Skeletal Assemblage**

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Learning Overview: After attending this presentation, attendees will have a better understanding of the accuracy and reliability of visual pair matching of lower limb bones within a commingled skeletal assemblage as well as the effect of unconscious bias during evaluation.

Impact Statement: This presentation will impact the forensic science community by bringing awareness to the strengths and limitations of visual pair matching as an association and segregation tool within commingled skeletal assemblages.

In cases of commingled human remains, visual pair matching is a commonly utilized method for sorting remains due to its efficiency and ease of use. However, the success of this method is largely based on observer experience, and few studies have tested its accuracy. As such, this study aims to address questions regarding the accuracy and reliability of visual pair matching of lower limb bones, as well as whether being aware of case context creates unconscious bias.

The sample consists of remains from individuals who were killed in the Battle of Tarawa during World War II. After recovery and identification efforts in the mid-1900s, unknown individuals were subsequently buried in the National Memorial Cemetery of the Pacific in Honolulu, HI. When these individuals were later disinterred by the Defense POW/MIA Accounting Agency (DPAA) for continued identification efforts, it was discovered that, in some cases, a moderate amount of commingling had occurred during both the recovery and early identification efforts.

For this study, pairs of femora, tibiae, and fibulae from ten unknown individuals, as received by DPAA, were analyzed. True pairs were based on the results of peer-reviewed analyses conducted at the DPAA laboratory by trained anthropologists. A total of 17 observers of varying experience and educational levels were given the 30 long bone pairs without any context and were asked to record: (1) if the antimeres pair match, (2) their confidence in their determination, and (3) a ranking of the top five skeletal features used in their determination. After at least one month, the observers were then asked to complete another evaluation of the remains. However, during the second analysis, the observers were provided basic information regarding the history of commingling for each pair of bones.

Results from both rounds of analysis yielded average pair-matching accuracies over 85%, with observer accuracy ranging from 64% to 100%. Intra-observer agreement between the first and second round of analysis, evaluated through kappa scores, showed moderate-to-good agreement overall; however, the variation in kappa scores for individual observers ranged from slight agreement to almost perfect agreement. Interestingly, intra-observer agreement does not seem to reflect years of experience or education.

This research highlights the overall accuracy and reliability of visual pair matching when given previously paired antimeres from commingled contexts. Furthermore, it discusses the influence of experience and context. While generally accurate and reliable, this research still demonstrates the potential benefit of more standardized visual pair matching methodologies in commingled contexts. Future research will explore the role of analyst confidence and observed skeletal traits in visual pair matching.

Pair Matching; Accuracy; Reliability

A19   Assessing the Impact of Opioid Use on Human Clavicles Through Clinical Computed Tomography (CT): A Pilot Study

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Learning Overview: This presentation will utilize clinical CT data from human decedents to investigate and illustrate potential morphological changes to the clavicles of young opioid users. After attending this presentation, attendees will understand the different classes of gross presentations of the costal tuberosity of the clavicle, the prevalence of each type found in opioid users versus non-opioid users, and that forensic anthropologists should consider opioid-induced morphological changes when assessing skeletal remains.

Impact Statement: This presentation will impact the forensic science community by providing the first known assessment of gross morphological changes to the clavicles in opioid users. This is especially important in a forensic anthropological context, as opioid users make up an increasing proportion of the decedents encountered, and the morphological impact of opioids on bone are currently unknown.

Opioid use and overdose deaths have been rapidly increasing in recent years.[1] Opioids are known in clinical settings to cause changes to bone quality and can present as osteoporosis-like pathologies.[2] In forensic anthropology, these pathologies may interfere with the assessment of skeletal remains (e.g., age estimation); however, evidence of any drug-related pathologies is not well documented and typically is only found in anecdotal form. Previous case studies described resorptive-like changes to the costal tuberosity along the inferior surface of the medial clavicles of known opioid users.[3] Prior research has described a variety of gross presentations of the costal tuberosity, also known as the rhomboid fossa or costal pit, ranging from smooth to elevated, depressed, or pitted.[4,5] In this pilot study, we utilized decedent CT scans to identify the pattern of these morphologies in the clavicles of documented opioid users to help inform forensic anthropological practices.

Clinical CT scans of 20 decedents (10 males, 10 females) ages 17–37 years were obtained from the New Mexico Decedent Image Database. Of those 20 decedents, 5 males and 5 females were known opioid users. The remaining decedents were age- and sex-matched controls with no history of opioid use. The right and left clavicles were segmented through manual thresholding and selection tools. Surface meshes were generated to create 3D models for visual scoring of the costal tuberosity for each clavicle using five classes: smooth, flat, elevated, depressed, and pitted. Cross-sections orthogonal to the bone surface were extracted for review of any internal changes. The clavicles of both the male and female opioid users presented with a mixture of classifications: smooth (two bilateral, three unilateral), flat (four unilateral), elevated (one unilateral), depressed (one bilateral, four unilateral), and pitted (two unilateral). The male controls presented with only depressed (four bilateral) and pitted (one bilateral), while the female controls presented with only smooth (one bilateral) and flat (four bilateral). In nearly all individuals, the cortex of the costal tuberosity region was visualized as more radiolucent when viewed in cross-section, indicating a potential region of ongoing bone remodeling.

While the sample size of this study is small, the results indicate opioid users, especially females, may display different classes of costal tuberosity morphologies than non-opioid users. This work also lays the foundation for future research into opioid-induced gross morphological changes to the human skeleton and their impacts on forensic anthropological casework.

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Forensic Anthropology; Opioids; Bone

A20 An Evaluation and Integration of Disparate Classification Schemes for Interparietal Bones in the Human Crania

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Learning Overview: After attending this presentation, attendees will be familiarized with scoring strategies for interparietal bones and newly developed, standardized composable diagrams capturing various expressions of interparietal bones in a single system for use by forensic anthropologists, skeletal biologists, and clinicians.

Impact Statement: This presentation will impact the forensic science community by addressing a significant gap in current understanding of the growth and development, distribution, and classification of interparietal bones.

Supernumerary (wormian, extrasutural) bones in the skull are some of the most recognizable cranial non-metric traits in the human skeleton. The study of supernumerary bones has extended well beyond anthropology to other disciplines, likely due to their abundance, variation in form, and association with various clinical conditions (e.g., pyknodysostosis, rickets).[1,2] However, disciplines interpret supernumerary bones differently due to divergent points of interest and focus, leading to disparate scoring strategies and limiting interdisciplinary research. Standardization is needed for better collaboration and communication but should not erase or alter existing systems. A compromise is the creation of a unifying model using an evidence-based approach, incorporating data from currently available systems into a single data collection/data sharing protocol.

To demonstrate the utility of standardization through evidence-based composable schematics, the authors focus on research into interparietal bones in the human occipital. Interparietal bones are of particular interest due to their various manifestations and because their documentation is essential for forensic anthropologists differentiating between supernumerary bones and perimortem trauma. Through a comprehensive review of the available literature, we identified research articles and chapters focusing on the appearance and classification of interparietal bones. Specifically, the authors examined trends in the study of interparietal bones over time, as well as differences in foci and the various approaches to classification of these manifestations. In addition to this literature review, the authors examined physical evidence of interparietal bone variations on crania, including those in research article figures and those available in nearby university skeletal collections.

Much of the research focused specifically on the growth and development of interparietal bones through examination of fetal occipital squamae and the identification of ossification centers. We found large discrepancies in the reported number (ranging anywhere from 1–10) and positioning of ossification centers. In terms of classification, we identified a number of disparate methods. Some of these use a dichotomous (absence/presence) system, often based on the presence of a complete transverse occipital suture, while others use the overall morphology of the interparietal bone, where any additional bone in the occipital squama is included and the structure emphasized (e.g., bipartite, quadripartite). Although some overlap between systems exists, the variations in foci are often too disparate for direct comparisons.

Therefore, the next step in our research was the development and deployment of composable schematic illustrations incorporating all of these methodological descriptors into one unifying system. These diagrams, digitally created and stored as Scalable Vector Graphics (SVGs), account for the location of all occipital ossification centers and variations in the expression of interparietal bones observed in adult crania or identified in the literature. The inclusion of all variations seen in our unifying model allows for comparison between systems not previously possible, without the erasure or modification of previous work.

Standardization is an important component of science. Our research captures multiple systems in one outlet and permits data collection, data sharing, and data management using evidence-based illustrations. In addition, the flexibility of our approach permits the inclusion of new morphologies without the need to change an old system. We present this research and argue similar standardization is needed for other supernumerary bones on the human cranium.

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Cranial Non-Metric Traits; Extrasutural Bone; Composable Schematics

A21   Is Bone Speed of Sound Affected by the Molecular Composition of Infant Bone?

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Learning Overview: The goal of this presentation is to investigate whether the molecular composition of infant bone affects bone Speed of Sound (SOS) values and, if so, evaluate which components are influential.

Impact Statement: This presentation will impact the forensic science community by contributing to the research evaluating the relationship between the components of bone quality and SOS values in infants.

Forensic anthropologists, either explicitly or implicitly, assess bone quality as part of a blunt force trauma analysis. In association with pediatric trauma cases, forensic pathologists and attorneys may ask the consulting forensic anthropologists for their expert opinion regarding a child's bone quality as this may have important bearing on the death investigation and legal proceedings. Yet, there is currently no non-invasive quantitative method to measure general bone quality in infants. Such a method could inform diagnoses of infant skeletal trauma in both the clinical and/or medicolegal settings. Studies of the adult population indicate that bone SOS values, as measured by quantitative ultrasound, are significantly correlated with Bone Mineral Density (BMD), mechanical properties associated with bone strength, and fracture susceptibility. As such, SOS values are reported to measure bone quality in the adult population. It is unclear whether the same is true for infants.

This is the third phase of a larger study investigating the relationship between SOS and bone quality in infants. Previous phases identified significant correlations between SOS and: (1) the volumetric BMD (vBMD) of infant bone as measured by micro-Computed Tomography (microCT), and (2) the ultimate bone strength and elastic toughness of infant bone as measured by mechanical testing.

The current phase evaluates the relationship between the molecular composition of infant bone, as measured by Raman spectroscopy, and SOS. The study sample consists of 47 infants between the ages of 30 weeks gestation and 10 months at the time of death that were autopsied at the Harris County Institute of Forensic Sciences. Post autopsy, SOS (m/s) was measured on the anteromedial aspect of the left tibial midshaft using a quantitative ultrasound device. Planks of the tibial midshafts coinciding with the SOS measurement site were subjected to Raman spectroscopic analysis to quantify bone composition. Univariate statistics were used to investigate the relationship between SOS and bone composition.

Results indicate that SOS is positively correlated with mineral-to-matrix ratio, explaining 10% of the variance in SOS ($r = .309$, $p=.029$). SOS was not correlated with any other bone composition parameters, including mineral-to-collagen ratio. These findings suggest that SOS increases as bone mineral content increases. A linear regression predicting SOS using mineral-to-matrix ratio values and vBMD with stepwise variable selection was performed to further explore the relationship between SOS, bone mineral content, and bone mineral quantity. Mineral-to-matrix ratio was excluded from the model, indicating that bone mineral content on the molecular scale did not significantly contribute to the variance in the SOS data beyond bone mineral quantity. This may suggest that bone quantity is more influential on SOS than bone mineral content. However, these results may also reflect the difference in scale in which SOS and bone composition were measured. Translated for medicolegal use, ultimate material strength and elastic toughness, as measured by mechanical testing, vBMD as measured by microCT, and bone mineral content as measured by Raman spectroscopy are positively correlated with SOS, indicating SOS is influenced by multiple components of bone quality in infants. There is still variance in the SOS measurements that remains unexplained by any of the tested methods. Future phases will investigate the source(s) of this unexplained variance, including possible effects of the presence and distribution of subperiosteal new bone on the methods used to quantify the components of infant bone quality.

Forensic Anthropology; Bone Speed Of Sound (SOS); Raman Spectroscopy

A22 Laser Me This: Using Laser-Induced Breakdown Spectroscopy to Detect Formalin in Human Remains

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Learning Overview: After attending this presentation, attendees will have learned a new approach to evaluating taphonomic changes to human remains.

Impact Statement: This presentation will impact the forensic science community by introducing attendees to the detection of formalin within preserved remains using Laser-Induced Breakdown Spectroscopy (LIBS).

Forensic anthropologists are tasked with evaluating taphonomic change as it informs death investigations. The use of LIBS in forensic anthropological casework has matched skeletal elements to the individual in mass fatality or commingled assemblages. However, the LIBS is both an analytical instrument and a technique increasing in popularity and utility in research laboratories as the spectrometer applies a high-powered, minimally destructive pulsed laser to a sample surface area and creates vaporized plasma for spectral analysis at various wavelengths (nm).[1,2] Despite these advances, researchers have not addressed the utility of the LIBS in the detection of human remains exposed to embalming fluids. Formalin is a fixative chemical (containing 40% aqueous formaldehyde) that can be used during embalming funerary practices.[3]

In this case study of fetal remains presumed to be associated with a defunct funeral home, the LIBS (System 150 Nano Series Pulsed Nd: YAG laser—RMA 0346-00) was used to analyze the intensity of formalin (10% neutral buffer) on human bone and soft tissue. A standard control was established by soaking a sterile cotton swab in formalin until saturation. This standard was compared to spectra of soft tissue, left temporal, right scapula, right femur, and soil (e.g., fill dirt) associated with these remains. The LIBS was sampled on the midpoint surface of each element and produced spectral analyses evaluated from 528nm to 716nm. The formalin control had 14 peaks that were used for comparison. The soft tissue spectrum yielded 100% peak similarities and suggested that the formalin was completely absorbed in the sample. In descending order, the temporal (64.3%), soil (21.4%), and scapula (7.1%) revealed spectral peaks consistent to the control (Fisher's Exact Test, $N = 6$, $p > 0.05$). The femur (0%) produced no similar peaks to the control but was previously encased in the soft tissue. This case study revealed that the LIBS can detect formalin (10% neutral buffer) with most of the similarities produced in the soft tissue, skeletal elements, and soil containing the remains.

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Laser; Human Remains; Preservation

A23 An Application of Dietary Isotopes to Estimate Temporal Context of Unidentified Human Remains in British Columbia, Canada

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Learning Overview: The goal of this presentation is to demonstrate the basics of dietary isotopic analysis and how it can be employed as an investigative tool in determining the medicolegal significance of unidentified human remains. Using carbon and nitrogen isotopes, we demonstrate how an individual's diet can be an initial indicator of temporality.

Impact Statement: This presentation will impact the forensic science community by demonstrating one of many potential uses for human isotopic analysis in forensic investigation. Isotopic analysis provides a cost-effective initial strategy for investigations involving skeletal unidentified human remains. Dietary isotopes indicated that 15 of 33 individuals were of an archaeological context. These interpretations were validated using radiocarbon dating.

Isotopic analysis has been used in archaeological and forensic contexts to examine diet, migration, trace evidence, and the origin of individuals.[1] On behalf of, and in collaboration with, the British Columbia (Canada) Coroners Service, this project sought to estimate the medicolegal significance of 33 cases of unidentified human remains using isotopes to indicate the subsistence pattern of these individuals. Carbon and nitrogen isotopes were analyzed as they are strong indicators of diet and could potentially differentiate an archaeological or contemporary population. As bulk isotopic analysis is more affordable than radiocarbon dating, this method may be a cost-effective initial strategy to estimate the medicolegal significance of unidentified human remains.

Archaeological populations in British Columbia typically maintained a marine diet resulting in higher $\delta^{15}N$ and $\delta^{13}C$ isotopic values, while contemporary populations typically maintain a terrestrial-based diet, resulting in lower $\delta^{15}N$ and $\delta^{13}C$ isotopic values.[1]

We extracted collagen from the 33 individuals and measured the carbon and nitrogen isotopes in the Archaeology Isotope Laboratory at the Department of Archaeology, Simon Fraser University, Canada. Using K-means cluster analysis, the sample was clustered into two archaeological or contemporary groups. Cases considered archaeological would be returned to local communities for reburial, while those determined to be contemporary indicate possible medicolegal significance. These results were validated using radiocarbon dating to determine the accuracy of the authors' interpretations.

Fifteen individuals were classified as archaeological context due based on their increased $\delta^{15}N$ and $\delta^{13}C$ values, while the remaining 18 individuals were classified as contemporary due to their lower $\delta^{15}N$ and $\delta^{13}C$ values. Radiocarbon dating confirmed that all individuals classified as archaeological were not of forensic interest.

The significance of this research is the demonstration that bulk isotopic analysis can provide a cost-effective initial strategy to the investigation of unidentified human remains when it is unclear whether they are from forensic or archaeological contexts.

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Stable Isotopes; Unidentified Persons; Anthropology

A24   How Low Can You Go? A Lower Bound Limit for δ^{15}N Values From Human Bone Collagen

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Learning Overview: After attending this presentation, attendees will understand a potential new tool for determining if osseous material is non-human animal based on nitrogen isotope delta values.

Impact Statement: This presentation will impact the forensic science community by demonstrating a lower bound limit for nitrogen isotope delta (δ) values for human bone collagen that can assist in determining if osseous material originated from a non-human animal.

One of the foundational questions in forensic investigations is identifying the material being examined and determining its probative value. For laboratories that routinely deal with small, non-diagnostic osseous material, this process often entails asking: is it human or non-human? The answer to this question has major implications within laboratory and medicolegal systems, as a non-human result will often remove the item from an evidentiary tract.

For non-diagnostic remains, multiple approaches for answering human vs. non-human questions are available in a large interdisciplinary laboratory setting. Histological analysis and DNA analysis (12S protocols) are often used. However, these analyses may not be feasible or appropriate due to resource limitations and/or available sample materials. Stable isotope ratio analysis has previously been used for assessing the origin of unidentified human remains, assisting with individuation, and supporting identifications in large, commingled assemblages.[1] The integration of isotope testing into the laboratory framework provides an opportunity to investigate its potential as an additional tool for determining if osseous material is human or non-human.

The present study investigates the use of nitrogen isotope δ values (δ^{15}N) to determine if a lower limit exists for human bone collagen. The examined data were generated from an accredited laboratory that prepares, analyzes, and interprets bone collagen δ^{15}N values as a routine part of casework. In total, approximately 4,068 samples had measured δ^{15}N values available for the study. This dataset was scrubbed to remove samples with unacceptable atomic C:N ratios (<3.1 or >3.5); second preparations/analyses of samples completed solely for research purposes; and known non-humans, resulting in a final dataset of 3,613 test results. Individuation of the final dataset was not performed to mimic real casework circumstances in which an individual may be sampled multiple times for testing.

The mean δ^{15}N value for the 3,613 samples was 11.38‰ with a standard deviation (σ) of 1.17‰. The range was 5.16‰ to 19.33‰. Using these statistics, we propose a lower limit of 3σ from the mean for "are probably" non-human and a limit of 4σ from the mean for "are" non-human. After accounting for measurement uncertainty (0.34‰) and rounding to the nearest 0.05‰, these proposed limits are 7.50‰ and 6.35‰, respectively. From the dataset, 43 samples fell below the "are probably" non-human limit of 7.50‰ for an error rate of 1.19% and only 5 samples fell below the "are" non-human limit of 6.35‰ for an error rate of 0.14%.

An initial test of these limits using 12 non-human elements and standards available in the laboratory found that 67% (n = 8) fell below the "are probably" limit, and 50% (n = 6) fell below the "are" limit. The limits will be further tested using all routine casework samples prepared in the laboratory between August 2023 and January 2024. Additionally, no modern human bone collagen δ^{15}N value found in the published literature to date has fallen below these limits. Future work will use more published data (e.g., a large dataset of non-human samples) to test the lower bound limits for δ15N values of 7.50‰ for "are probably" and 6.35‰ for "are" non-human to refine their use and application.[2-4]

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Stable Isotopes; Anthropology; Fragments

A25 Taphonomic Effects on the C:N Ratio and δ^{13}C and δ^{15}N Isotopes in Bone Collagen

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Learning Overview: After attending this presentation, attendees will understand the taphonomic effects of season and deposition type on stable isotope values in bone collagen using fetal and juvenile domestic pig bone samples.

Impact Statement: This presentation will impact the forensic science community by providing data on the preservation quality of collagen in bone using the C:N value, as well as identifying variation in the δ^{13}C and δ^{15}N values in bone deposited in different seasons and in deposition types.

Bone provides mechanical and functional properties to the skeletal elements within the human body. Structurally, the organic, proteinaceous part of bone creates a protective framework around the inorganic, mineral part of bone. This structure gives bone resistance against diagenetic processes such as bacterial invasion. The C:N ratio in bone has been used to measure the quality of the organic material in bone, which is almost entirely made up of the protein collagen.[1] In addition, the δ^{13}C and δ^{15}N values from bone collagen have been used to reconstruct past diets and determine suitability of collagen for radiocarbon dating.[2] In various conditions, microbial processes affect the rate of bioerosion depending on length of time and the conditions of the environment.[3] In turn, stable carbon and nitrogen isotope values in bone collagen may be influenced by the environment in which the bone is buried or deposited. In a forensic context, observing the conditions in which the C:N ratio may be affected by taphonomic factors is important to understand not only taphonomic effects of varying burial conditions, but also the reliability of δ^{13}C and δ^{15}N values.

The purpose of this study is twofold: (1) to explore collagen preservation in bone using the C:N ratio and its variation according to depositional and seasonal environments in a temperate zone; and (2) to examine individual δ^{13}C and δ^{15}N variation within these different seasonal and depositional environments. The current research draws on samples of *Sus scrofa domesticus* (fetal n=16 and juvenile n=13) deposited seasonally over the course of two years in Raleigh, NC. Raleigh, NC, is in the Piedmont region, which is defined by the Köppen-Geiger climate classification system as a Cfa climate: temperate, without a dry season, and with a hot summer.[4] Isotopic values were measured using an elemental analyzer isotope ratio mass spectrometer. A one-way Analysis Of Variance (ANOVA) was performed to compare the effect of season and deposition on C:N, δ^{13}C, and δ^{15}N. A paired sample t-test was performed on all results to compare each pair of seasons (summer and winter, summer and fall, summer and spring, spring and winter, fall and winter, spring and fall) and each pair of depositions (bag and control, blank and control, bag and blanket fetal remains, and buried and surface juvenile remains).

The ANOVA and paired sample t-test results from this study indicate that conditions of burial associated with seasonality, such as moisture and temperature, affect the stable isotope values obtained from bone. Season was a significant factor in the δ^{13}C value in juvenile bone (p-value=0.011), the C:N ratio between summer and winter (p-value=0.023), and in fetal bone between summer and fall (p-value=0.029), and fall and spring (p-value=0.017).

Season was not a significant factor in the δ^{15}N value for fetal (p-value=0.557) and juvenile (p-value=0.228) bone, and deposition was not a significant factor in C:N ratio (p-value=0.405) or the δ^{13}C and δ^{15}N values for both fetal (p-value=0.727; p-value=0.386, respectively) and juvenile (p-value=0.753; p-value=0.689, respectively) bone. Conditions of seasonality affect stable isotope analyses on bone collagen, and taphonomic alteration should be considered when conducting these analyses. Further research is needed to consider if the variation observed in this study is applicable in other settings and how to control for this variation.

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Stable Isotope Analysis; Taphonomy; Preservation

A26 Using Forensic Anthropological Landmarks to Optimize DNA Extractions From Post-Cranial Remains

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Learning Overview: After attending this presentation, attendees will understand how the various bone cell types (osteoblasts, osteoclasts, and osteocytes) that contribute to the formation, maintenance, and changes that forensic anthropologists rely on relate directly to forensic DNA analyses. Additionally, there will be discussion of the bone tissue types (cortical, trabecular, and lamellar) and how these also relate to mitochondrial, autosomal, and Y-chromosomal DNA analyses. There will be a brief discussion of the specific sampling method, which was designed to limit the amount of destruction to bone tissue while maintaining the integrity of DNA extraction, quantitation, and analysis.

Impact Statement: This presentation will impact the forensic science community by providing an effective method for human bone sampling that reduces destruction to bone tissue, cost, and turn-around time associated with low DNA levels while increasing efficiency and accuracy. The method is effective for mitochondrial DNA (mtDNA) analysis, Short Tandem Repeat (STR) profile construction, and Y-chromosomal analysis and is currently being investigated for use in Single Nucleotide Polymorphism (SNP) panel construction for use in Forensic Genetic Genealogy (FGG).

It is well known in the fields surrounding forensic human identification that not all bones are ideal sources of postmortem DNA. To date, studies have focused on the primacy of postmortem (taphonomic) factors such as soil pH or exposure to water or sunlight, in an attempt to explain differences in DNA preservation. No current studies of human skeletal remains exist that incorporate deep understanding of the cellular components of bone as a potential reason for differential DNA preservation between or within post-cranial elements.[1-12] This research seeks to fill that void. By testing nearly 400 extraction sites representing all bone groups and three bone tissue types, this study seeks to find an explanation for differential DNA preservation that spans all demographic and taphonomic factors and uses far less bone tissue than in previous studies. Here, we show that knowledge of the cellular processes and populations specific to skeletal development and maintenance leads to far more targeted and accurate DNA extractions. By combining this novel sampling strategy with well-established DNA analytical methods, we can avoid excessive destruction of human skeletal material, reduce associated costs and turnaround time, and increase reliability of forensic DNA testing.[13–17]

Unlike previous studies, this project began from a targeted cellular and microstructure-based approach. Combining knowledge of bone cell and tissue types with traditional forensic anthropological methods of human identification, this project sought a new explanation for differential DNA preservation in intra-and inter-elemental extraction sites. The project began with a very targeted and strategic approach to sampling, followed by traditional chemical DNA extraction, amplification and quantitation of both autosomal DNA and mtDNA, and concluded with forensic STR profile construction.

This study provided a rare opportunity to investigate the role of the different osteological cell types: osteoblasts (including their differentiation into bone lining cells), multinucleated osteoclasts in the process of bone matrix resorption at the time of death, and fully encapsulated osteocytes, as a potential reason for differential DNA preservation and extraction quantity and quality in modern forensic cases.[19–29] From this launching point, detailed analysis was performed of the variance in DNA preservation and accessibility across a single, recently deceased individual's skeletal remains. This analysis into the number of starting molecules for mitochondrial, autosomal, and Y- chromosomal DNA, as well as the ability to obtain a complete Combined DNA Index System (CODIS) profile, allows us to understand how differential DNA presence, and therefore accessibility, can be predicted and measured across the post-crania. Finally, the results clearly showed that this sampling method is more accurate, significantly less destructive, and thus more cost and time efficient than methods used previously. The method can be used by forensic practitioners as well as biological anthropologists involved with genetic and genomic research of ancient humans and hominins.

By starting from a cellular and biochemical launching point, this research attempts to bridge the knowledge gap between forensic anthropology, forensic DNA analysis, and biomedical understanding of human skeletal biology and to make that knowledge accessible and applicable to those professionals on the front lines of the real-world problem of identifying unknown human skeletal remains.

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Anthropology; DNA; Bone

A27 Validation and Quality Control of 3D Coordinate Data From Human Crania for Forensic Casework

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Learning Overview: After attending this presentation, attendees will have a new approach to performance check digitizers according to laboratory accreditation standards for the purpose of obtaining 3D coordinate data from human crania.

Impact Statement: This presentation will impact the forensic science community by demonstrating how to compute the measurement uncertainty and repeatability for Interlandmark Distances (ILDs) obtained from 3D coordinate data in accordance with laboratory accreditation standards.

Direct digitization of anatomical landmarks more accurately captures geometrical information than traditional linear approaches. Other studies have demonstrated high repeatability with relatively low intra- and inter-observer error in locating most anatomical landmarks on crania with direct digitization as well as between other 3D modalities.[1-5] Laboratory accreditation, however, requires annual performance checks for measurement devices, including 3D coordinate machines.[6] Further, 3D coordinate data obtained for forensic casework are often transformed to ILDs to be used in discriminant analysis function software to interpret population affinity. Therefore, the purpose of this study was to design a quality control, performance check procedure that ensures ILDs extracted from 3D coordinate data are below the threshold of measurement uncertainty required by laboratory accreditation standards.

Eleven traditional craniometric measurements and their corresponding 18 anatomical landmarks were collected from 25 complete crania at the Defense POW/MIA Accounting Agency (DPAA).[7] To assess the full range of measurement uncertainty, Type I, II, and III landmarks were included.[8] Crania were measured twice at one-week intervals and digitized with a Microscribe® G2X digitizer a total of six times between two sessions (three times per session) at one-week intervals.[9] Landmarks were identified using small dot stickers when obtaining traditional measurements and were removed between the two one-week intervals. Measurement uncertainty has two components: random and systematic. The systematic uncertainty of the digitizer is reported by the manufacturer as 0.23mm and the measurement variance is 0.0529mm. The random measurement uncertainty was calculated as the combined standard uncertainty, or the square root of the variance of all measurements, and then expanded to encompass a 95% confidence interval. If the expanded uncertainty across the repeated measures was less than 2mm, it was within the acceptable margin of error for traditional craniometric measurements. A mixed effects Analysis of Variance (ANOVA) model for repeated measures was employed to assess the repeatability of the measurements between the two modalities. Finally, a repeated measures Multivariate Analysis of Variance (MANOVA) test was used to assess within-group differences across all measurements collectively.

The expanded measurement uncertainty of all 11 ILDs was below 2mm, with a range of 1.18 (frontal chord) to 1.72mm (asterionic breadth), and within a 95% confidence interval. Frontal chord distance also showed the most consistent values of uncertainty for each cranium digitized. In general, the measurements showing the greatest variation in uncertainty were parietal chord, maximum cranial length, and asterionic breadth. None of the craniometric measurements were significant for the univariate repeated measure random effects demonstrating good repeatability across 11 measurements collected. The MANOVA procedure also showed no significant differences between repeated measures across all craniometric ILDs ($p=0.3528$). These results demonstrate: (1) good repeatability across the measurements collected, and (2) the digitizer used has relatively low measurement uncertainty within acceptable standards. Finally, this procedure provides consistent measures that demonstrate its efficacy for collecting 3D coordinate data within performance check standards.

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Validation; 3D Coordinate Data; Measurement Uncertainty

A28   A Multivariate Analysis and Evaluation of Classification of Human Skeletal Vertebrae for an Applied Context

Jolen Anya Minetz*, University of Illinois, Paxton, IL; Stephen Ousley (posthumously), Erie, PA

Learning Overview: This presentation explains a new osteometric method for classifying vertebrae and will demonstrate how multivariate analyses and quantitative classification of individual vertebrae within a vertebral column can be applied in a Forensic anthropology context.

Impact Statement: This presentation will impact the forensic science community by demonstrating the utility of a multivariate analysis of vertebrae in an applied context.

The purpose of this osteometric vertebral classification method is to assist with the sorting and analysis of fragmentary or commingled skeletal remains. The human vertebral column is a morphologically complex group of elements. Current methods rely on morphological characteristics to classify isolated vertebrae qualitatively. This research provides a bridge between morphological assumptions for vertebral designations and quantitative classification. These osteometric methods and statistical analyses provide quantifiable information relating to the accuracy of classifying individual vertebrae.

The sample used for this analysis consists of osteometric vertebral measurements from intact vertebral columns from 59 individuals. To assess the overall potential for these vertebral measurements to classify vertebrae, regional grouping models based on vertebral column segments (cervical 3-7, thoracic, and lumbar models) were developed and analyzed. The data was tested for multivariate normality and homogeneity of Variance-Covariance Matrices (VCMs) to comply with the assumptions required by the statistical analyses used for classification. This analysis utilized Linear Discriminant function Analysis (LDA) and K-Nearest Neighbors (KNN) classification methods. LDA requires homogenous VCMs, so when the VCMs were not homogenous, KNN was used for classification. LDA classification analyses for two cervical models (C3-C7 and C3-C5) were conducted using all available variables. The C3-C7 model has a prediction accuracy of 0.7366, and the C3-C5 model has a prediction accuracy of 0.7108. The full models for the thoracic and lumbar vertebral segments could not use LDA for classification since the VCMs for these full models were not homogenous, so KNN was used. The KNN algorithm is a supervised machine learning classification technique; the optimal number of k neighbors for each model was determined by proportionally splitting the data into training (70%) and testing (30%) samples. The classification for the full thoracic model (T1-T12) using the KNN algorithm (k=28) results in a prediction accuracy of 0.5103. The classification for the full lumbar model (L1-L5) using the KNN algorithm (k=9) results in a prediction accuracy of 0.5672. The sensitivity and specificity of each vertebral group prediction within each model was evaluated.

This method has been developed to assist with the sorting and analysis of commingled and fragmentary skeletal remains, which have a variable number of potential measurements. The results in this abstract illustrate the potential applicability of these methods in a broad and passive way; however, in an applied situation, the LDA and KNN methods should be used more dynamically (i.e., create the most appropriate models, using the most appropriate methods, for each situation) in order to accommodate the process of eliminating groups of vertebrae to reduce the model to the most relevant groups of vertebrae for the final analysis. For example, an unseriated mid-thoracic vertebra might start with a broad T1-12 model, but through an iterative deductive process, it may end with a T5-T7 model with probabilities associated with each potential vertebral group classification. This research demonstrates that by using osteometric methods and statistical analyses, the accuracy of individual vertebra classification is quantifiable.

Vertebrae; Classification; Multivariate Osteometric Analysis

A29 Investigating Applications of Forensic Fractography of Bone Over the Postmortem Interval

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Learning Overview: After attending this presentation, attendees will be informed about fractographic features observed in bones exposed to outdoor conditions in the semi-arid climate of West Texas.

Impact Statement: This presentation will impact the forensic science community by contributing regionally and seasonally relevant data on the utility of forensic fractography of bone throughout the Postmortem Interval (PMI).

Fracture surfaces may provide important information about bone failure.[1] Fracture surfaces of brittle materials exhibit features relating to the origin, speed, stability, and direction of the propagating crack.[1] Research shows fractographic surface features can reliably indicate crack initiation and propagation in fresh bone, however there is limited data on their utility throughout the PMI.[1-3]

The objectives of this study were to: (1) investigate whether fractographic surface features are observed in bone throughout the PMI, (2) evaluate whether their frequency varies with PMI length, and (3) investigate their utility for reconstructing crack propagation throughout the PMI.

Forty partially de-fleshed deer femora were acquired from a local wild game processor. Five bones were never exposed to outdoor conditions (trial 0). The remaining 35 bones were placed within caging in a designated outdoor research area in Lubbock, TX, in January 2023. Five bones were removed every 2 weeks for 14 weeks (trials 1-7). A drop tower apparatus was used to deliver blunt force impacts to the anterior midshafts of each femur.

After impact, bones were macerated and fragments >10mm were examined for fractographic features, including bone mirror, bone hackle, arrest ridges, wake features, and cantilever curl. Fragments were examined using the unaided eye, a magnifying lens (3x magnification), and a stereomicroscope (0.7x–4.5x magnification). Fractographic features were then used to determine crack propagation direction and nucleation.[2]

Environmental data was obtained from the West Texas Mesonet weather network. During experiments, the average daily minimum temperature was 2.22ºC, maximum temperature was 19.44ºC, minimum relative humidity was 21%, maximum relative humidity was 71%, precipitation was 0.032cm, and solar radiation was 5.43kWh/m. Accumulated degree days following outdoor placement were as follows: trial 1 (537), trial 2 (1,164.5), trial 3 (1,892), trial 4 (2,618), trial 5 (3,379.5), trial 6 (4,243.5), and trial 7 (5,075).

The number of fragments produced per bone ranged from 0 to 31 (average = 9.3). Fractographic surface features were present in all trials. Cantilever curl was most frequently observed (90% of experiments), followed by arrest ridges (62.5%), bone hackle (52.5%), wake features (40%), and bone mirror (30%). In one bone (trial 0), no fractographic surface features could be identified. While most features were present in similar frequencies from trial 0 to 7, bone mirror was identified less frequently in later trials.

Orientation of fractographic features aligned with biomechanical expectations for anterior loading of the femoral midshaft. When present, bone mirror was always located posteriorly, consistent with crack initiation on the posterior tensile surface. Cantilever curl was located anteriorly 90% of the time, consistent with termination on the anterior compression surface.

This study contributes data on fractographic features in bone exposed to outdoor conditions during the mild winter and spring months in West Texas. The results show fractographic features are present and can be used to reconstruct crack propagation and nucleation throughout the recent PMI. The similar frequencies of fractographic features across different PMIs suggest that their presence alone is not indicative of PMI. These results are consistent with results obtained in human bones subjected to simulated warm weather conditions up to 40,600 accumulated degree hours.[3] The next phase of research will repeat experiments during the hot summer to fall months to investigate potential seasonal effects on expression of fractographic features.

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Anthropology; Trauma; Blunt Force

A30   Measuring Volatile Organic Compounds (VOCs) From Decomposing Human Tissue

Sheana S. Ramcharan*, SUNY University at Buffalo, Williamsville, NY; Luis L. Cabo, Mercyhurst University, Erie, PA; Jack D. Williams, Department of Chemistry and Biochemistry, Mercyhurst University, Erie, PA; Michael S. Foulk, Mercyhurst University, Erie, PA

Learning Overview: After attending this presentation, attendees will understand the different decomposition research conducted and its shortcomings due to different taphonomic agents. This presentation highlights the need for a controlled environment to account for as many variables as possible and proposes a new way of conducting this research in a controlled laboratory environment as opposed to an outdoor setting using whole bodies on body farms. This presentation also discusses the relationship between microbes and their off gases (VOCs).

Impact Statement: This presentation will benefit the forensic science community by focusing on a new experimental design with a proof of concept that can be replicated in a laboratory setting. Body farms are not in abundance and whole-body research has many limitations. This method can be used to test and control all variables known to impact human decomposition and was still tested on humans instead of on animal proxies; this will allow for more research to be conducted on human decomposition and will allow for better Postmortem Interval (PMI) estimation.

Accurate estimation of the PMI is a key element of forensic investigations, influencing aspects as diverse as victim identification, event reconstruction, and alibi corroboration. Current forensic pathology methods, based on a variety of short-lived physiological and biochemical markers, are effective at low PMI ranges of a few days. However, after the onset of more advanced decomposition stages, current PMI estimation methods must rely on the degree of decomposition of soft tissues, or entomological data. Most PMI research focusing on soft tissue decomposition utilizes designs based on the deposition of whole bodies at outdoor body farms, usually grouping decomposition into five broad macroscopic stages (fresh, bloat, active, advanced, and skeletonized). However, these designs do not allow the control of many relevant taphonomic variables (e.g., experimentally fix their values), their results are difficult to extrapolate to other geographic and environmental contexts, and make it difficult to reliably standardize the collection of some decomposition outputs, such as VOCs, which are gas byproducts produced as microbes break down organic macromolecules during decomposition.

This study proposes a more accessible design for collecting and analyzing VOCs, including at outdoor settings, utilizing isolated muscle tissue samples to be later safely stored, maintained, and analyzed in a controlled, hypoxic laboratory environment. One-gram samples of human muscle tissue were placed into 52 hermetic headspace vials filled with either sterilized or non-sterile natural soil, which were then maintained in an incubator at 25°C for 60 days. This design allowed us to sample and analyze the produced gases without opening the vials. Two sample vials from each treatment were extracted every five days, and their VOCs analyzed through Solid-Phase Microextraction (SPME) Gas Chromatography/Mass Spectrometry (GC/MS) to examine their evolution with time in each one of the soil treatments.

Preliminary findings indicate significant differences in VOC off-gassing between sterilized and non-sterile soil samples, serving to outline differences in the relative contributions of the soil and tissue microbiomes to the decomposition process. VOC composition was also observed to change over time, likely reflecting the evolution of the microbial community.

This tightly controlled experimental approach is a proof of concept and initial attempt to sample and characterize the VOCs emitted by decomposing human tissue. The results of this line of research show great promise to contribute to PMI estimation and areas such as buried body detection, soil chemistry, and the evolution of microbial communities during human decomposition.

Anthropology; Volatiles; Taphonomy

A31   Tracing PMI Through Bone Metabolomics With GC/MS and LC/MS/MS

Elisa Roggia*, University of Central Lancashire, Piemonte, Italy; Luke William Gent, University of Central Lancashire, Preston, England; Andrea Bonicelli, University of Central Lancashire, Preston, England; Noemi Procopio, University of Central Lancashire, Preston, England; Eugenio Alladio, Department of Chemistry, University of Torino, Piemonte, Italy

Learning Overview: The goal of this presentation is to show how metabolomic approaches can be used for Postmortem Interval (PMI) estimation on skeletonized tissues and will highlight the accuracy in the estimation that can be achieved by conducting such studies and how parameters such as burial depth affect the estimations.

Impact Statement: This presentation will impact the forensic science community by offering a new approach for PMI estimation of skeletal remains buried for up to six months. Attendees will learn about the effect that the burial environment plays on the metabolomic signature and about the accuracy that this method offers.

The estimation of the PMI has a key role in forensic investigations.[1] The postmortem modifications affecting the human body are complex and known to be affected by various intrinsic and extrinsic factors, making the PMI estimation challenging and debated. Recent studies have shown the potential of metabolomics for estimating PMI starting from skeletal remains.[2,3] This study aims to investigate the correlations between bone metabolites and PMI in a six-month time window, as well as to investigate the influence of depth on the metabolomic signature.

The experiment was conducted at the Taphonomic Research in Anthropology: Centre for Experimental Studies (TRACES), the animal taphonomy facility of the University of Central Lancashire, UnitedKingdom. Overall, 12 pig mandibles purchased from a local butcher were divided into four quadrants. All the fragments obtained were considered to be biological replicates, as preliminary analyses on the fresh bones showed that their metabolomic profile was comparable. A total of 48 bone replicates were buried at four different depths (0, 10, 30, and 50cm); for each depth, two replicates were deposited approximately 70cm from each other. Every month (up to six), a couple of replicates for each depth were recovered and frozen until further processing. Subsamples were obtained using a drill by cutting a wedge shape fragment, which was then powdered using SPEX® Sample Prep 6775 Freezer Mill. Samples were then prepared for the metabolite extraction weighing 40±1mg of bone powder in 2ml Pre-Filled Bead Mill Tubes. The extraction was performed by adding methanol/water solvent (8:2) to each tube, then homogenized and centrifuged. The extraction just described was run two times and the extracted liquid was then transferred in two sets of Eppendorf® tubes (one set for Gas Chromatography/Mass Spectrometry [GC/MS], one for Liquid Chromatography/Tandem Mass Spectrometry [LC/MS/MS] analysis). Samples were then dried using the Sample Concentrator and stored at -20 degrees. For the reconstitution MOX and BSTFA solvents were used in equal volume; each addiction was followed by a 15-minute run on the ThermoMixer® at 60 degrees/400rpm. Finally, all the samples were transferred into glass vials with an insert and secured with their cap. For the analysis, samples were run in Agilent® 8879 GC (MSD 30m x 250µm x 0.25µm column), with autosampler, combined with 5977C GC-MSD, paired with Mass Hunter™ software. Untargeted metabolomics was then carried out also using a Thermo™ Orbitrap™ instrument.

Identified compounds with both GC/MS and LC/MS/MS revealed the lack of significant correlations between the metabolomic profile and the burial depth, showing that depth does not have a relevant influence on PMI estimation when using bone metabolomics. Regarding the PMI estimation, Partial Least Squares Regression (PLS-R) was performed on the data acquired with the GC/MS and showed a Mean Absolute Error (MAE) value of 0.47 months, highlighting the potential that this method may offer for PMI estimation of skeletal remains with relatively short PMIs. In addition to the GC/MS data, LC/MS/MS results will be also presented to the attendees, and the difference between the two platforms and relative results will be discussed at the conference. By comparing these two techniques, we expect to obtain wider metabolomic coverage.

Overall, it can be concluded that the bone metabolomics approach is a valid resource for relatively short PMI investigation from skeletal remains in a cheap and reliable manner, and further studies may elucidate its applicability to real forensic scenarios.

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Postmortem Interval; Bone Metabolomics; Skeletal Remains

A32 Using Evidence of Microbial Modifications on Postmortem Bone Surfaces to Narrow Down Time Since Death Estimations

Caroline Rowe*, University of Minnesota Twin Cities, Minneapolis, MN

Learning Overview: After attending this presentation, attendees will understand how to differentiate bacterial from fungal bone surface modifications on the cortical surfaces and the internal microstructure of postmortem bone. Attendees will also learn how these modifications, in conjunction with microbial sequencing patterns, may assist in narrowing down the Postmortem Interval (PMI).

Impact Statement: This presentation will impact the forensic science community by increasing the methods available that are useful for assisting forensic investigators with the determination of the PMI.

Determining the PMI during a forensic investigation is critical for numerous reasons, including predictions regarding cause of death, validating alibis, and the identification of victims.[1] However, resources for assisting investigators with this task are limited, thus the development of additional tools that forensic investigators have for predicting PMI is critical. One such tool that has recently been exploited is the sequencing of microbial communities during the stages of decomposition. Because human decomposition is sustained by shifts in ever-present microbial communities, the knowledge of microbial succession can provide a reliable postmortem clock.[2] One class of microbes found consistently throughout the stages of decomposition are those that are capable of lysing collagen, which may result in the bioerosion of bone.[3] These collagenolytic bacteria are thought to be released from the enteric and thoracic cavities as decomposition progresses.[4] While previous research has isolated morphology unique to modifications created by collagenolytic microbes, including fungi and bacteria, on the internal microstructure of postmortem bone, there is no existing research that documents microbial modifications on the cortical surface of postmortem bone.[5]

This study will document the morphology of modifications made by collagenolytic microbes on the cortical surface of bone. This study will then use those data, along with previous reported data identifying microbial bioerosion on the internal microstructure of bone, to determine the timing between the first appearance of collagenolytic microbes on a carcass and evidence of bioerosion on the cortical surface and the internal microstructure of postmortem bone.

This study first isolated, established, and confirmed morphology consistent with that of microbial modifications on the cortical surfaces of bone by inoculating bones with either collagenolytic bacteria or allowing them to naturally accumulate fungus in a shallow burial environment. Bones were examined for modifications periodically over four weeks. The second part of this study determines the stage of decomposition when collagenolytic microbes first appear relative to the time it takes for these microbes to create modifications on both the cortical surface and within the microstructure of the bone. Sixty rat carcasses were left to decompose in a shallow burial environment with long bone elements removed from two rats per day for 30 days and until skeletonization occurred. Bones were cleaned and examined for bone surface modifications using scanning electron microscopy. The same bones were then prepared for examination under light microscopy for evidence of bioerosion within the microstructure of the bones. As long bones were initially removed, the enteric and thoracic cavities of the rats were cultured on skim milk agar plates to identify the presence of collagenolytic bacteria.

Preliminary results show that it is possible to differentiate between modifications created by bacteria and fungi on the cortical surface of postmortem bone. Bacterial modifications presented as areas of staining around nutrient foramina as well as the formation of bone sequestra during the transition to skeletonization. Fungal modifications presented as staining in the shape of hyphae, which intensified throughout decomposition. Tunneling of the fungi into the bone, which has been previously documented on the internal microstructure of bone, did not appear within the first 30 days, but was present on random bone samples that had been buried for three months.[5]

Results show that the timing between the appearance of collagenolytic microbes and the appearance of modifications on the internal microstructure was approximately two days, versus four days on the cortical surface of the bone. These preliminary results suggest that the presence of microbial modifications may be used, along with information regarding the timing of first appearance of collagenolytic microbes during decomposition, to assist in narrowing the PMI of a corpse.

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Microbial; Postmortem Interval; Decomposition

A33 The Megyesi Total Body Score Method: Inter-Observer Agreement and Sources of Bias

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Learning Overview: After attending this presentation, attendees will learn how the accuracy of the Total Body Score (TBS) method by Megyesi et al. and its recommended modifications by Moffatt et al. is affected by scoring using only photographs versus scoring using photographs with the addition of case-related written documentation.[1,2] Attendees will learn about inter-observer agreement and possible origins of scoring bias in this sample.

Impact Statement: After attending this presentation, attendees will be aware that the findings of this study strongly suggest that the flaws of macroscopic Postmortem Interval (PMI) estimation methods cannot be explained by subjectivity factors, such as lack of clarity, or levels of detail in the scoring methodology and documentation. The flaws of these methods are more likely caused by fundamental factors, such as the inability of the TBS scale to represent decomposition progression in sufficient detail, or the inability to represent the relationships between TBS and Accumulated Degree Days (ADD) through mathematical models.

The PMI is an important part of forensic investigations. The first PMI estimation method from macroscopic decomposition stages was proposed by Megyesi et al.[1] This method is based on TBS and ADD. TBS is determined by combining the numerical scores assigned to decomposition stages of three anatomical regions (Head/Neck, Trunk, Limbs) from photographs. The TBS is converted to ADD, which is then compared with weather-station temperature data to obtain the PMI estimate, similar to the use of physiological times in forensic entomology. This study's objective is to test the accuracy of the Megyesi et al. method, and its recommended modifications by Moffatt et al., in an American Northeast sample, when TBS is estimated from photographs alone or when they are supplemented with case-derived written documentation.[1,2]

The study sample consists of 44 forensic cases from the Mercyhurst University Department of Applied Forensic Sciences Database (MU-DAFS DB). All selected cases were surface deposits of adult individuals, with appropriate photographs and preserved body regions. The first observer scored each case back-to-back, once only from photographs, then with both photographs and case notes. A second observer scored a subsample of 25 cases to test inter-observer agreement. All scores were then plugged into the respective equations provided by the Megyesi and Moffatt methods to obtain ADD and PMI estimates. The different types of estimates from both methods and observers were compared through Wilcoxon tests. Spearman correlations and percent agreement were utilized to quantify agreement between methods and between observers. The addition of notes did not result in any significant differences either in the resulting ADD (Megyesi, $p = 0.765$; Moffatt, $p > 0.999$) or PMI estimates (Megyesi, $p=0.762$; Moffatt, $p > 0.999$) in any of the methods. Indeed, the estimates obtained with and without the addition of extra documentation showed extremely strong correlations with one another (Megyesi: ADD: $r=0.98$, PMI: $r=0.99$; Moffatt: ADD: $r=0.98$, PMI: $r=0.98$). Moderate to high agreement rates were also found between observers, with variations depending on the anatomical area. From only photographs, the anatomical section with the highest inter-observer agreement was the Head/Neck region (80%), followed by Limbs (68%), and Trunk (64%). Although utilizing photographs and the addition of notes did not influence the final ADD and PMI estimates, it appeared to decrease inter-observer agreement. When observers incorporated case documentation during scoring, inter-observer agreement was 72% for Head/Neck, 64% for Limbs, and 56% for Trunk. Overall, supplementing the use of photographs with additional information, in the shape of detailed case notes and documentation, did not improve the PMI estimates obtained from either of the methods. However, the addition of the supplementary information appears to increase observer bias, decreasing inter-observer agreement. This strongly suggests that the shortcomings of these methods are not explained by subjectivity factors, or deficits in the clarity or level of detail of their scoring methods and documentation, but likely by more fundamental factors, such as the ability of the TBS scale to represent decomposition progression in sufficient detail or of their mathematical models to represent the relationships between TBS and ADD.

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Anthropology; Taphonomy; Postmortem Interval

A34   Using Artificial Intelligence: Deep Learning for Human Decomposition Staging

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Learning Overview: After attending this presentation, attendees will have a better understanding of how deep learning vision methods can be used to automatically assign the stage of human decay from digital photos.

Impact Statement: This presentation will impact the forensic science community by demonstrating an approach that utilizes deep learning to automate identification of the stage of human decomposition from a digital photograph.

Knowing the Stage Of Decomposition (SOD) based on morphological features can inform the potential Postmortem Interval (PMI) of the decedent and aid in identification of the remains. Presently, establishing the SOD is commonly done manually by trained experts via an existing evaluative method, such as Megyesi et al., which stages human decomposition by assigning a point value by body region and giving a total body score in an effort to estimate objectively the SOD and PMI.[1] This study aims to automate Megyesi et al.'s method for determining the SOD by building a deep learning model from photos of approximately 800 human donors undergoing decomposition at the Anthropology Research Facility, located at the University of Tennessee, Knoxville.

The described approach employed the Xception Convolutional Neural Network (CNN) model architecture trained on the human decomposition image dataset.[2] To align with Megyesi et al.'s staging method, which divides staging into three body segments (i.e., head, torso, and limbs), three Xception models, trained on head images (head-CNN), torso images (torso-CNN), and limbs images (limbs-CNN) were developed and evaluated. Specifically, each model classifies an image into one of the following four stages of decay used by Megyesi et al: (1) fresh or SOD1, (2) early decomposition or SOD2, (3) advanced decomposition or SOD3, and (4) skeletonization or SOD4.

To evaluate the performance of each CNN model, the macro-averaged F1 score (maF1), which is an unweighted mean of the F1 scores calculated per class (i.e., SOD) commonly used for multi-class classification problems, was calculated. The maF1 scores for each CNN model were as follows: 0.89 for head-CNN, 0.88 for torso-CNN, and 0.7 limbs-CNN. Similar SOD prediction performance was achieved by the head-CNN and torso-CNN as indicated by their maF1 scores. However, the limbs-CNN maF1 score was notably lower than the maF1 scores of the head-CNN and torso-CNN, which could be due to the decay variability present in limbs (e.g., part of a leg was fresh or SOD1, while the remaining part was showing advanced decomposition or SOD3) as well as the photography itself. This variability can confuse the Artificial Intelligence (AI) and hence lower its classification performance. Early results of the maF1 scores have indicated model ability to predict the SOD from images, but future work will build upon this foundation to further improve model performances.

This proof-of-concept study suggests that contemporary AI methods may be successfully applied in forensic anthropology. Specifically, it may be possible to automate the manually intensive task of determining the SOD and applying it to existing and ongoing investigations and examinations by law enforcement and medical examiner offices. Additionally, it may also support research on PMI estimation and other downstream forensic tasks. The current deep learning model would only apply for human remains in temperate conditions. Further work of training the model on other forensic collections representing varied climatic conditions may be needed.

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Forensic Anthropology; Human Decomposition; Deep Learning

A35   Testing Decomposition Scoring Methods at FOREST in Cullowhee, North Carolina

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Learning Overview: After attending this presentation, attendees will have a better understanding of how published decomposition scoring methods to estimate the postmortem interval do not sufficiently model the patterns of decompositional changes observed at a human decomposition facility in Cullowhee, NC. Difficulties with two published methods versus the morphological changes observed will be discussed.

Impact Statement: This presentation will impact the forensic science community by adding to the ongoing discussion of regional differences demonstrated at human decomposition facilities.

The purpose of this study was to examine the performance of two of the primary decomposition scoring methods, Megyesi et al. and Connor et al., using decomposition data generated from the human decomposition facility at Western Carolina University, the Forensic Osteology Research Station (FOREST).[1,2] FOREST is on WCU's campus in Cullowhee, NC. Cullowhee is within an area classified through Köppen-Geiger as a warm-temperate, fully humid, warm summer (Cfb) region.[3] The average temperature is 12.97°C (55.35°F), and the area receives approximately 130.23cm (51.27 inches) of rain annually.[4] Donors often undergo periods of extensive moisture changes due to the rain and temperature shifts at FOREST, and scavenging often leads to differential skeletonization between individuals and across single sets of remains.[5,6]

For this project, 20 donors from the 2022 calendar year were chosen: winter donors were placed in February, spring donors were placed between April and May, summer donors were placed between late June and August, and fall donors were placed between September and November. Donors that were included had not been enrolled in any other skeletal modification studies nor had they arrived beyond the earliest stages of decomposition; autopsied donors were included, though, as FOREST often receives individuals who were organ donors or autopsied. Donor remains were scored for both the Megyesi et al. and Connor et al. methods from daily and weekly photographs taken for six months or until the donor was recovered from FOREST, whichever happened first.[1,2] Total Body Score (TBS) and Total Body Desiccation Score (TBDS) were calculated, respectively, for the given photograph ranges, and Accumulated Degree Days (ADD) were also tallied per donor for the given range utilizing temperature data from the nearby NOAA station at the Jackson County Airport, which is approximately 3.54km (2.2 miles) from FOREST. ADD estimates were calculated for the Megyesi et al.[1] method to examine the accuracy and precision of actual ADD; as no equations are provided in Connor et al.[2] to calculate the predicted ADD from this method, qualitative data were utilized to examine the fit to the decompositional processes at FOREST.[1,2]

Results found that only 1 of the 20 scored donors had an actual ADD within the 95% confidence interval for predicted ADD from the Megyesi et al. method, making it neither accurate nor precise for scoring decomposition at FOREST. The conciseness of this method was helpful for quickly scoring donors, but the broadness of each category was insufficient to capture the variation in decomposition. In particular, the combination of scoring upper and lower limbs together complicated scoring as donors at FOREST often retain mummified tissue on the lower limbs for several months beyond that of the upper limbs, likely due to differential scavenging patterns. For the same reason that the Megyesi et al. method was not fitting the decompositional pattern at FOREST, the Connor et al. method was more helpful.[1,2] The division of scoring for upper and lower limbs, as well as the expanded categories, provided more options. However, the dearth of variety in the color category and the lack of qualifiers for the moisture category often left questions about how to score the unique colorations and tissue moisture observed at FOREST. For example, donors at FOREST often have orange patches on their skin in the early stages of decomposition that are not captured in the provided color category. Additionally, portions of the donor's faces, mainly eyes and mid-face, are targeted by scavengers, while the rest of the face may still have fresh tissue. These complicating factors make neither of these methods a good fit for regular use in the decompositional environment of the FOREST.

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Decomposition; Human Remains; Postmortem Interval

A36   It's a Dry Heat: Testing Existing Postmortem Interval (PMI) Equations in Clark County, Nevada

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Learning Overview: After attending this presentation, attendees will have a better understanding of how existing equations used to estimate the PMI for human remains perform in the hot desert environment of Clark County, NV. Specifically, the accuracy of three different methods used to estimate PMI for decomposing human remains encountered in outdoor and aqueous environments are discussed, with recommendations for continued practice.

Impact Statement: This presentation will impact the forensic science community by demonstrating the need for environment- and region-specific methods for estimating the PMI. These improvements will aid stakeholders of the medicolegal community in their investigations by providing more accurate estimates of time since death, improving chances of positive identification, and promoting collaboration between practitioners seeking justice for all decedents.

The state of Nevada is divided into two very different environments: in the north are "cool mountain forests" and in the south, where Clark County is located, a "scorching lowland desert."[1] With the possibility of reaching extreme temperatures well over 100°F, estimations of PMI for decedents found in advanced stages of decomposition can be challenging for forensic anthropologists.[1] Furthermore, the commonly used equations for estimating PMI in forensic anthropology were not designed to estimate decomposition rates in desert environments, like that of Clark County, posing additional challenges.

Previous research has evaluated the Megyesi et al. equation, comparing its accuracy between three different decomposition facilities in Texas and Tennessee.[2,3] Results of their comparative study found that the estimated and actual accumulated degree days did not correlate well, overestimating for remains directly in the sun and underestimating those in the shade.[3] However, their study was conducted using the original equation, not the improved equation proposed by Moffat et al.[4]

This study, modeled after previous research, compares the accuracy of three PMI equations in the desert environment of Clark County, NV.[3] With permission from the Clark County Office of the Coroner/Medical Examiner, their existing database was used to identify cases for inclusion. For this study, the following parameters were used: (1) positive identification of the decedent; (2) decedent/remains found outdoors; (3) listed date found; (4) listed date last known alive or reported missing; and (5) photographs of all body regions to be scored (i.e., head and neck, truck, and limbs). Out of 688 cases reviewed, 52 met the parameters for inclusion. Photographs for each case were scored following the guidelines published by Megyesi et al. with the improvements recommended by Moffatt et al.[3,4] For skeletonized cases, Behrensmeyer was also used to estimate PMI (n = 6).[5] Finally, cases found submerged in Lake Mead (n = 3) were evaluated following Heaton et al.[6] Spearman's Rank Correlation was calculated for the estimated PMI produced using the Moffatt et al. equation and the actual PMI in days.[4] There was a positive correlation between the two groups, $r(50) = 0.76$, $p<0.001$. However, due to the small sample size, as well as over half of the cases scoring in the "Early Decomposition" phase, a two-sample, two-tailed t-test was also performed. There was a statistically significant difference between the two means ($t[102] = 2.52$, $p = 0.01$). All cases evaluated following Behrensmeyer fell within the estimated PMI range, except for one that was underestimated.[5] Finally, all aqueous cases evaluated following Heaton et al. grossly underestimated the PMI.[6] These results suggest that the PMI estimates produced using Moffatt et al.'s equation may not be the most accurate for cases in advanced stages of decomposition in southern Nevada. For cases where a PMI estimate by a forensic anthropologist in Clark County, NV, is requested, results suggest that Behrensmeyer should be used for the most accurate results.[5]

These data evaluate existing methods of estimating PMI in forensic anthropology for quality assurance, highlighting areas for improvement within the field. Though a preliminary study, demonstrating the underperformance of these methods for the hot and dry desert environment of Clark County supports the development of region-specific equations, which will improve our accuracy in PMI estimations. By continuing to improve our methods, we increase our chances of accurately estimating time since death, aiding investigators in the positive identification of decedents. Importantly, this supports the ultimate goal of seeking justice for all.

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Taphonomy; Postmortem Interval; Reliability

A37   Postmortem Interval in the Pacific Northwest—King County, Washington

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Learning Overview: After attending this presentation, attendees will be familiar with the general timeline of the decomposition process in King County, WA, and its applications for estimating the Postmortem Interval (PMI).

Impact Statement: This presentation will impact the forensic science community by addressing the scarcity of research concerning PMI estimation in the Pacific Northwest. The anthropology department at the King County Medical Examiner's Office (KCMEO) conducted a retrospective case review to establish broad-scale timeframes for the decomposition process within its jurisdiction—a region extending from the Cascade Mountains to the Puget Sound and including settings ranging from urban to rural.

This case review examined 102 cases involving anthropology consultations at KCMEO between 2001 to 2022. Only cases in which the decedent was positively identified were included, and PMI was approximated by comparing the date of recovery to the date the individual was last known alive. Cases that were thermally altered, unidentified, or exhumed were not reviewed. Of the 102 cases reviewed, three fell into the "fresh" category and were excluded from further analysis. Investigation, autopsy, and anthropology reports as well as scene photographs were used to classify each case into decomposition stages and calculate a Total Body Score (TBS).[1,2] Additional information recorded includes the location (latitude and longitude), an inventory of remains, the presence of clothing/covering, depositional environment (including indoor versus outdoor contexts), and observable taphonomic changes. Box plots were used to explore the rate at which decomposition occurred across different settings.

Results of this research indicate that, relative to published data from other regions of the United States, decomposition rates in King County are delayed.[1-4] Observed rates of decomposition in King County are comparable to those reported from a study in British Columbia, however, likely reflecting the influence of a shared moderate oceanic climate that is distinct from regions where the bulk of decomposition studies have occurred.[5] Although the total ranges of PMI for each stage of decomposition overlap, their interquartile ranges exhibit greater separation. Within this dataset, early-stage individuals (corresponding to TBS scores of 8–18) are typically seen from a few days to three weeks but may persist for upward of one month. Of note, no early-stage individuals were recovered outside in the cases reviewed for this study. Individuals exhibiting advanced decomposition (associated with a TBS of 18–26) had PMIs that generally fell between one and two months but could occur as early as two weeks or persist for more than six months. Skeletonization (TBS of 24–35) can be seen as early as three weeks but is more common after a period of approximately three months. Although possibly the result of sampling error, the available data indicate that advanced decomposition is attained more quickly in indoor settings, but that skeletonization occurs more rapidly outside. Bleaching and exfoliation of the bone in the extreme stage (TBS >31) are only seen in cases recovered from outdoor settings. Bleaching typically occurs after one year of exposure, while exfoliation and/or cortical cracking is not observed until approximately 18–24 months.

It is a truism that decomposition rates exhibit regional variability, yet the majority of human decomposition facilities in the United States have been established in areas with similar Köppen climate classifications.[6] This research not only provides baseline data for decomposition rates in the climatically distinct Pacific Northwest, but also illustrates the utility of retrospective studies for the study of PMI in the absence of formal human decomposition facilities.

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Postmortem Interval; Taphonomy; Anthropology

A38 A Drop in the Bucket: An Analysis of Southwest Florida Weather Station Variables to Inform Postmortem Interval

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Learning Overview: After attending this presentation, attendees will have learned the importance of site-specific soil data collection and weather station instrumentation placement in the estimation of anaerobic Postmortem Interval (PMI).

Impact Statement: This presentation will impact the forensic science community by identifying the need to obtain crime scene soil data due to the variability of data extracted from open-source weather stations. These findings influence the calculation of the PMI estimated by forensic anthropologists and entomologists.

Existing PMI estimation methods recommend the use of weather data obtained from National Weather Service (NWS) stations closest to the crime scene.[1-3] However, recent research suggested that selecting NWS stations based on closest proximity may not provide climate data effective for accurate PMI estimations.[4] Specifically, Dabbs found that NWS stations up to 9km away from the scene were more effective at establishing an accurate PMI than those stations nearest in proximity by comparing the NWS temperature data to site ambient weather loggers (60cm above ground) that captured the microclimatic differences.[4,5]

The NWS' instrumentation standards (e.g., temperature sensors should be 5 feet above ground and 100 feet from a paved or concrete surface) are more defined than alternative weather stations. A relatively new weather station platform, WeatherSTEM™ (WS), provides both ambient weather and soil data but its applicability in PMI estimation has not been tested. In this study, we investigated the utility of WS stations and the effect that station installation variables have on PMI estimation. Further, we supplement existing NWS PMI studies by examining ambient humidity and temperature relative to Site-Specific Soil (SSS) data (i.e., temperature and moisture) within Florida Gulf Coast University's Buckingham Environmental Forensics Facility (BEFF).

We compared data from the BEFF WS to the five closest stations that yielded ambient weather and soil data. Then, as an alternative to the microclimate data loggers used in previous studies, we collected the SSS data using Vernier® LabQuest® 3 temperature and moisture probes during an exhumation at BEFF and tested the efficacy of these data as applied to Vass' anaerobic equation. The data revealed a difference between average BEEF WS and SSS temperature and moisture, where SSS temperature was 3°C higher and soil moisture was 59.5% less saturated than BEFF WS. Among the other WS stations, three of the five (60%) stations were situated within two yards of dense vegetation or fixed structures (e.g., a building). Two stations (40%) were situated within irrigated orange groves or gardens. The altitude among stations varied significantly ($p < 0.05$) with the lowest and highest altitudes being 7 feet and 161 feet above sea level, respectively. One of the five (20%) WS stations had a soil sensor at the same depth (50cm) as the BEFF and the burial we used to test Vass' equation while the other WS units captured soil data at depths ranging from 30cm to 58cm ($p < 0.05$). Despite these variations in station factors, only the SSS data produced an accurate PMI range for the exhumed decedent. This study shed light on the utility of the WS and emphasized the need for forensic practitioners to collect SSS data from the scene to accurately estimate PMI in subaerial contexts.

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Postmortem Interval; Burial; Accuracy

A39   **Under the Weather: A Report of Faunal Scavenger Activity During Major Weather Systems Within a Taphonomic Facility in Southwest Florida**

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Learning Overview: After attending this presentation, attendees will better understand the influence of major climatic events on faunal activity at an outdoor taphonomic facility.

Impact Statement: This presentation will impact the forensic science community by introducing attendees to faunal presence and activity related to major weather systems at a taphonomic facility in Southwest Florida. This research has potential implications on Postmortem Interval (PMI) estimation as these faunae have been identified as scavengers at similar taphonomic facilities.

Faunal presence and activity at taphonomic facilities is well-documented with wild boar (*Sus scrofa*), bobcat (*Lynx rufus*), squirrel (*Sciuridae*), raccoon (*Procyon lotor*), rat (*Rattus*), mouse (*Mus*), and dogs (*Canidae*) identified as common scavengers within these facilities.[1-3] However, the presence of these faunae at the Florida Gulf Coast University (FGCU) Buckingham Environmental Forensics Facility (BEFF) and faunal activity changes during major climatic events within taphonomic facilities, in general, is less understood. Major climatic events, such as Hurricane Irma (Category 5) in 2017, produced extreme atmospheric changes (e.g., barometric pressure, temperature, and relative humidity) that influenced faunal scavenger activity.[3] For example, Hurricane Irma reduced deer (*Odocoileus virginianus*) populations and altered migration patterns, with deer leaving marshes and shrub habitats in favor of pine forests in the aftermath of the storm.

In this study, we documented the impact of two hurricanes (Category 1 Hurricane Nicole = indirect hit; Category 5 Hurricane Ian = direct hit) on the presence and frequency of faunal scavengers within the BEFF. These data were collected via FGCU's Human Identity and Trauma Analysis (HITA) WeatherSTEM™ unit and 11 Xtellar trail cameras (with motion-triggered infrared sensors). We visually inspected the weather station video feed and trail camera photographs that were time and date delineated, recorded the presence of the faunal scavengers within the facility, and controlled for overlapping data (i.e., animal movement on multiple cameras). We used the six-month interval prior to the storms' impact as our control.

Through our analysis of the weather station videos and trail camera photographs, we identified four common faunal scavengers: wild boar (*Sus scrofa*), bobcat (*Lynx rufus*), squirrel (*Sciuridae*), and raccoon (*Procyon lotor*). *Sus scrofa* was the most abundant faunal scavenger recorded, with the greatest abundance occurring during Hurricane Nicole and the least abundance recorded the week prior to Hurricane Ian. This activity was positively correlated (0.217) with the timing of the storm systems ($p < 0.05$). We observed no change in bobcat, squirrel, or raccoon abundance within BEFF in this study. The temperature, barometric pressure, and relative humidity changed significantly ($p < 0.005$) from the control. These findings can inform PMIs estimated by forensic anthropologists, especially in Florida, as *Sus scrofa* abundance is estimated to be 500,000 to 1 million (in Florida) and is considered the most widely distributed invasive ungulate across the United States.[4] As observed in this study, *Sus scrofa* was the most prolific faunal scavenger observed at the BEFF.

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Postmortem Interval; Hurricane; Scavenging

A40   Exploring the Scavenging Behavior of Bobcats, *Lynx Rufus*, in the Middle Tennessee Area and Its Forensic Implications

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Learning Overview: After attending this presentation, attendees will have learned about the scavenging patterns of bobcats, *Lynx rufus*, and their taphonomic effects on a carcass in the Middle Tennessee area. Additionally, there will be an overview of the decomposition research facility of the Middle Tennessee State University (MTSU) Outdoor Forensic Facility (MOFF) provided to the attendees.

Impact Statement: This presentation will impact the forensic science community by highlighting the scavenging activities of bobcats, which have generally been known as hunters. A deeper understanding of bobcats' scavenging effects will allow forensic investigators to identify their activities and will furthermore help reconstruct the context of crimes more accurately when bobcat-associated trauma is found on a victim.

Knowledge about local scavengers and their scavenging activities is required to determine if any trauma/damage observed on a victim was inflicted by animals or humans.[1] Although bobcats are generally known as hunters, their scavenging activities have recently been reported.[2] Fain and Jeong also confirm that bobcats are among the primary scavengers active in the Middle Tennessee area.[3] The goal of this study is to examine the scavenging patterns of bobcats and their taphonomic effects on carcasses.

Experiments took place at the MTSU Outdoor Forensic Facility (MOFF) in Murfreesboro, TN, an outdoor decomposition research facility administered by the MTSU Forensic Science Program. Seven deer carcasses were placed into plots across 15 months (January 2022–April 2023). Each plot was surrounded by 3-foot-high metal fences and monitored by two motion detection cameras. Bobcats' activities were examined with the following factors considered: the time of day/year, weather conditions, and type of activities.[1] The types of activities fell into five categories: Feeding, Covering/Scent marking, Resting, Appearing with no activity, and Unknown. The peak hours of bobcat activity reported by Rippley and colleagues (04:00 a.m.–10:00 a.m. and 6:00 p.m.–12:00 a.m.) were tested.[2]

The results showed that bobcats are readily willing to scavenge in this area. There were 71 occurrences of bobcats recorded by the cameras, with 38 instances being of them feeding on the carcasses. There were 27 occurrences where bobcats appeared with no activity, and the other three categories had fewer than five occurrences each. Two of the seven carcasses had no bobcat recordings at all, and one carcass received visits from a bobcat with no feeding involved. Out of the remaining four carcasses, three were fed on by a bobcat within eight days of placement. The duration of bobcat scavenging ranged from 4 days to 46 days. Limbs appeared to be the initial target of scavenging; however, no distinct preference between the forelimbs and hindlimbs was noticed. Regarding weather conditions, bobcats did not seem to display a strong aversion to colder seasons/temperatures, precipitation, or carrion insects. This study suggests revised peak hours of 5:00 p.m.–7:00 a.m. during which 64 out of the total 71 occurrences happened (90.1%).

This is one of the few studies examining bobcat scavenging from a forensic perspective. The results will provide forensic investigators with practical knowledge about bobcat scavenging activities in this area, helping them to: (1) distinguish bobcat-associated evidence from human-induced trauma, (2) reconstruct the context of peri- and postmortem events more accurately, and ultimately (3) resolve forensic cases on a more informed basis.

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Taphonomy; Scavenging; Anthropology

A41 A Longitudinal Observation of Pattern and Trajectory of Packrat Scavenging Among a Cohort of Human Donors

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Learning Overview: After attending this presentation, attendees will understand the longitudinal pattern and trajectory of packrat scavenging among a cohort of human donors.

Impact Statement: This presentation will impact the forensic science community by supplementing the case study-based body of knowledge with controlled, longitudinal observation of the pattern and trajectory of packrat scavenging among a cohort of human donors.

The destructive nature of packrat (*Neotoma cinerea*) progenerated scavenging of human remains has received considerable attention in archaeological literature, but specificity is less understood in forensic ecological context.[1,2] Data pertaining to packrat scavenging in both contexts is limited to retrospective point observation derived from midden dissection and forensic case study.[1-3] While specific reference can be found in forensic taphonomy literature, data are more typically nested within a greater discussion of "rodent" behavior.[3] While rodent scavenging of both soft and osseous tissue has been described in the literature, absent are data derived from longitudinal observation of the pattern and trajectory of packrat scavenging within a cohort of human donors[4,5] A higher resolution model of packrat scavenging is reported, with an emphasis placed on synchronous longitudinal patterns of hard and soft tissue removal, with the goal of describing the distinct trajectory of intragroup scavenging behavior.

The impact of packrat scavenging was investigated at Colorado Mesa University's Forensic Investigation Research Station high-altitude satellite facility (FIRS-TB40). FIRS-TB40 is in the Rocky Mountain Region of Colorado 9,500 feet (2,896m) Above Mean Sea Level (AMSL). The local environment is characterized by steep slopes, alpine forests, open moraine grasslands, hypoxia, precipitate weather fluctuation, punctuated precipitation patterns, a high Ultraviolet (UV) index, wildfires, and heavy annual snowfall with persistent overburden. Distinct environments foster distinct adaptive radiation, manifest in competitive and cooperative models for resource procurement, and environmentally mediated behavioral patterns and motivating factors in resource exploitation. Summarily these variables are expected to yield a polyvalent outcome on local scavenger behavior.

Packrat scavenging behavior was observed within a cohort of 12 human donors opportunistically placed between March 2020 and October 2021. Field observation persisted through November 2022. Human donors were placed unclothed, supine, on a 45° slope in 10'x10' steel cages. Donors were monitored by game cameras programmed to collect both time-lapse and motion-activated photographs. The presence of packrats was established through game camera capture, midden construction, game trails, scat, and tracks.

Temporal specificity was not observed in packrat scavenging. While scavenging was observed throughout the year, voracity increased markedly in late fall and persisted throughout overwintering. Scavenging began in early decomposition, temporally preceded avian scavenging, and presented on a smaller anato-spatial scale. Scavenging accelerated as postmortem interval increased. Primary modes of packrat scavenging included: (1) opportunistic exploitation of unique resources and lipid dense diet; (2) resource-centric shelter (i.e., intracorpus nesting); (3) superficial tissue removal and skeletal gnawing; and (4) territorial resource defense. Superficial tissue removal was the predominant mode of scavenging and was characterized by: (1) layered peeling of the epidermis from the dermis, followed by separation of the dermis from the hypodermis, where tissue removal terminated; (2) peeling of tissue layers from superficial bone; (3) peeling of dense ceraceous layers of superficial tissue from the hands, feet, face, and abdomen; and (4) consumption of deep tissue throughout intracorpus overwintering. Osseous involvement was limited to ridge gnawing and cortical destruction of the manual and pedal phalanges. The introduction of incisal striae—an expected byproduct of packrat scavenging—was not observed within the sample.

Longitudinal observation serves to inform the pattern and trajectory of packrat scavenging inherently absent from archaeological and case study derived data. This higher-resolution model serves to establish temporal markers associated with modes of tissue destruction and establish an environment specific trajectory from which regional variation and continuity may be derived.

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Scavenging; Human Remains; Taphonomy

A42   Multiple Scavenging Agents: The Effect of Termite and Canine Scavenging on Forensic Cases in Northern Thailand

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Learning Overview: After attending this presentation, attendees will have a better understanding of how to differentiate between termite activity, canine scavenging, bone weathering, perimortem trauma, and anatomical variants.

Impact Statement: This presentation will impact the forensic science community by providing examples of multiple taphonomic effects resulting from living and non-living agents that could complicate the determination of animal scavenging, perimortem trauma, and anatomical variants. Better understanding of these defects could enhance the efficacy of death investigation.

Forensic taphonomy is application of taphonomic analysis in forensic perspectives to estimate elapsed time since death, reconstruct the events happening to the remains, and distinguish between defects caused from human behavior and biological, physical, chemical, or geological agents. The latter aspect is one of the most important foci of forensic taphonomy in forensic anthropology circumstances. It could aid in differentiating between perimortem trauma to the bones and breakage that occurred after death. Therefore, the purpose of this study was to thoroughly describe and interpret the effect of termite and canine scavenging on human skeletons as the first report of multi-scavenging agents, especially termites, in the northern region of Thailand in order to provide better understanding of bone defects affected by different taphonomic agents.

Remnants and traces of tunnels and nests created by termites was observed on the cranium, mandible, and part of the right scapula that were found in the wooden area of Northern Thailand. Tunnel remnants were built on the surface and through cavities of the cranium. Skeletal macro- and microscopic modifications were examined. Obliteration of bone and boreholes were found on the left parietal bone macroscopically and could possibly be misunderstood as parietal foramina. The largest borehole was measured at 3.71mm in its maximum diameter, while the maximum diameter of the smallest one was measured at 2.52mm. Examination utilizing stereomicroscope revealed osteophagic activity of termites, including edge gnawing, star-shaped features, and clusters of subparallel striations on the surface of the cranium. In addition to termite activity, defects resulting from canine scavenging on bones were also noted. Bone destruction on the tip of left and right mastoid processes demonstrated signs of canine chewing. Tooth pit, which could be measured 6.42mm for its maximum diameter, was also observed on the lateral surface of the left mastoid process. Pupal cases of *Chrysomya nigripes* were also found endocranially with the mass of a termite's nest. It was evident that canine and *Chrysomya nigripes* scavenging occurred before that of termites since the tunnels were obviously built on top of the damaged tip of the right mastoid process and the pupal cases were found underneath the termite's nest inside the cranium. Careful microscopic examination could aid in distinguishing between boreholes (termite activity), tooth pit (canine scavenging), parietal foramen (anatomical variant), and penetrating wound (sharp force trauma). Bone weathering, such as sun bleaching and cracking were also found.

This study provides examples of taphonomic effects resulting from the living, emphasizing termite and canine scavenging as first reported in Thailand, and non-living agents that could aid in reconstructing events that happened to the skeletons. Additionally, these features could obscure or mimic perimortem trauma characteristics; therefore, correct determination of these defects could maximize the efficiency of death investigations.

Anthropology; Taphonomy; Scavenging

A43   Soft Tissue Scavenging Patterns of Mice on Human Remains

Alexander J. Smith*, Forensic Investigation Research Station, Colorado Mesa University, Grand Junction, CO

Learning Overview: After attending this presentation, attendees will better understand the soft tissue scavenging patterns of mice on human remains.

Impact Statement: This presentation will impact the forensic science community by building on the existing knowledge of a common scavenger and the potential impact of that scavenger on both human remains and a death scene overall.

While rodent scavenging on human remains has been documented repeatedly through recent forensic literature, most of this documentation covers individual cases, not patterns observed in multiple cases. Within this, there is limited documentation of mouse scavenging, and again this comes in the form of case studies.[1-3] From existing documentation, mice generally consume soft tissue rather than gnawing on bone. They generally prefer tissue form the hands, feet, face, and areas where other defects are present.[1-3] Scavenging is initiated throughout the decomposition process from fresh to skeletonized, though activity generally targets soft tissue with little to no impact to bone. Common features include scalloped edges and serrated tissue.[1-3]

Mice are frequently observed on motion-activated game cameras at the Forensic Investigation Research Station (FIRS) in Whitewater, CO. At the facility, donated human remains are placed on the surface, exposed to the environment, and allowed to decompose naturally. The mice documented at the facility are frequently observed on, in, and around human remains, both to scavenge soft tissue and nest within desiccated torsos. While most donors frequented by mice showed no signs of scavenging activity, 11 individuals were confirmed to be scavenged by mice. Almost all game cameras deployed within the facility in the past ten years have documented mouse activity, and most remains recovered from the facility for cleaning and curation have evidence of mouse nesting within the torso.

Scavenging at the FIRS presented most often in the limbs and was confined to the soft tissue. While the initial scavenging of some donors began in areas where the tissue was already opened (autopsy, insect activity, etc.), mice often opened new defects. Tissue consumption was layered, showing some preference for the adipose tissue over muscle tissue. While some muscle tissue was consumed, generally the mice did not eat all the way to the bone.

Mouse scavenging was most common in the spring (March–May), with more than 70% of confirmed cases at the FIRS beginning within this time frame. Generally, scavenging began on the limbs, then moved proximally. This sometimes left a trail pattern of impacted tissue along the limbs and torso. The defect margins showed fraying and scalloping. Scavenging was rarely deep enough to reach bone and no bone gnawing was observed. Mice were predominately observed at night and spent more time around the remains than they spent scavenging. Generally, mice scavenged in groups of two or more. Mice were also observed scavenging during the window of other active scavengers, though not simultaneously.

While some elements of the scavenging observed at the FIRS were consistent with documented mouse scavenging, there were some inconsistencies. There were no defects observed on the face and while distal limbs were scavenged, hands and feet were not impacted, contrasting previous documentation. Mice scavenged everything from fresh remains to mummified remains, which is consistent with documented cases. While there are a couple existing examples of mice consuming large amounts of tissue, most cases elsewhere document far less tissue consumed than was observed in most cases at the FIRS.[1] In the most extensive case at the FIRS, mice consumed tissue from nearly the full length of all four limbs.

Mouse activity can interfere with the state of human remains on a scene and even confuse cause of death in some cases. There is not a lot of information available about mice as scavengers despite the prevalence of omnivorous mice in a variety of environments. Detailed documentation of their scavenging patterns and behaviors can help increase understanding of these scavengers as it pertains to death investigations.

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Mouse Scavenging; Soft Tissue; Human Remains

A44 Corpse Concealment by Use of a Foundry Furnace: An Experimental Study on a Swine Carcass

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Learning Overview: In cases of homicide, the absence of the victim's body can present a formidable obstacle for forensic pathologists, particularly when tasked with determining the potential use of non-traditional methods, like melting the body. This presentation will illustrate how a judicial experiment can help substantiate an investigative theory in a homicide trial when scientific literature lacks conclusive data. This way, even when it appears unlikely to secure definitive evidence, a judicial experiment can help bring justice to missing bodies.

Impact Statement: This presentation will have a significant impact on the forensic science community by demonstrating the effects of melting metal on a carcass until its complete destruction. This presentation will cover various aspects, including the duration of the process, characteristics of the remains, odor perception, and smoke production, all within the context of a single case study. Furthermore, this presentation can serve as a resource for the forensic community, contributing to the advancement of knowledge in this field based on the data presented.

The authors conducted a study to assess the feasibility of concealing a corpse in a brass furnace during a homicide trial and to investigate the effects of melted metal on a deceased body. Due to the lack of existing literature on this topic, an experimental study was performed using a swine carcass in the presence of the court. A preliminary study indicated that, with certain precautions, there was no risk of an explosion.

The experiment was conducted at a scale of 1:6 using a roughly oval melting furnace measuring 84x42cm in dimensions and 35cm in depth, containing 400kg of brass, and maintaining a stable temperature between 960–990°C. Above the furnace, a movable aspiration hood connected to a collection tank was installed. A young female specimen of Sus scrofa domesticus weighing 13.2kg, which had died of suspected bacterial septicemia, was selected. To simulate human clothing, the sample was wrapped in towels.

No explosions occurred during the entire experiment. Upon placing the carcass inside the furnace, intense flames were generated from the body and towels; however, they gradually diminished after five minutes. Throughout the experiment, the entire carcass floated on the surface of the molten metal. Initially, almost transparent smoke emanated from the furnace, which progressively darkened over time, although it never reached an intense level within the building. After ten minutes, a distinct burnt keratin smell was detectable, but it dissipated after a few minutes. The side of the carcass not in direct contact with the brass gradually burned and carbonized, resulting in a reduction in volume and a diffuse muscle contraction affecting its position. Conversely, the side of the body in direct contact with the brass did not generate flames and merely underwent gradual consumption. The complete combustion and consumption process took approximately 2.5 hours. To determine the maximum time of combustion without external handling, the carcass remained untouched. At the conclusion of the experiment, the combustion remnants were separated from the brass. After an hour, they had cooled down sufficiently to be handled and weighed 4.6kg, consisting of ashes, dust, and larger debris. Some fragments of the bone structure could still be recognized within the debris, but they could be easily destroyed by applying light finger pressure. Samples of dust and debris were collected; however, considering the high temperatures involved, the authors did not seek DNA traces on them based on forensic literature.[1]

The experiment demonstrated the possibility of concealing a corpse in a foundry furnace and provided insights into the effects of direct contact between melted brass and an organic body, comparable to a human organism. The process involved a combination of combustion effects similar to carbonization (for the side of the body not in direct contact with the melted brass) and accelerated combustion (for the side in direct contact).[2] The consumption of the body occurred more rapidly in the areas in contact with the melted metal. Therefore, it is hypothesized that the more the body is moved within the furnace, the faster carbonization and body destruction occur. This hypothesis was later confirmed: the remains were mixed four times, and after each mixture, they became progressively less recognizable. Studies on cremation suggest that the total weight of remains should have been approximately 0.5kg.[3] However, the measured total of 4.6kg of remains was not surprising as it consisted of a mixture of heavy melting brass debris and swine ashes. Further studies could enhance the understanding of the effects of melted metal on human bodies and provide more in-depth insights into them.

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Homicide; Burn; Incineration

A45   Determining Forensic Significance: Pilot Observations of the Taphonomic Patterning of Greek-Orthodox Cemetery Inhumations in Cyprus

Samantha Cohen*, University of Glyndwr-Wrexham, Wales, United Kingdom; Xenia Paula Kyriakou, University of West London, London, United Kingdom

Learning Overview: This presentation will review the taphonomic changes observed in skeletal remains buried in a Greek-Orthodox cemetery, reflect upon the funerary rites practiced within this religious framework, and discuss how these contribute to the postmortem modification of the human body after burial in a way that informs the forensic significance of unknown remains found in Cyprus.

Impact Statement: This presentation will impact the forensic scientific community by providing information on the taphonomic state of human remains found within the specific burial environment of Greek-Orthodox funerary practice. Attendees will learn about those traits that can help distinguish between remains buried in cemeteries and those found in clandestine graves, thus providing a framework for determining forensic significance.

Research on burial taphonomy allows for the differentiation between cemetery remains and remains from clandestine graves. The ability to make this distinction is very important in determining the forensic significance of unknown and uncontextualized human remains, a task that is one of the most challenging in forensic anthropology. The current literature is, however, limited in terms of geographical variations that may also reflect socio-cultural and religious differences in the treatment of the dead. Existing literature on this topic has been primarily based on material from North America.[1] This significantly limits the direct application of these methods elsewhere, outside of North America, where the dead are treated under a different suite of funerary rites and burial practices. The current Greek-Orthodox mortuary framework observed on the island of Cyprus today has been consistent for more than 100 years.

Following the method by Pokines, the taphonomic state of 62 adult (skeletonized only) cemetery inhumations was evaluated to determine the type and distribution of skeletal modifications related to Greek-Orthodox funerary rites and burial practices.[2] The results varied and the prevalence of some traits were both similar and differed from those observed by Pokines.[2] For example, whereas evidence of embalming is typical in remains from cemeteries in North America, only one (1.6%) individual exhibited this trait in the Greek-Orthodox sample. On the other hand, like North American samples, the Greek-Orthodox sample also exhibited a high prevalence of adhering sediment (n=60; 97%), followed by brain preservation (n=53; 85.5%) and coffin wear at pressure points (n=52; 83.9%). Similarly, staining from water minerals was absent in the Greek-Orthodox sample; however, evidence of wine staining was found in 85.5% (n=53) of the inhumations instead. Additionally, the presence of adhering roots (n=42; 67.7%) and hair (n=30; 48.4%) was also noted.

The results suggest that cultural variation in mortuary behavior may be a determining factor in accurately distinguishing between cemetery-buried and clandestine grave remains. However, more research is still required in order to map out these variations across time and space on a global scale. In conclusion, research conducted in cemetery environments of different socio-cultural and religious settings has the potential of further informing forensic significance and allowing for the identification of unknown remains found not only in clandestine graves but also in those remains that may have been displaced from their original burial place following a natural catastrophe. The global prospects of this research are high, and thus more research outside of North America is highly recommended.

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Burial Taphonomy; Forensic Significance; Human Identification

A46   The Resilience of Cut Mark Characteristics: An Experimental Study on the Effect of Corrosive Chemicals

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Learning Overview: After attending this presentation, attendees will understand the impact of corrosive chemicals on bones and how they can negatively impact the analyses of cut marks.

Impact Statement: This presentation will impact the forensic science community by illustrating how some traits used for cut marks examinations to differentiate serrated and non-serrated knives could be altered by corrosive chemicals, affecting the reliability of the analysis.

In the United Kingdom, the prevalence of knife crime is at an all-time high, with sharp instruments being the most frequent cause of homicidal death in England and Wales as of March 2022.[1] Knife marks classification systems have become more standardized in recent years; however, analyzing how the selected traits are resilient to different taphonomic factors is important to develop reliable methodologies.

As the United Kingdom is also the country with the highest reported number of acid attacks per capita, more research regarding the effect of chemical exposure on trauma lesions is particularly relevant.[2] Several studies regarding the morphological effect of acid/alkali substances on hard tissues have been conducted, although the aim was predominantly on restorative dentistry or medicine. In addition, most knowledge regarding chemicals for body disposal is based on case reports; therefore, there remains a lack of forensic experimental research on how chemicals can affect the reliability of trauma analysis.

The authors present a study whereby cut-mark characteristics were exposed to several chemical substances to determine whether there were significant alterations and if these could affect the classification of the implements used (serrated/non-serrated knives).[3] The traits examined were: (1) shape of the cut mark; (2) shape of the cross profile; (3) rising profile; (4) feathering; (5) bone shards; and (6) mounding.

One single-edged, micro-serrated knife was used to create 180 cut marks on 18 defleshed porcine ribs (Sus scrofa domesticus). According to previous analysis, the implement was selected as the most recognizable due to the distinctive traits left behind.[3] The samples were divided equally across six experimental conditions, including five laboratory-grade chemicals: hydrochloric acid, sulfuric acid (96–98% and 32%), sodium hydroxide, sodium hypochlorite, and a tap water control. A stereomicroscope with a total magnification range of 4.73–7.5x was used to observe the cut marks and categorize their traits prior to and post-chemical exposure. The samples were submerged and controlled at intervals: 4, 8, 12, 24, 48, and 72 hours. Macroscopic observation of bone morphology was also recorded at each observational stage.

The study demonstrated that hydrochloric acid caused the most morphological destruction to the samples, destroying any evidence of cut marks and their associated characteristics at 48 hours. The second most destructive chemical was 32% sulfuric acid, followed by 96–98% sulfuric acid and sodium hydroxide. Sodium hypochlorite and tap water had minimal effect on the bone and the visibility of cut marks. Evidence of sharp force lesions remained detectable for up to 72 hours in all conditions except hydrochloric acid.

The most variation was seen to elliptical-shaped cut marks (SD= 10.799) and V-shaped cross profiles (SD=7.480) in 96–98% sulfuric acid. However, no statistically significant association could be made between the cut marks' shape and cross profile shape at the end of the study ($p > 0.05$), meaning that it was impossible to recognize that cuts were created by a serrated knife.

To improve the real-life application of the study, the authors suggest future research on non-defleshed ribs to evaluate the impact of muscle and fat on cut mark preservation. Testing a more extensive range of knives is also recommended to improve the reliability of the results.

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Cut Mark Analysis; Forensic Anthropology; Taphonomy

A47   The Resilience of Cut Mark Characteristics: An Experimental Study on the Effect of Fire Exposure

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Learning Overview: After attending this presentation, attendees will understand the impact of fire on bones and how it can negatively impact the analyses of cut marks.

Impact Statement: This presentation will impact the forensic science community by illustrating how some traits used for cut mark examinations to differentiate serrated and non-serrated knives could be altered by burning and cremation, affecting the reliability of the analysis.

In murder investigations, the analysis of cut marks left on human bones can play a crucial role in forensic investigation. The examination can be particularly challenging when the remains have been altered by taphonomic factors, especially in arson cases or when the bones have been cremated.[1]

This study aimed to identify the resilience of cut mark characteristics after exposure to direct fire for different time intervals. The effects of temperature on soft tissue and bone have been forensically evaluated, considering that cremation and fire are noted to be one of the oldest disposal methods for human remains in both cultural and criminal.

Fifteen defleshed porcine ribs (*Sus scrofa domesticus*) have been used in place of human bones, and ten cut marks per bone were created using a standard serrated kitchen knife in one back and forward motion, mimicking a stabbing action. One single-edged, micro-serrated knife was used to create the cuts on the ribs; the implement was selected as the most distinguishable due to the typical traits left behind. The marks were then examined under a low-powered stereomicroscope using a total magnification of 4.73–7.5x, and the traits were recorded. For the present study, the authors used six characteristics previously identified as indicative of the correct classification between serrated and non-serrated knives.[2] The traits examined were: (1) shape of the cut mark; (2) shape of the cross profile; (3) rising profile; (4) feathering; (5) bone shards; and (6) mounding.

After the initial observations, groups of three ribs were burned on an outdoor pyre for different times: 5, 10, 20, and 40 minutes. In addition, a group had been burned for 20 minutes, then rapidly cooled in water, recreating a scenario where firefighters extinguish flames. After the exposure to the fire, the bones were again examined with the stereomicroscope, and the traits were recorded to be compared with the original observations.

Results showed that the resilience of the traits did not notably change with the duration of the exposure to the flame. Four out of six traits exhibited a definitive survival rate: the overall shape demonstrated a 100% survival rate with all samples remaining unchanged, whereas feathering, rising, and mounding demonstrated 0% resilience after exposure to the fire. The traits that do not follow this trend are shards and cross-profile shape. Shards' survival varied from 0% to 33.33%, and unlike what would be expected, the resilience did not decrease with the duration of the burning process, suggesting that the way the flames reached the bones may have played a significant role in the experiment. Another recorded anomaly is the cross-profile shape: following the classification used, the cross-profile shape was initially identified as Y or V; however, after the burning, a new category (U shape) was identified.[1] As the introduction of this category may have impacted the results in identifying the resilience of the original cross-profile shapes, the authors recommend further investigation in order to evaluate this new type and its significance.

In conclusion, the authors demonstrate that even if using a standard methodology for classifying cut marks on human remains is important, it is vital to understand how often they survive a fire exposure and if they can be correctly recognized after thermal alterations.

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Forensic Anthropology; Taphonomy; Thermal Damage

A48   In Full or in Part: The Effect of Incomplete Remains on Trauma Analysis

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Learning Overview: After attending this presentation, attendees will better understand how completeness of skeletal remains can affect forensic anthropological trauma analysis.

Impact Statement: This research impacts the forensic science community by presenting the relationship between skeletal completeness and forensic anthropological trauma findings.

Forensic anthropologists may conduct trauma analyses on human remains months to decades after the original autopsy. Their skeletal analysis of trauma may be further constrained by: (1) the incompleteness of the skeletal remains related to cases that are unsolved, unidentified, and/or a victim of domestic violence; or (2) the medical examiner/coroner requested the analysis of specific skeletal elements to the exclusion of the rest of the skeleton. While the utility of forensic anthropological trauma analysis is well documented, there is a gap in the research concerning whether the quantity of skeletal elements (completeness) impacts identification of trauma type (blunt, sharp, ballistic, or strangulation) relative to the state of decomposition.[1-3]

We evaluated closed, adjudicated forensic anthropology trauma analysis reports (n = 97) in Florida from 2018–2020. Of the cases from 2018 (n = 30), 2019 (n = 28), and 2020 (n = 39), 26 females and 71 males were represented in a unimodal age distribution where the sample mean age range was 41–50-years-old. On average, these remains were in a fresh state of decomposition (45%), followed by active decomposition (29%), and skeletonization (26%), though a majority of these decedents were complete with greater than 90% of elements present for analysis.

Overall, an average of 86 elements (42%) were evaluated by the forensic anthropologist. Interestingly, trauma was identified more commonly in cases with fewer elements present (r = -0.326, p <0.01) for males, with age not significantly correlated for males (p = 0.088) or females (p = 0.202). These data revealed that males received a more detailed analysis because their skeletons were more complete (47%) than females (26%) within the sample. Yet, the frequency of trauma type was the same for both sexes, with the absence of trauma being most often observed followed in descending order by blunt, sharp, and ballistic trauma (p <0.001). Of those individuals without trauma (n = 13 females, n = 47 males), an average of 108 bones (or 52%) were evaluated. In contrast, when trauma was identified, the forensic anthropologist was asked to evaluate only 24% of the skeletal remains. Importantly, 42% of female cases and 24% of male cases involved a focused analysis of the neck organ block (i.e., hyoid, thyroid, cricoid cartilage, tracheal rings). This study revealed that the forensic anthropologist's detection of trauma was not impacted by the state of decomposition, trauma type, or quantity of elements evaluated.

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Trauma; Human Remains; Decomposition

A49   A Case Presentation of Fractures to the Wrists and Hands in a Suicide and Homicide

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Learning Overview: This presentation will compare two case studies of wrist injuries to demonstrate the importance of careful consideration of trauma when it comes to the interpretation of fracture patterns. It further demonstrates fractures from gun recoil, beyond the site of impact. Following this presentation, attendees will be able to identify the overall similarity in fractures between the cases and the need for further investigation into patterns in wrist injuries.

Impact Statement: This case comparison emphasizes the similarities in injury between those sustained during a homicide and those from a suicide. This presentation will impact the forensic science community by highlighting the need for thorough trauma analysis to identify other injuries that better represent the cause and manner of death.

Equivocal death investigations are challenging and often remain unresolved. In cases involving fractures of the forearms, wrists, and hands, the etiology may not always be clear. In states like Florida that have the death penalty option, the number of injuries is often critical evidence for demonstrating an aggravating factor. Under Florida law, the jury must find at least one aggravating factor, such as the crime being "especially heinous, atrocious, or cruel" to consider the death penalty.

While published literature supports finding injuries to the wrists and hands from gun recoil and from professional fighting in a sports setting, not much exists regarding these injuries in a forensic context.[1-4] The purpose of this study is to explore two case studies with similar patterns of injury resulting from homicide and suicide.

The cases come from the University of South Florida (USF) Donated Skeletal Collection, a well-documented collection with extensive data regarding donor life history, autopsy reports, and the official cause and manner of death. Both donors selected for study exhibit fractures to the wrists and hands, which highlights the similarity between fractures caused due to the recoil of a gun and those sustained through brawling.

In the first case study, the donor's manner of death was homicide, and the cause of death was multiple gunshot wounds to the chest. In addition to ballistic trauma, he exhibited several fractures consistent with a physical assault that occurred just prior to being shot. This decedent presented fractures to the cranial vault, craniofacial region, and the right fourth metacarpal. The second case study was that of a donor whose manner of death was suicide, and the cause of death was a gunshot wound to the right side of the head. In addition to extensive fracturing of the cranium from the ballistic trauma, he exhibited fractures to the right second metacarpal and the right radius. Donor records indicate that the individual was right-handed, which is consistent with both the position of the entrance wound, as well as the fracturing to the hand and wrist following the gunshot. The perimortem wrist injuries were the result of gun recoil. The second metacarpal would have been used to pull the trigger, which could explain why it fractured instead of the other surrounding metacarpals.

The similarities in injury between the two cases show that these injuries could possibly confuse the manner of death. In cases of gunfire injuries to the head, most postmortem autopsies do not X-ray the hands and arms. While an external exam of the tissues should note injury to this region, documentation in this area is frequently lacking, especially in decomposing cases. The wrist injuries in the case of the suicide could easily be misinterpreted as injuries sustained while trying to fight off an attacker. Other evidence of skeletal trauma is necessary to determine manner of death, which highlights the need for thorough recovery at scenes involving advanced decomposition or skeletal remains and the utility of direct skeletal observations in all cases.

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Fracture; Anthropology; Manner of Death

A50   Tibia Size and the Contribution to Variance in Fracture Outcomes

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Learning Overview: After attending this presentation, attendees will understand the relationship between global tibia size and fracture characteristics through an examination of fracture outcomes in human tibiae experimentally loaded in the same blunt force bending mechanism.

Impact Statement: This presentation will impact the forensic science community by providing data and analyses that explore why fracture outcomes vary in the same skeletal element impacted under matching loading conditions and whether tibia morphology explains the variance in fracture characteristics.

Understanding individual-specific variables that contribute to variation in fracture outcomes is critical to forensic skeletal trauma analysis. Fracture characteristics (e.g., number and type of fractures) have been shown to demonstrate variation under the same loading conditions.[1] Identifying which variables contribute to and explain the variance in fracture characteristics is critical to support analyses of skeletal trauma and prevent potentially inaccurate interpretations. Previous research has demonstrated that in human tibiae, age had a significant, but weak, relationship with both number and type of fractures, while sex had no relationship with either when considered independent of age.[2] However, the role of bone-specific variables and their contribution to fracture characteristics has yet to be explored. Therefore, the objective of this research is to examine relationships between global bone morphology and fracture characteristics in human tibiae.

Sixty human tibiae (females, n=31 [29–102 years old, M=68]; males, n=29 [24–96 years old, M=59]) were experimentally loaded at 6m/s in a lateral-medial direction in a 4-point bending scenario. Prior to testing, the following measurements were obtained for each tibia: Length (excluding medial malleolus), Maximum Proximal Epiphyseal Breadth, Distal Epiphyseal Breadth, and Maximum and Minimum Diameters at the nutrient foramen. After testing, each tibia underwent fracture analysis, consisting of identification of fracture type, group, and qualifiers, and number of fracture types per tibia, following the methods outlined in the AO/OTA Fracture and Dislocation Classification Compendium.[3] The number of fractures reflects the count of identified fracture types and not each individual fracture. For example, a single transverse fracture and an intact wedge fracture would both be documented as one fracture. Additionally, while longitudinal fractures are not currently represented in the AO/OTA classification system, they were documented as their own fracture type and included in the number of fractures per tibia when present.[3] The analyses included Random Forest (RF) models to explore the relationship between tibia size variables and their ability to predict type, group, or location of fracture. A Principal Component Analysis (PCA) was implemented to discover if the multivariate relationship of the quantitative tibia variables can be linked to any of the fracture morphology variables. Lastly, a Multiple Correspondence Analysis (MCA) was conducted to explore the relationships between the fracture type, group, location, and categorical demographic variables.

The RF models revealed no predictive power in the tibia size variables to predict the type or group of fracture. Even with demographic variables included, the predictive power did not meaningfully increase. The PCA results show complete overlap for the type, group, and location of fractures. In contrast, the MCA analyses revealed clusters when categorical demographic variables were used; longitudinal fractures grouped together, multifragmentary fractures grouped together, and simple and wedge fractures grouped together. However, there were no obvious patterns within Body Mass Index (BMI), sex, and age categories. In consideration of these and previous findings, future work will incorporate tibia cross-sectional variables and evaluate their predictability of fracture outcomes in both univariate and multivariate analyses.

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Trauma Analysis; Trauma Interpretation; Experimental Research

A51   An Analysis of Lower Extremity Fracture Patterning in Fatal Motor Vehicle Collisions

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Learning Overview: After attending this presentation, attendees will recognize fracture patterning of the lower extremities in fatal Motor Vehicle Collisions (MVCs), as well as the role that select intrinsic and extrinsic factors play in initiating lower extremity fractures in this context.

Impact Statement: This presentation will impact the forensic science community by providing attendees with a better understanding of a suite of Lower Extremity Long Bone (LELB) fracture patterns resulting from fatal motor vehicle collisions. Further, it will contribute toward improving interpretations of fracture patterns for forensic anthropology case work related to motor vehicle accidents.

LELBs are the second-most common injury resulting from MVCs, though forensic investigations most often focus on more fatal injuries of the head, neck, spine, and pelvic regions.[1] LELB fractures are well documented in engineering and automotive safety research and accompany clinical investigations of LELB injuries in MVC survivors. These studies assist forensic anthropologists in interpreting blunt force trauma and fracture patterning, while a gap in the forensic literature exists on this topic. Aiming to fill this gap in forensic science, the present study focuses on investigating the frequency and patterning of femur, tibia, and fibula fractures in fatal front and side impact MVCs.

This retrospective study examined patterning in the presence, type, and location of LELB fractures, the relationship between fracture frequency, morphology, impact direction, and the influence of intrinsic and extrinsic variables to fracture frequency and patterning. Cases were selected from the Office of the Medical Examiner's database at the Western Michigan University Homer Stryker M.D. School of Medicine, Department of Pathology that matched inclusion parameters. These parameters included: that the death investigation occurred between 12/31/2016 and 11/16/2020, at least one fracture is present in the LE, that the decedent was located in a car, SUV, van, or truck, the collision occurred with a front or side impact, and that the decedent was 15 years old.[2] Cases where decedents were ejected or the vehicle rolled over were excluded. In total, 67 decedents of front (n=51) and side (n=16) impact collisions were included. For each case, fracture data were collected from radiographs, and demographic and extrinsic collision variables were collected from available medicolegal records.

Fracture patterning was predicted for each impact type through a thorough review of the available literature on LELB fracture in MVCs. In the study sample, fracture patterning demonstrated some agreement with the predicted patterning of ankle, knee, and femur fractures.[3] Side impact fracture patterning coincided less with the predicted pattern of primarily tibial fractures. In both impact directions, no statistically significant relationship was found between intrinsic (decedent) and extrinsic (vehicle and collision) factors and fracture presence and location in Pearson's Chi-squared tests.

Front and side impact collisions differed in the most-often fractured LELB. Femoral fractures accounted for the majority (41%) of front impact fractures, while the majority of side impact fractures were of fibulae (48%). Utilizing a Pearson's Chi-squared test, a statistically significant (p=0.05) relationship between anatomical location of fracture and LELB was found in front impact collisions—indicating that there are common mechanisms causing LELB fracture between individuals and incidents. The same was not found in side impact collisions. With respect to fracture type, comminuted fractures were most common in front impact collisions, while oblique fractures were most common in side impacts

The present research demonstrates that there is variable fracture patterning in fatal MVCs based on impact direction and other factors. This conclusion is supported by the common understanding that impact direction influences fracture propagation and patterning. This work compliments recent forensic anthropological interest in fracture patterning due to motor vehicle collisions (i.e., Hulse, Stull, and Weaver 2018), and contributes to forensic literature on this subject.[2]

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Forensic Anthropology; Fracture; Motor Vehicle

A52   **Establishing a Skeletal Atlas of Elder Abuse: An Examination of the Relationship Between Bone Density and Skeletal Fractures in Accidental Falls and Suspected Abuse**

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Learning Overview: After attending this presentation, attendees will be aware of the relationship between bone density and skeletal fracture occurrence associated with the abuse/neglect of elderly individuals as distinguished from those commonly found in accidental ground-level falls.

Impact Statement: This presentation will impact the forensic science community by examining the contribution of osteoporosis to differential skeletal fracture occurrence in cases of suspected abuse and in documented accidental falls, contributing data to the ongoing effort to unmask elder abuse more broadly.

Ten percent of adults aged 65+ will experience some form of abuse annually.[1] Physical abuse and caregiver neglect account for most cases, yet remain difficult to prove.[2] Skeletal expressions of abuse offer key indications of inflicted and untreated injuries but are masked by assignation to accidental falls. Funded by the National Institute of Justice, our research aims to produce a standard for the diagnosis of elder abuse in the skeletal system.[3] We previously identified differences in the skeletal patterns of injury between accidental falls and cases of abuse/neglect.[4,5] To further refine a standard of expected skeletal injury associated with abuse in elders, we must consider the degree to which aging affects the structure and density of the bone in relation to fracturing. Osteopenia, the early loss of bone density, and osteoporosis, a condition of progressive deterioration of bone tissue, affect approximately 54 million individuals in the United States and are associated with increased fracture risk.[6-9] Radiographic standards have been established for the measurement of bone density and assessment of osteoporosis.[10-14] To explore the relationship between bone density and skeletal fractures in abuse/neglect vs accidental falls, cases from the Maricopa County Office of the Medical Examiner were reviewed.

Digital radiographs of 175 individuals over the age of 60 who had sustained skeletal trauma were examined and scored by three observers with varying levels of experience according to Barnett and Nordin 1960.[11] This method was chosen as an investigatory starting point based on low rates of reported inter-observer error and ease of implementation by a range of professionals, of particular interest for the future application of our criteria. Bone density measurements were estimated from the hands, femora, and vertebrae, and osteoporosis was diagnosed where the threshold for minimum bone loss was met. Of those examined, 66 were cases of abuse/neglect based on previously established criteria and 109 were cases of witnessed falls.[4,5]

To assess potential differences in bone density between those individuals manifesting skeletal fractures from an accidental fall and those for whom abuse/neglect is suspected, we performed independent t-tests to compare the average bone density scores for left and right metacarpals and femora, respectively. The results suggest no significant differences in metacarpal density (hand: $t=1.1$, $df=85$, $p = 0.28$), but a significant difference in femoral bone density between abuse and accidental cases (femora: $t=2.53$, $df=109$, $p = 0.01$). The World Health Organization defines osteoporosis as a bone mineral density value more than 2.5 standard deviations below the mean.[15] We examined differences in osteoporosis between accidental and abuse groups and found a disproportionately high frequency of osteoporosis among individuals sustaining fractures in accidental falls ($X^2=8$, $df=1$, $p=0.005$).

To better understand the potential for implementation of this simplistic measurement of bone density in a forensic context, we performed a paired t-test of inter-analyst correspondence using a sample of the data (n=129 pairs). The results of the paired t test suggest no significant differences between analysts (hand: $t=-0.096$, $df=65$, $p = 0.92$; femora: $t=-0.096$, $df=105$, $p = 0.06$; alpha $=0.05$).

Fractures remain the most common musculoskeletal condition requiring hospitalization among individuals aged 65 and older in the United States, and rigorous diagnostic criteria must be developed to differentiate accidental injury from abuse.[6] Radiographic assessment of skeletal fragility and injury is the standard initial approach to fractures in both living and deceased individuals.[16] To develop a standard of expected skeletal injury associated with accidents and/or abuse, we must first be able to provide a baseline of bone health against which we may evaluate noted fractures. Results from this ongoing study represent an extensive effort to develop and improve diagnostic criteria for a skeletal atlas of elder abuse.

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Elder Abuse; Fractures; Bone Density

A53　　Assessing Variability in Kerf Floor Shape Utilizing a Revised Set of Shape Categories

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Learning Overview: This research utilizes a modified classification system to assess saw kerf floor shapes in bone. After attending this presentation, attendees will better understand challenges in assigning kerf floor shape categories, as illustrated with inter-observer assessments, and patterns between kerf floor shapes and saw characteristics.

Impact Statement: This presentation will impact the forensic science community by highlighting some of the limitations and utility in kerf floor shape analysis to predict saw class characteristics in dismemberment cases.

In cases of dismemberments, forensic anthropologists are asked to assess bony kerf variables to provide information about the tool used. Kerf floor shape has been proposed to reflect specific saw class characteristics. In this study, we created a set of 12 kerf shape categories based on previous studies and observations. We aimed to test inter-observer agreement in kerf floor assignments and to evaluate the relationship between kerf shapes and saw characteristics.

This research utilized a subsample of incomplete saw cuts in human bone made available by Mercyhurst University and originally collected as part of a National Institute of Justice grant project (2005-IJ-CX-K016).[1] The cuts were made on fully macerated long bones using a controlled sawing method. A subsample of 90 specimens, created by 19 saws, were semi-randomly chosen, ensuring approximately equal representation of each saw. Stereomicroscopic photos were taken of the kerf profiles and three observers scored the 90 kerf floors. Percent agreement between observers and patterns in kerf floor shape were assessed to investigate the utility of floor shape in classifying saw characteristics.

Agreement between the two inexperienced observers and the experienced observer was 60.0% and 75.6% for the 12-shape scoring system. Kerfs with a "W" or truncated "W" shape had 100% agreement across all observers, illustrating the ease of differentiating this shape from flat or curved kerfs. Collapsing the kerfs with flat or slightly rounded floors increased agreement to 90.0% and 88.8%. Patterns between kerf floor shape and saw class characteristics were limited. All W-shaped kerfs were created by hand saws with alternating crosscut teeth, with one exception: a mechanical circular rip saw produced two W-shaped kerfs, but the peaks of the "W" were shallow and easily differentiated from the other W-shaped kerfs. Similarly, all hand-alternating crosscut saws produced W-shaped kerfs except for four kerfs produced by two saws (a dovetail and general carpenter saw). Beyond being created by rip saws, no patterns were discerned between the various flat and rounded kerf floors and saw characteristics; they were created by hand and mechanically powered saws of various sets and teeth-per-inch. A new category developed, relatively flat kerfs with a midline divot, was only observed in mechanical reciprocating rip saws with a raker set, although not all mechanical reciprocating raker rip saws created this divot feature.

This preliminary research expands upon our understanding of kerf floor shape and saw characteristics. Saws are highly variable, however, with various combinations of power, set, tooth shape, teeth-per-inch, blade width and length, and intended cutting material. As such, it is important to further assess kerf shape patterns in a diverse array of saws if forensic interpretations are to be drawn from kerf floor shape in casework.

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Anthropology; Dismemberment; Sharp Force

A54   A Comparison of Fatal and Non-Fatal Postcranial Gunshot Wounds

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Learning Overview: The goal of this presentation is to focus on the skeletal differences between postcranial fatal and non-fatal Gunshot Wounds (GSWs).

Impact Statement: This presentation will impact the forensic science community by allowing for better recognition of fatal vs. non-fatal skeletal GSWs in the postcranial region.

Gun violence has a devastating effect on the United States with over 48,000 firearm deaths in 2021.[1] In addition, there have been over 385,000 injuries between 2006 and 2010.[2] Approximately $734.6 million is spent each year on treating GSWs.[2] The difference between a fatal GSW and a non-fatal GSW is complex and varied.

C.A. Pound Human Identification Laboratory case folders from 1974 to 2020 were examined for trauma. There were 268 total GSW cases: 93 had postcranial trauma; thorax = 48, neck = 20, abdomen = two, pelvis = zero, and limbs = three. Seventeen had trauma to more than one region, two were unknown, and two were antemortem. The majority (31%) of cases had trauma to five+ elements, with the greatest frequency to the ribs (45%) followed by thoracic vertebrae (18%). In 63%, one shot occurred.

Radiographs of living trauma victims located in Florida were pulled (with Institutional Review Board [IRB] approval) from the University of Florida's Clinical and Transitional Science Institutes Integrated Data Repository. Cases came with radiographs and doctors' notes. One hundred and five cases were pulled: thorax = 54, neck = one, abdomen = 19, pelvis = 15 (including three to the penis), limbs = three, multiple regions = six, and seven unknowns. Fifty-six percent had trauma to no elements. In 81% of cases, the individual was shot once. The non-fatal GSWs had a higher chance of no skeletal trauma, with fatal GSWs involving more elements per case. The non-fatal had more trauma to the pelvis and abdomen. The fatal population had a higher frequency of comminution. The non-fatal population had less catastrophic trauma. The scapula had the most similar types of fracturing between all the elements.

The majority of GSWs are non-fatal within the literature, with the exception of one study from Finland.[3,4] Several studies conclude that the cranium and thorax are the most common sites of fatal GSWs.[4-7] This can be seen in the fatal sample where the majority are cranial followed by thorax. Non-fatal GSWs are often found in the limbs, abdomen, and pelvic region.[8-11] The non-fatal sample had few cases with limb GSWs, but this may have been due to the random nature of the selection. The sample did have several cases involving the pelvis and abdomen, but the majority were from the thorax, specifically rib fractures. One study found that approximately 36% of cases had only one shot per individual and 61% had trauma only in one region, which is keeping with both the fatal and non-fatal sample.[3] Single GSWs and GSWs to single elements are commonly found in fatal GSWs with one study finding 41% having trauma to one element.[12] One study found a correlation between multiple GSWs and requiring a hospital stay or death.[3] However, another study found no correlation between multiple GSWs and greater morbidity.[13] Both sample populations had more males than females, which is fairly universal with over 90% of non-fatal GSWs and 81% of fatal GSWs being male.[1,8,9,11] Males tend to have riskier behavior.[14]

Further study could be done to expand on the results here such as adding the skull to see how different survivability is compared to other regions. In addition, most of the doctors' notes were from initial review of the trauma. It would be interesting to see if additional trauma was found during surgery versus radiographs. Overall, fatal GSWs tend to involve more elements with more severe trauma, and abdomen and pelvic GSWs are seen more often in non-fatal GSWS than fatal.

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Gunshot; Trauma; Non-Fatal

A55 The Patterning of Fracture Characteristics in Gunshot Wounds in Long Bones

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Learning Overview: After attending this presentation, attendees will have a better understanding of the fracture characteristics that are typically associated with gunshot wounds in long bones.

Impact Statement: This presentation will impact the forensic community by contributing data on gunshot wound fracture patterning in the long bones. Gunshot wounds in tubular bones are understudied relative to those of the cranial vault, though they are increasingly common in medicolegal contexts.

Gunshot wound analysis remains a challenge in forensic anthropology. The potential utility of skeletal gunshot wounds in medicolegal contexts is limited by the fact that descriptive characterization of fracture patterns in ballistic trauma has focused on the skull, with few studies dedicated to long bone injuries.[1,2] Publications on long bone gunshot wounds are derived from medical research and orthopedic interventions, where descriptions of fracture patterns of forensic interest are somewhat limited. A more comprehensive characterization of fracture initiation and propagation in gunshot wounds to tubular bones is still needed.

In recent years, Computed Tomography (CT) scans have been considered a complement to traditional autopsy, enhancing reconstruction of gunshot wounds and fracture patterns.[3] With the increasing availability of CT scans, they now represent an important source of high-quality data for the study of gunshot wounds with broader applications in forensic research.

In this study, postmortem CT scans were used to characterize gunshot wounds in long bones and to explore patterning of lesion characteristics that could be indicative of projectile trajectory. CT scans from 33 individuals (mean age=37; SD=15) with gunshot wounds in long bones were selected from the New Mexico Decedent Image Database (NMDID). Scans were processed with automated thresholding segmentation protocols to visualize the skeletal tissue using 3D Slicer 5.3.0 and InVesalius 3.0. Fragment realignment for fracture reconstructions was completed using Blender 3.6 LTS. Qualitative characteristics of the wounds were recorded, such as the shape and size of bone defects, and the presence or absence of radiating, concentric, spiral, and butterfly fractures. Patterning of characteristics were explored using hierarchical cluster analysis.

Three types of fractures were observed in the data: indirect fractures (bullet did not impact the bone); entrance wounds of direct impacts; and exit wounds of direct impacts. Indirect fractures without penetrating injuries were characterized by the absence of bone defects and the presence of transverse, butterfly, and spiral fractures. Direct impacts to long bones were characterized by oblong-shaped bone defects with radiating and concentric fractures forming a butterfly pattern. Differentiating between entrance and exit wounds was complicated by challenges with fracture reconstruction and the presence of image noise as a result of metallic artifacts from projectile fragments. This led to difficulty observing important features detailed in other studies, including irregular defects, beveling, delamination, and edge sharpness.

Results demonstrate clusters of characteristics that could be associated with indirect, entrance, and exit wounds. However, conclusions about the characterization of entrance and exit wounds associated with the fracture category groups could not be drawn since the confirmed projectile trajectory information from the autopsy records was not available for this sample. If these clusters can be reliably associated with entrance and exit wounds, these results can be used for reconstructing projectile trajectory and potentially circumstances of death in cases of gunshot wounds to the postcranium.

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Gunshot Trauma; Long Bones; CT Scans

A56   An Analysis of Bevel Angle in Gunshot Trauma to Long Bones

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Learning Overview: After attending this presentation, attendees will understand that bevel angle in gunshot entrance wounds to long bones has a degree of variance that likely cannot be attributed to impact angle or caliber alone. This suggests that entrance beveling is influenced by bone morphology, as well as microstructure, indicating a need for more studies that investigate how bevel angle forms.

Impact Statement: This presentation will impact the forensic science community by providing information on bevel angle variance and formation. While bevel angle is an important indicator of directionality in gunshot trauma, information on how it forms and potential variability in bevel angle is limited. There are few actualistic studies concerned with variability in bevel angle formation. The data here suggest that bevel angle can be highly variable and is likely influenced by bone morphology.

Bevel morphology has consistently been used to indicate directionality in gunshot trauma; however, few studies have examined how the bevel angle is created. Previous research on gunshot trauma to the cranium suggests that bevel angle morphology is variable. This study directly examined bevel angle formation and variability through actualistic research on porcine femora. We hypothesized that handgun caliber would influence the bevel angle, and there would be variability in the angle of the bevel around the circumference of the entrance wound.

Bevel angle was measured in 32 porcine femora, 15 of which were shot with a 45-caliber handgun and 17 of which were shot with a 9mm handgun. The soft tissue was removed from all but two limbs, which were held as controls for the mm sample. After tissue removal, the limbs were shot by a trained marksman at a distance of 15 feet. The femora were suspended within a plexiglass container to assist in the retention of all bone fragments.

When possible, measurements were taken in three locations to assess the degree of variance in bevel angle. To obtain these measurements, the fragments were pressed into a contour gauge while keeping the fragment perpendicular to the gauge. The resulting contour was transferred to paper. A digital angle finder was then used to measure the apex of the bevel—where the outer surface of the bone transitions to the entrance bevel. Additional comparisons were made between the caliber type to assess if caliber type altered bevel angle. The results indicate that there is a high degree of variance within each gunshot wound. For example, the mean range for all 30 of the test impacts was 18.87 degrees (median value = 18.3). In addition, no significant differences were found in bevel angle between caliber types ($p=.355$; $\alpha=.05$). The degree of variance in each entrance wound bevel angle suggests that the morphology of the bone in the area—its thickness, density, and shape—likely plays a larger role in the formation of bevel morphology than caliber or impact angle. Thus, the lack of difference between the two caliber types suggests caliber size and speed in this case do not play key roles in bevel angle formation.

This research suggests that morphology, in particular bone thickness and shape, play a significant role in the formation of bevel angle. Additionally, it shows that caution should be used when trying to interpret angle of impact solely from the bevel morphology of an entrance wound.

Trauma; Gunshot; Long Bones

A57   A Comparison Between Full Metal Jacket and Hollow Point Projectile Effects on Bone

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Learning Overview: Attendees to this presentation will acquire a higher understanding of how bone reacts to different types of projectiles by the analysis of the entrance and exit defects morphology, which may assist in the reconstruction of the events.

Impact Statement: This presentation will impact the forensic science community by providing morphological traits that may be consistent with different types of ammunition in gunshot trauma defects in the bone.

The location, size, and shape of the gunshot defects in a body depend on the amount of kinetic energy lost by the bullet in its path through the tissue, the loss of energy, and the characteristics of the tissue.[1] However, the type of projectile and its behavior when impacting the tissues plays an important role in the effects in the tissue.

The bullet is the part of the cartridge that hits the target. Jacketed bullets contain a core covered by a thin layer of some other material, referred to as the jacket. If the entire bullet is fully enclosed, and the jacket is crimped shut at the base of the bullet, that's referred to as a Full Metal-Jacketed Bullet (FMJ). The jacket covering the tip prevents its deformation upon impact. If the jacket leaves part of the tip uncovered and hollowed out, the bullet is referred to as a semi-jacketed Hollow-Point (HP) bullet and expands to double the bullet diameter on impact.[2,3]

Different projectiles can produce different defects depending on the interaction of the bullet with the tissues. Bullet deformation and fragmentation are significant contributors to permanent cavity formation.[4,5] Additionally, radiating, concentric, delamination, and avulsion fractures may be associated with gunshot defects.[6]

The aim of the present study is to evaluate the entrance and exit defects morphology in bone caused by two types of ammunition: FMJ and HP bullets.

FMJ and HP projectiles were impacted at different distances and angulations on 32 specimens of swine (*Sus scrofa*) skulls with soft tissue present and skeletonized.

The results showed that HP defects in dry bone didn't present as many associated fractures as specimens with soft tissue present. Entrance wounds on skeletonized samples caused by FMJ ammunition created radiating fractures. On the contrary, HP caused defects presented as several radiating fractures. Concentric fractures appear only in entrance defects with soft tissue present. These fractures are caused by intracranial pressure created by the bullet as it passes through the skull and compresses the soft tissue.

This study confirmed some of classical statements about the features of gunshot defects such as size and shape and opened up new perspectives on the analysis of fracture patterns that may assist in the reconstruction of the events.

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Shotgun Defect; Skeletal Trauma; Projectile

A58 Antemortem Trauma and Trauma Reporting in the Texas State Donated Skeletal Collection

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Learning Overview: After attending this presentation, attendees will understand trends in antemortem trauma in a modern United States population. In addition, attendees will understand the importance of trauma reporting for the recognition and interpretation of antemortem trauma in forensic case work.

Impact Statement: This research will impact the forensic science community by illuminating trends in antemortem trauma through the statistical analysis of observed skeletal lesions. This presentation will improve interpretation of antemortem trauma by providing instances of trauma with known and documented explanations.

Trauma analysis is an important component of forensic anthropological investigation. It has the potential to provide information about perimortem events that can inform the determination of cause and/or manner of death.[1] When compared to perimortem trauma, antemortem trauma analysis is underreported in forensic anthropological literature. Understanding the patterning of antemortem trauma can assist with the interpretation of health and injury along the life course, as well as provide individualizing information to assist with positive identification efforts.[1,2]

This research project assessed the presence, absence, and accumulation of antemortem trauma in the Texas State Donated Skeletal Collection.[3] This sample examines 62 individuals for trauma patterns assessed based on body location, sex, age group, and socioeconomic status. In addition, the accuracy of antemortem record keeping by donors and their families was assessed to determine the amount of skeletal trauma that goes unreported.

Eight of the 62 (13%) individuals had no instances of antemortem trauma observed on the present skeletal elements. The body was divided into regions and assessed for percentage of trauma by fracture count. The head and neck contained 8.36% of trauma, the trunk had the highest rate of trauma at 65.07%, the upper extremities had 8.06%, and the lower extremities had 18.51%. When fracture counts were analyzed by element, it was found that rib fractures were highly underreported and made up 38.97% of all fractures. Both thoracic and lumbar vertebrae made up 6.54% of fractures each, with the third-highest rate of fractures (4.9%) seen in the tibiae. When trends in the number of fractures within demographic groups were analyzed for significance with unpaired T-tests, the difference between middle and older adult approaches significance ($P=0.0656$). Because the sample only contained two young adults, they were removed in statistical analysis. No other grouping (sex, occupation, or socioeconomic status) showed significance when examining number of fractures per person.

In the assessment of accuracy of antemortem donor paperwork, it is noted that one individual could not be assessed for matching records due to the incompleteness of remains. Out of the 61 individuals assessed, 13 were self-reported (~21%) and 48 (~79%) were Next-of-Kin reported. Records were assessed as complete, partial, or not a match to what was observed on the remains. The difference between reporting type accuracy was not statistically significant ($P=0.7721$). However, self-reported records had fewer instances of no match at 46% when compared to the Next-of-Kin reported records at ~75%.

The ability to characterize the embodiment of antemortem trauma in a contemporary documented skeletal collection has applications in bioarcheology, medicolegal death investigation, and the medical field. This research indicates there could be correlation between amount of antemortem trauma and age and provides evidence for the underreporting of rib fractures. Further research is required to determine patterns in rib fractures as well as fracture frequency by age group.

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Anthropology; Trauma; Skeletal Remains

A59 **Standards Development in Forensic Anthropology: Updates from the OSAC and ASB**

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Learning Overview: After attending this presentation, attendees will be updated on the status of standards and best practices drafted by the Organization of Scientific Area Committees (OSAC) Forensic Anthropology Subcommittee and those published by the American Academy of Forensic Sciences' Standards Board (ASB) Anthropology Consensus Body.

Impact Statement: This presentation will impact the forensic science community by increasing awareness of forthcoming and existing standards and best practices in forensic anthropology, including publication by the ASB and placement on the OSAC Registry. This presentation will further highlight the need for participation and engagement by the forensic anthropology community in the development of consensus standards.

The OSAC was established in 2014 to facilitate the development of national standards for all areas of forensic science, including forensic anthropology. Each OSAC subcommittee is tasked with drafting standards documents, which are then submitted to a Standards Developing Organization (SDO) for further development and eventual publication. The Forensic Anthropology Subcommittee submits draft standards to the ASB, which was established in 2015 as an SDO accredited by the American National Standards Institute to publish forensic science standards; once a standard is published, the Forensic Anthropology Subcommittee reviews it for technical merit, makes a recommendation for its placement on the OSAC Registry, and submits it to the Forensic Science Standards Board for final approval.

This presentation will describe the differences between standards and best practice documents; summarize recent standards development activities by the OSAC Forensic Anthropology Subcommittee and the Anthropology Consensus Body of the ASB, including ASB standards currently on the OSAC Registry; list ASB standards eligible for the OSAC Registry; inform attendees of proposed forensic anthropology standards that are currently open for public comment or in comment adjudication at the OSAC or ASB; and briefly summarize the topic areas addressed by drafts of standards and best practice recommendations that are currently in progress at the OSAC and/or ASB. As of August 1, 2023, one Forensic Anthropology document is currently listed on the OSAC Registry, and eight other published ASB standards are eligible for the Registry. Three standards are undergoing further development at the ASB and are anticipated to be published in the next nine months. Additionally, the ASB is drafting four best-practice recommendations, and six standards are currently under development by the OSAC Forensic Anthropology Subcommittee, including one that is a collaborative effort with Task Group members representing the OSAC, ASB, and the American Board of Forensic Anthropology (ABFA).

Standards; ASB; OSAC

A60   Assessing the Place and Feasibility of Activism and Advocacy in Forensic Anthropology

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Learning Overview: After attending this presentation, attendees will have a better understanding of the field's consensus on activism and advocacy. Attendees will have an idea of the variation in the conceptualization of attitudes about activism and advocacy held by their peers in the field.

Impact Statement: This presentation will impact the forensic science community by spurring discourse and intellectual engagement with activism and advocacy within forensic anthropology. This is especially important during the current climate where these topics are sometime perceived as divisive. This presentation will elucidate the nuances and the similarities in the opinions that forensic anthropologists share regarding these topics.

This research explores the attitudes surrounding the place, practicality, and feasibility of activism within the field of forensic anthropology. It examines the comfort of forensic anthropologists in performing activist and advocacy work and evaluates how these are conceptualized within the field. For this ongoing study, data were collected through a combination of surveys (n=38), questionnaires (n=11), and interviews (n=9). Only surveys that were at least 75% complete were included in the current study. This research will provide insights into the conceptualizations of advocacy and activism and their trajectory within forensic anthropology and present the shared and diverse opinions of the field. This will further assess relationships between attitudes toward activism and multiple variables such as a practitioner's level of experience, pedagogical era, and the context in which one practices. These data will be used to answer three research questions: (1) Is the conceptualization of activism and advocacy consistent across the field? (2) Is the conceptualization of activism and advocacy influenced by the context in which the forensic anthropologist practices? And (3) How comfortable are forensic anthropologists with integrating activism and advocacy into their practice?

Out of the 38 surveys, 31.6% are students, 36.8% are primarily practicing in academia, 15.8% are primarily practicing within the government, and 3.2% are practicing within the medicolegal context (in medical examiners or coroners' offices). In response to the questions regarding the conceptualization of activism where respondents were asked to identify whether a given scenario qualifies as activism or not, the most common of the five response options was "Not Activism" (26.6%). However, when comparing responses of "Not Activism" to those that indicate that the given scenarios represented some level of activism, responses that indicated any activism were more popular (71.8%). When rating how comfortable the practitioner would be in performing the same scenarios, the most common answer was "Very Comfortable" (46.1%). There are slight differences in the frequency and type of responses by practice context and many responses to scaled survey questions included supplemental context to the answer. These results may indicate a general consensus in the conceptualization of activism in the extremist connotation across the field, which is supported by the responses from the interviews and questionnaires. Also reflected by the survey data and supported by interviews and questionnaires is the breadth of opinions, attitudes, and comfortability with advocacy and activism in forensic anthropological practice.

Anthropology; Activism; Advocacy

A61   Forensicanthrotok: The Depiction of Forensic Anthropology on TikTok

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Learning Overview: After attending this presentation, attendees will understand the public perception of forensic anthropology on TikTok, the discipline's use of the social media platform, and the interconnections of forensic anthropology with various TikTok subcommunities.

Impact Statement: This presentation will impact the forensic science community by providing information regarding the presence of forensic anthropology on TikTok and discussion regarding effective use, ethical issues, and public interaction with the discipline via the platform. This research presents initial findings from a mixed method, digital ethnographic investigation of TikTok videos to understand how anthropological concepts are discussed on social media, particularly by extremist communities.

Social media platforms like TikTok have dramatically increased in popularity in recent years, particularly due to restrictions associated with COVID-19. Much like other platforms, an algorithm provides content to users based on their prior activity (e.g., videos watched, liked, and shared). Sometimes seemingly unrelated videos are shown to users despite a lack of prior interaction with the topic. As such, a broad audience may view and interact with forensic anthropological content. Additionally, different ways to engage in material (e.g., duets, shares, comments) allows users to not only share anthropological information but to utilize this information for different purposes, whether educational, ideological, or entertainment.

The present research seeks to understand the ways in which forensic anthropological content is produced and interacted with on TikTok. Hashtags directly related to some aspect of forensic anthropology (e.g., #forensicanthropology, #cranium, #biologicalanthropology, #dentalmorphology) were selected from a database of TikToks collected as part of a larger investigation of the relationship between anthropology and extremism on social media. TikTok metadata and videos were collected using the Python® package TikTokAPI where the 500 most "popular" TikToks were downloaded for each hashtag over a one-month period.[1] TikTok comments were downloaded using the Python®-based code TikTokCommentScraper.[2] The analyzed sample for this presentation included 5,117 TikToks drawn from 22 hashtags.

Due to the heavily skewed nature of the data, non-parametric descriptive statistics were calculated. The median value for the number of likes is 8,540 (Quartile 1: 142; Quartile 3: 77,550). The median value for the number of shares is 45 (Quartile 1: 1; Quartile 3: 505.5). Last, the median value for the number of comments is 87 (Quartile 1: 5; Quartile 3: 1,017.8). This compares to median values of 16,700 likes (Quartile 1: 828; Quartile 3: 156,600), 184 shares (Quartile 1: 8; Quartile 3: 8,864), and 204 comments (Quartile 1: 20; Quartile 3: 1,359) from the broader sample of 543,798 TikToks. These results indicate that those TikToks related to forensic anthropological concepts have a generally smaller engagement compared to other TikToks in the database; however, TikToks still receive thousands of likes and views and, therefore, have a broad reach. Some of the topics discussed included the television show *Bones*; educational material; day-in-the-life vlogs of anthropology students; "aesthetic" depictions of forensic anthropology; and comedic jokes regarding forensic practice and education. Additionally, many TikToks either: (1) address issues of biological essentialism of gender, or (2) utilize forensic practice to invalidate trans identity or the distinction between sex and gender.

A network analysis using the R package *quanteda* was conducted to understand the 100 most commonly associated hashtags and, by extension, communities of the sampled TikToks.[3] Notably, the most associated communities included Anthro TikTok, Academic TikTok, ConspiracyTikTok," ArtistsofTikTok, and FurryTikTok. Many of these communities' hashtags were within the 50 most popularly associated hashtags. These results highlight the significant overlap of forensic anthropology with seemingly disparate digital communities and the discipline's possible far-reaching influence.

With the constant expansion of social media platforms and the quick turnover of trends in digital space, it is important to understand how digital communities engage with forensic anthropology and how the discipline ethically and effectively engages with these platforms and audiences. By introducing ways in which forensic anthropology exists on TikTok, this presentation will discuss issues including curriculum for responsible presentation of the discipline and imagery of human remains, ethical public engagement (e.g., discussion of bioprofile estimation; avoiding images containing intact, skeletal, and decomposing human remains), and combating weaponization of forensic anthropology in prejudicial rhetoric.

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Anthropology; Social Media; Ethics

A62   Forensic Anthropology and Structural Vulnerability in Casework

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Learning Overview: After attending this presentation, attendees will gain insight into the burgeoning literature that analyzes the expansion of forensic anthropology practice, specifically in the realm of public health and social vulnerability reporting. This presentation will critically examine the idea of applying archaeological and social anthropology lenses to interpreting crime scenes, material evidence, skeletal trauma, and skeletal pathology to extrapolate information about the victim's lived experiences. To promote further discussion of this emerging topic at our primary conference, this presentation will address ethical obligations of forensic anthropologists as last responders, practical challenges, and the current strengths and challenges that have surfaced in recent professional discussions and related scholarship.

Impact Statement: This presentation will impact the forensic science community by laying the groundwork for further discussions within the field on how forensic anthropology data drawn from cases could be used to inform other sectors related to the medicolegal system.

Forensic anthropologists within the United States have traditionally been expected to extrapolate information regarding biological profile, trauma, pathology, or postmortem interval to assist in victim identification, crime reconstruction, and cause and manner of death. However, as anthropologists, those in the forensic subfield can still choose to pursue archaeological and sociocultural training that may inform forensic anthropology.[1,2] Recent scholarship has emphasized the unique potential of these skills for expanding forensic anthropological practice.

Specifically, recent publications have underscored the potential for forensic anthropologists to contribute to policymaking and data reporting for social vulnerability determinants. This application of anthropological analysis exists in other subfields but not within standard forensic anthropology practice. Through exploring the ability, limitations, and potential of forensic anthropological analysis to infer information about access to resources and health status, practitioners may find the role of forensic anthropology changing to provide data that is evidentiary of human rights law (e.g., access to housing) or serves public health initiatives.

Longstanding studies in structural violence discuss social inequity in terms of access to resources such as housing and health care.[3,4] These same principles are seen in international human rights law (e.g., Universal Declaration of Human Rights, International Covenant on Economic, Social, and Cultural Rights) that is framed in similar language and also has a deep interdisciplinary scholarship base. In the United States, accessibility and access to resources remains a fundamental concern for policymaking and community response, with organizations such as the Centers for Disease Control and Prevention (CDC) using a social vulnerability index to identify communities that need additional support. Medical anthropologists, bioarcheologists, and other bioculturally focused practitioners are often familiar with some of these literatures (we suggest forensic anthropology also engage with broader public health and human rights law issues).[5,6]

In essence, the United States approach to forensic anthropology has largely focused on the individual—enabling their identification and case resolution. While these goals remain important, we wish to highlight the potential for the discipline to refocus on population-level trends among the decedents we analyze. In this presentation, we ask: What patterns emerge among their causes of death, circumstances of death, and the lived experiences they may embody? What social structures are implicated in those patterns? How might forensic anthropological reporting inform public health and policy to effect change, improve quality of life, and seek to prevent future deaths?

Recent forensic anthropological research and casework explores forensic anthropologists' ability to extrapolate information from individual cases or bodies of casework regarding a population's access to resources and health status—particularly when contextualized for a specific geographic region.[7-9] Here, we synthesize and critically evaluate literature related to Winburn, Wolf, and Marten's proposed Structural Vulnerability Profile (SVP).[10] Some scholarship calls attention to the relationship between SVP reporting and human rights/humanitarian anthropology, while other literature highlights concerns that should be addressed before pursuing an SVP.[11,12] This presentation reviews the literature to summarize main arguments, clarifies the original intent of an SVP proposal, and encourages further discussion among practitioners.

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Forensic Anthropology; Structural Vulnerability; Ethics

A63 Justice for All Missing: The Mississippi Repository for Missing and Unidentified Persons

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Learning Overview: After attending this presentation, attendees will have a better understanding of who goes missing in the state of Mississippi and how the Mississippi Repository incorporates socioeconomic data to help resolve missing persons cases.

Impact Statement: This presentation will impact the forensic science community by highlighting the need to develop region- and state-specific databases that rely on multiple sources, including missing persons data, health data, and socioeconomic factors for missing persons work.

In the United States, a lack of missing and unidentified persons repository data at both the state and national level continues to impact the identification of missing and unidentified people, particularly from historically marginalized communities.[1-3] As of July 2023, the National Missing and Unidentified Persons System (NamUs) reported 23,446 open missing persons cases, only 24% of the open cases in the FBI's National Crime Information Center database.[4,5] These statistics highlight a discrepancy in missing persons data, and such inconsistencies are compounded for marginalized groups, who are less likely to be reported missing, less represented in media coverage, and have fewer resources allocated to their cases.[6-8] However, state data collection and analysis can help rectify these inequities by providing more accurate data that can be shared nationally. The Mississippi Repository for Missing and Unidentified Persons was developed to help identify, resolve, and archive Mississippi's cases of missing and unidentified persons. The Mississippi Repository is a statewide database and collaborative tool that seeks to increase public access to missing persons information, partner with neighboring states to facilitate data sharing of missing and unidentified persons information, and visualize socioeconomic and medicolegal disparities affecting missing persons using geospatial analysis.

The Mississippi Repository comprises over 800 current and resolved missing persons profiles gathered from local law enforcement agencies, non-profit missing persons advocacy groups, and social media platforms. Preliminary analyses have shown that Black, Native American, and Multiracial individuals were overrepresented in the Repository compared to their general population percentage in Mississippi. Of all the missing persons resolved, 62% of those found deceased were Black. Moreover, Black men and women were twice as likely to be found deceased compared to their White counterparts. These disparities are also present in looking at health outcomes. Compared to White Mississippians, Black Mississippians have the highest mortality rate from cancer, diabetes, heart disease, homicide, hypertension, renal disease, and stroke.[9] These stark Black-White disparities in Mississippi are geographically structured as well. The Mississippi Delta and other counties with high Black populations have the worst rankings on premature death, self-reported health, and percentage of low-birth-weight newborns in the state.[10] Combining missing persons data with socioeconomic factors will provide a greater understanding of who goes missing and how we can help identify and resolve these cases in Mississippi and across the Southeastern United States.

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Missing Persons; Social Justice; Rural Communities

A64   The Cold Case Initiative: Bringing Cases up to Current Investigative Standards

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Learning Overview: The goal of this presentation is to present an inclusive investigative model for Unidentified Human Remains (UHR) and missing persons at the state level, including field, lab, and archival methods.

Impact Statement: The National Institute of Justice (NIJ) guidelines for establishing cold case units calls for inclusive models combining homicide, missing person, and unidentified human remains investigations. The research presented here was a state-wide initiative that serves as a template for other agencies and programs seeking to establish similar units. This presentation will impact the forensic science community by providing best practices for locating and identifying lost investigative materials, with a focus on including traditionally underserved demographic groups and minority populations.

Throughout Pennsylvania, thousands of unresolved homicides dating back to the 1960s represent families and victims who have been denied justice. Among the most difficult to solve are the hundreds of Missing Persons (MP) presumed dead and the 500+ individuals who are victims of homicide or died alone, resulting in unknown identities. Such cases pose significant prosecution challenges with thousands of hours spent searching for human remains and clandestine burials.

The Institute for Forensic Anthropology and Applied Science at the University of South Florida (IFAAS) began a collaboration with the Pennsylvania State Police (PSP); numerous district coroners and district attorney offices; the Philadelphia Police Department, the Philadelphia District Attorney and Medical Examiner Offices; the Pennsylvania Attorney General's Office, and several other Eastern District police departments. The purpose of the Cold Case Initiative was to bring select UHR cases from open homicide investigations up to current investigative standards, facilitating they meet protocol standards and methods required to solve long-term open cases. This initiative led to numerous positive outcomes, positive identifications, and was successful in laying the groundwork for a statewide effort. The methods, results, and policy recommendations are discussed in this presentation.

In 2016, Philadelphia had a total of 41 UHR cases listed in NamUs, and all other counties throughout Pennsylvania had a combined total of 62 UHR cases. As a result of this initiative, an additional 178 UHR cases from Philadelphia and 29 from surrounding counties for a total of 207 UHR were added to the existing lists into NamUs and National Crime Information Center (NCIC). Still, these impressive results represent only a portion of the total number of unresolved cases.

This investigation found significant bias among minority communities in terms of missing person investigations. A consistent gap in records shows that cases involving females and minorities have not been investigated (e.g., there is no documented female missing person investigation in the city of Philadelphia from 1985-1992, and only one missing Black female is listed prior to 1980). The majority of listed missing persons cases since that time are females from minority communities; yet the majority of UHR listed by coroners and medical examiners are male, illustrating the disconnect between reported MP and UHR cases. Two of the UHR cases we attempted to exhume in 2016 were unknown Black children. Neither were in the reported graves upon exhumation and currently their skeletal remains are "lost." It was further discovered that one female child had never been in the missing persons system despite police reports about her disappearance in 1984.

The results of this project also establish areas for further investigation that will be addressed in the presentation such as locating John/Jane Doe graves in unmarked cemeteries; the need for vital statics to provide death certificate data on burial locations, the use of facial reconstructions to engage the public for generating investigative leads, and the occurrence of undocumented UHR located in university research and teaching collections.

Cold Case; Identification; Missing Persons

A65   The East Marshall Street Well Project: Community-Driven Research to Restore Identity and Dignity

Tal Simmons*, Virginia Commonwealth University, Richmond, VA

Learning Overview: After attending this presentation, attendees will understand the role of descendant communities in providing direction, guidance, and oversight to humanitarian forensic research concerning recent historical remains. Community engagement objectives and preliminary results of the project will also be discussed.

Impact Statement: This presentation will impact the forensic science community by raising awareness of the need not only to consult and engage with but also be guided by descendant communities when conducting research with humanitarian objectives.

During construction of a new building on the Virginia Commonwealth University (VCU) medical campus in 1994, a brick-lined well, or anatomical sink, was discovered containing human remains. The university president gave archaeologists a single weekend to remove the deposits with a backhoe and retrieve the remains. Archival research indicates that these individuals most likely were grave-robbed from local cemeteries of enslaved Africans and paupers (ca.1840-1860) by resurrectionists, used at the medical school for anatomical dissection and amputation practice, and subsequently discarded in the Well. In 1994 when the Well was rediscovered during construction, VCU did not comply with the law and had the remains hurriedly removed with a backhoe over a weekend, never filed the appropriate paperwork with the Virginia Department of Historical Resources, and shipped the remains to the Smithsonian for analysis—all without the knowledge of the local community. The community only became aware of what had occurred in 2011, with the screening of a film (*Until the Well Runs Dry: Medicine and the Exploitation of Black Bodies*) by Dr. Shawn Utsey, of VCU's African American Studies department.[1] VCU then began a program of community consultation concerning what should be done with the Ancestral remains.

Published analyses (2012) by anthropologists from the Smithsonian Institution's Museum of Natural History determined that there were a minimum of 54 individuals, including nine children under the age of 16 years, primarily of African ancestry.[2] As awareness in the community grew, the Family Representative Council (FRC) was established in 2015 to stand in for the descendant community and set research, memorialization, and interment goals for the Ancestral Remains.[3] After some seven years of ongoing community consultations, the skeletal remains of the Ancestors from the East Marshall Street Well (EMSW) were returned to Richmond in 2019 and VCU's Forensic Anthropology Laboratory in 2022; DNA analysis to re-associate the bones into individuals and to establish their ancestry, phenotype, and relationship to people living in the community today is ongoing. This presentation will explore the ethical treatment of human remains, the agency of the community in determining the fate of the Ancestral remains, and the community's oversight of the research and involvement of scientists. The session will also present the results of the research thus far and the continuing goals of the project. Additional community engagement has occurred in the form of an "Ethical Treatment of Human Remains" curriculum for high school students, which was piloted to two Richmond Public Schools in the spring of 2023.

Over 1,000 commingled bones were recovered from the EMSW, of which approximately half are being sampled and analyzed for DNA in order to re-associate them to form as complete individuals as possible; ribs, vertebrae, and patellae were excluded from sampling, as well as clavicles that were previously subjected to unauthorized isotopic sampling. Method development enabled the DNA sampling to be restricted to a 40mg bone powder sample obtained using a Dremel® tool with a diamond bit. Although the work is still ongoing, results from comparisons of (1) anthropological pair matching with bone-to-bone DNA matching and (2) anthropologically determined population affinity and genetically determined ancestry will be discussed. In addition, phenotype estimates from the genotypes obtained will be discussed. Current work involves using mitochondrial DNA (mtDNA) to both understand the diverse ancestry of the current community and attempt to relate individuals living today to those ancestors recovered from the EMSW.

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Commingling; Ethics; Human Rights

A66 Mapping Medicolegal Disparities and Indigenizing Harm Reduction Strategies for Missing and Murdered Indigenous Persons in Idaho

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Learning Overview: After attending this presentation, attendees will better understand the intersectional factors of the Missing and Murdered Indigenous Persons (MMIP) crisis in North America. Attendees will be able to incorporate factors such as social services and health care support via Geographic Information Systems (GIS) mapping in order to assess the vulnerabilities, resource needs, and cultural knowledge fundamental to finding solutions in MMIP cases.

Impact Statement: This presentation will impact the forensic science community by offering strategies for collating and contextualizing MMIP cases through the incorporation of Indigenous cultural knowledge, public health theory, and culturally informed harm-reduction strategies.

Indigenous persons are disproportionately reported as missing or unidentified, necessitating a specialized response. In Idaho, Indigenous peoples account for 3.38% of the total missing persons entries, compared to 1.76% of total entries nationwide, and in proportion to their population, are 2.1–10 times more likely to go missing.[1] These numbers likely misrepresent the true nature of the crisis due to the unknown number of cases that go unreported or unentered into databases.[1] Inconsistencies in reporting and lack of collaborative efforts among law enforcement and forensic practitioners with tribal leaders precludes culturally specific strategies and preventative measures.[2-3]

Since there is no national unified system for the reporting of missing/unidentified persons, nor a federal mandate for law enforcement to compile their data centrally, databases are often incomplete, decentralized, inconsistent, challenging to access, and understaffed. None have standards to collect tribal data and lack the inclusion of relevant socioeconomic data that could allow a contextualized analysis. Jurisdiction also impacts reporting and resources for MMIP cases, as tribal police may engage in their own data collection (often with few resources). Further, in regard to Indigenous stakeholders, there is consensus that policy or law enforcement definitions of "missing persons" lack cultural/communal understanding, leading to mistrust, inter/multi-agency miscommunication, under-resourced tribal dispatches, and a lack of culturally appropriate services to Indigenous victims and families.[1-5] All of these factors fail to support the data collection needed for longitudinal/trend analysis necessary for guiding community-specific action.[2,3,5]

To illustrate the potential of contextualizing MMIP data, this study presents a GIS database and map of MMIP in Idaho. Data are compiled from open-source databases such as Idaho Missing Persons Clearinghouse, the National Missing and Unidentified Persons System (NamUs), National Center for Missing and Exploited Children (NCMEC), the Charley Project, LGBT+ Accountability for Missing and Murdered Persons (LAMMP), and the DNA Doe Project. These repositories include demographic and individualizing information, city of origin, and last known location. A map is developed using MMIP geographic markers (last known location, location of unidentified remains) and overlaying polygonal census/health data.

This presentation demonstrates spatial patterning of missing persons and correlates that to three different factors of vulnerability; specifically, the visibility of the area, population size, and law enforcement presence all factor into the rate of missing persons. The rate of missing persons is contextual depending on county resources: counties with the highest presence of law enforcement also hold the most missing person cases, while other counties are among the lowest for population and have some of the highest rates of missing persons These results show the correlation between social services and social vulnerability in that certain factors predispose individuals of marginalized groups to experience colonial violence, racism, and discrimination, which are the foundation of the MMIP crisis. Finally, Indigenized harm reduction strategies are proposed by combining these multi-layered data with Indigenous ways of knowing.[2-3,5]

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Missing Persons; Indigenous; GIS

A67 The Validity of Radiographic Comparisons: Preliminary Findings by Body Region

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Learning Overview: By attending this presentation, attendees will learn which anatomical regions are more challenging to use for radiographic comparisons based on the preliminary results of an experimental study. Attendees will also learn how many concordant points are more predictive of positive identification by anatomical region and the effects of specific categories on the probability of positive identification.

Impact Statement: This presentation will impact the forensic science community by addressing a substantial gap in best practices in positive identification via radiographic comparisons. We will present preliminary predictive statistical models used to assess the validity of identifications from radiographic comparisons.

Forensic anthropologists are often called upon to assist with personal identification, many times using antemortem radiographs of a decedent as a reference. Radiographic comparisons are quick, easy, inexpensive, have broad utility, and are therefore commonly employed for positive identification. Moreover, radiographic comparison is considered a basic competency, expected of forensic anthropologists who are certified by the American Board of Forensic Anthropology. However, relatively little empirical research has been conducted on the reliability and validity of such analyses. This research gap creates a problem of objectivity for radiographic identification, particularly when it needs to be defended in court. Radiographic comparisons can be performed on any region of the body that contains mineralized tissue or bone and that has an antemortem image from the suspected decedent. The accuracy of radiographic comparisons is critical because they are used to make positive identifications in forensic cases and may be the only evidentiary means by which to make such an identification. However, the validity and reliability of these common methods have yet to meet the Daubert guidelines and National Academy of Sciences (NAS) report recommendations. Validity has only been demonstrated for specific regions of the body (e.g., AP chest). A more standardized, objective protocol and robust methodological approach to radiographic comparisons are required. In this presentation, we present preliminary results of the validity of radiographic comparisons as they pertain to predictive accuracy based on anatomical location and number of features using an experimental approach.

We generated a large dataset from a contemporary sample of radiographic data via partnership of the University of Nevada, Las Vegas (UNLV); Clark County Office of the Coroner/Medical Examiner (CCOCME); and Forensic Anthropology Center at Texas State University (FACTS). We used two means to collect radiographs: (1) archived case files from CCOCME from individuals identified using radiographs, and (2) whole-body donations at FACTS. We hypothesized that unique human skeletal morphological variation is sufficient to produce valid personal identifications and the identification probability can be calculated, regardless of the anatomical location being used. Over two years, ten participants of varying levels of experience and education each conducted nearly 500 radiographic comparisons of sex-matched image sets grouped by seven anatomical locations (AP skull, AP chest, AP elbow, AP hand/wrist, AP lumbar and pelvis, AP knee, and lateral foot) to identify correct matches. Participants were given basic introductions to radiographic comparisons and the comparison interface. They were instructed to identify as many concordant points as they deemed sufficient to make a confident match. The concordant points were divided into four categories: (1) normal anatomy, (2) developmental anomaly, (3) pathological, and (4) surgical. Ten percent of the sets presented to the participants were non-match sets.

Preliminary data were analyzed using a generalized logistic mixed-effects model to identify the factors that were most likely to predict an accurate identification. Results indicate that the minimum number of concordant points necessary to increase the probability of a positive identification is statistically significantly moderated by minimum education attained and anatomical region. Across participants and regardless of education level, the AP chest was statistically significantly the most difficult region for comparisons. In addition, participants who used more than one identificatory category (e.g., normal anatomy) statistically increased the probability of a match, as were those who used a minimum of ten points of concordance. The effect of specific identificatory categories on comparison predictive success will be discussed. Finally, these preliminary results indicate that continued exposure to radiographic comparisons improved mean accuracy rates over time for all body regions except for AP chest. These results will be used to highlight recommendations for radiograph best practices, minimum education, and training.

X-Ray; Validation; Positive Identification

A68   A Part-to-Part Comparative Analysis to Assess Intra-Individual Similarity and Inter-Level Distinctions for Personal Identification

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Learning Overview: After attending this presentation, attendees will have gained a deeper understanding of the utilization of the lumbar vertebrae from Antemortem and Postmortem Computed Tomographic (AMCT and PMCT) scans for personal identification and the limitations of using cross lumbar level comparisons.

Impact Statement: This presentation will impact the forensic science community by providing a cross-level lumbar vertebral comparison for personal identification. Our previous work has shown the reliability of part-to-part comparison on accurately identifying individuals based on their L1-L5 lumbar vertebrae with promising results showing no false positives and no false negatives.[1] The current study examines the cross-level reliability of comparing the different lumbar levels.

In the field of forensic anthropology and medicolegal death investigations, the accurate identification of human remains is of paramount importance. As part of a personal identification project sponsored by the National Institute of Justice (NIJ 2019-DU-BX-0031), the authors examined the utilization of lumbar vertebral models from AMCT scans and the comparison of those models to PMCT scans. That study strictly compared vertebrae from the same lumbar levels, with all lumbar level 1 (L1) vertebrae compared to only other L1s continuing through the L5s. However, in the event of mass disasters or discovered remains, it is common for bones to be found *ex situ* or even individually scattered about the scene. In this comparative analysis, we delve into the utilization of AMCT and PMCT scans to discern if individual variations across lumbar levels can be leveraged to aid in the identification of human remains in forensic scenarios.

Thirty-seven paired AM and PM de-identified thoraco-abdominal CT scans were imported into the Mimics® Innovation Suite v. 24. The L1-L5 vertebrae of each subject were isolated and modeled via hand-segmentation and thresholding. The AM L1 of all subjects were compared against all the L2-L5 vertebra of all the PM scans using part-to-part comparison. A threshold of +/- 1mm was set for the analysis. This process was conducted for every lumbar level where every AM vertebra was compared against every PM L1-L5 vertebrae. A match-ratio was recorded for each analysis, which resulted in over 27,000 unique data points.

A Receiver Operator Curve (ROC) was calculated for every comparison. The Area Under the Curve (AUC) for AM L1 against PM L2, L3, L4 and L5 was 0.928, 0.775, 0.643 and 0.608, respectively. The AUC for AM L2 against PM L1, L3, L4 and L5 was 0.927, 0.923, 0.716 and 0.612, respectively. The AUC for AM L3 against PM L1, L2, L4 and L5 was 0.88, 0.974, 0.909 and 0.714, respectively. The AUC for AM L4 against PM L1, L2, L3 and L5 was 0.751, 0.753, 0.895 and 0.814, respectively. The AUC for AM L5 against PM L1, L2, L3 and L54 was 0.696, 0.637, 0.665 and 0.79, respectively.

The study's findings, measured through the AUC values, demonstrated the potential of cross-level lumbar vertebrae comparison for personal identification. Notably, the AUC values for different vertebrae comparisons indicated varying degrees of accuracy, with some pairs showing high discriminatory power (e.g., AM L3 against PM L2, L4, and L5), while others exhibited lower discriminative ability (e.g., AM L1 against PM L4 and L5). Overall, the results highlight the potential of lumbar vertebrae in forensic human identification, with the highest accuracy observed in the comparisons between AM L3 and PM L2, L4, and L5. Notably, lumbar levels were most similar to their adjacent neighbors. With L3 being the most "central" vertebrae, it had an overall higher match to L2, L4 and L5. L1/L2 matches strongest with each other with L1 and L5 matching the least. However, as each comparison had an AUC higher than 0.5, which signifies that even at the worst values, the match is still greater than chance and significant.

In conclusion, the study's findings demonstrate the accuracy of cross-level lumbar vertebrae comparisons for personal identification. The highest discriminatory power was seen in the comparisons between AM L3 and PM L2, L4, and L5, highlighting the significance of the central vertebrae (L3) in matching with its adjacent neighbors. Moreover, the results demonstrated that even at their lowest values, the matches still surpassed chance, reinforcing the robustness of using lumbar vertebrae for identification purposes.

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Personal Identification; Forensic Radiology; 3D Modeling

A69   Automated Comparative Chest Radiography Using Deep Neural Networks

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Learning Overview: After attending this presentation, attendees will be familiar with machine learning techniques based on deep neural networks applied to the task of comparing chest radiographs (N=2,753) for identification purposes.

Impact Statement: This presentation will impact the forensic science community by addressing a significant gap in current methodological approaches to positive identification, particularly levels of subjectivity encountered when performing comparative medical radiography.

When assisting in decedent identification, forensic anthropologists use several approaches, including passive approaches leading to identification (e.g., bioprofile) and more active efforts linking an antemortem record from a tentative identity to the postmortem record of the decedent. Many "active" methods lack quantification, automation, or standardization. In most cases, analysts identify features of concordance (or discordance) in a point-by-point comparison between antemortem and postmortem records to support or refute the identification decision. While such comparisons have withstood the test of time, advancing methods to increase the accuracy, effectiveness, and efficiency of decedent identification is essential to the goals of medicolegal death investigation. One facet of innovation is quantifying how human examiners recognize radiographs from the same individual; another equally important facet is assessing the uniqueness of radiographic features and the specific areas of focus used by human examiners when making an identification.

To investigate this, 2,753 anonymized chest radiographs representing 680 individuals were compiled from the National Institutes of Health (NIH) Chest X-Ray Database and casefiles from the Michigan State University Forensic Anthropology Laboratory. This dataset will advance comparative medical radiographic identifications by providing probabilistic estimates for the confidence of matches and automate the comparison process. To that end, the dataset was divided into training (N = 1,498) and testing (N = 1,255) samples. Images were temporally organized, with earlier radiographs used for training and radiographs taken at a later date reserved for testing. This approach ensures effective learning and accurate assessment by the models.

To prepare the dataset, four Regions Of Interest (ROIs) were digitally annotated in each radiograph: (1) thoracic vertebrae one through five; (2) complete vertebral column; (3) clavicles; and (4) the entire radiograph. Deep learning classification models were developed using three Convolutional Neural Networks (CNNs): ResNet, DenseNet, and EfficientNet architectures with different hyperparameters.[1-3] A closed-set identification protocol was employed (i.e., for each test image [single radiograph], other images corresponding to the same identity were present in the training set). The models were trained using images in the training set. During the evaluation stage, each test image was input to a model, and the output consisted of a ranked list of matching identities with corresponding confidence scores. The results of the initial models are promising. Using only a single ROI, the DenseNet-169 model had the best performance, correctly identifying 74.42% of the test samples using the T1-T5 region. When using the complete vertebral column, EfficientNet-B3 outperformed other models, with an identification accuracy of 73.71%. DenseNet-201 had an identification accuracy of 69.72% when using the clavicles only, and DenseNet-169 had an identification accuracy of 69.72% using the entire chest radiograph. When the confidence scores of multiple ROIs (using multiple regions of a radiograph) were fused, the identification accuracy of both DenseNet-169 and DenseNet-201 reached 80.00%. Interestingly, most models relied more heavily on the T1–T5 and vertebral column regions than the clavicles. The DenseNet-201 model was able to identify 1,255 radiographs in 17 seconds with 74.42% accuracy, whereas trained human examiners need 30 to 60 hours to find a match from 1,000 radiographs but with 100% accuracy.[4]

Most of the current comparative medical radiographic techniques do not quantify the strength of evidence concerning a positive match. In this study, two aspects were evaluated: (1) computed identification accuracies of multiple deep learning models tasked with reconciling potential matches in a large sample of radiographs, and (2) areas in the chest radiographs utilized by these models to achieve those accuracies. Answering questions like these requires considerable collaboration and a multidisciplinary approach. Our research team comprises experts in biometrics, skeletal morphology, machine learning, and computer engineering, demonstrating the effectiveness of a multidisciplinary approach.

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Anthropology; Positive Identification; Machine Learning

A70   **Visual Comparisons of Frontal Sinus Radiographs: Documenting Accuracy and Exploring the Effects of Experience**

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Learning Overview: This presentation will provide results from a recent study in which forensic and medical practitioners were asked to evaluate pairs of radiographic sinuses for matches. After attending this presentation, attendees will better understand the accuracy rate of radiographic frontal sinus visual comparisons and the effects of training and experience on results.

Impact Statement: This presentation will impact the forensic science community by providing accuracy rates and a better understanding of factors involved in radiographic frontal sinus comparisons. These data may be used to support forensic testimonies and develop future training sessions.

The use of frontal sinus radiographic comparisons for forensic identification is well known in medicolegal fields. Visual assessments are the most commonly employed method, although several quantitative methods of sinus matching have been proposed in an attempt to decrease potential subjectivity. In this study, we aimed to assess accuracy rates in visual comparisons of frontal sinus radiographs and to explore the effects of training and experience on accuracy and confidence rates.

An electronic questionnaire was distributed asking participants questions about their education level, certifications, training, profession, and experience in radiographic comparisons. This was followed by 25 pairs of frontal sinus radiographs of which participants were asked whether they believed the two sinuses came from the same individual and to rate their confidence level (1–10). The radiographs were obtained from the American Association of Orthodontists Foundation (AAOF) Craniofacial Growth Legacy Collection. Of the 25 pairs, observers were presented with 18 in which both radiographs were from the same adult individual but taken a year or more apart. Radiographs were cropped to only display the frontal sinus so other craniofacial information would not play into observer decisions. Seven pairs had sinuses from two different individuals. These "mismatched" pairs were selected based on results from another on-going study, in which Elliptical Fourier Analysis (EFA) was used to quantitatively match sinuses of 128 individuals; the mismatched sinuses used in this study were ones EFA incorrectly assigned as a match. Consequently, the mismatched pairs presented to observers should represent challenging mismatches. All radiographs presented were antemortem and taken in the mid-to-late 20th century, meaning the presence of soft tissue and radiographic quality also made the comparisons challenging.

In total, 145 complete responses were received. Respondents included professionals and students in forensic and medical disciplines. Even with 65% reporting zero previous experience conducting radiographic comparisons, 89.9% (94.5% median) of the total comparisons (n=3,625) were scored correctly. Eighty-three respondents (57%) scored 92% or better (of those 29 scored 100%). Experience, training, and specialties were shown to affect results. The highest percent-correct scores were found among forensic odontologists (n=3; 98.7% accuracy) and forensic pathologists (n=3; 96% accuracy). Among forensic anthropologists, board-certified individuals scored significantly higher (95.8%; n=20) than those not certified (91.4%; n=28) (p=0.031). A significant positive Spearman correlation was found between an observer's accuracy and their average confidence level ($p<0.001$; $r=0.754$). Incorrectly scored radiographs were biased toward wrongly excluding a match (12.6% of matches erroneously reported as mismatches; 3.6% of mismatches erroneously reported as matches). It appears that slight differences in orientation played a role in the two lowest-performing radiographic pairs. Visual assessment of frontal sinus pairs also outperformed preliminary results from the quantitative outline analyses, given that practitioners can better evaluate explainable differences and internal features such as septa. These results confirm that visual comparisons of frontal sinus radiographs are highly accurate, even considering varied experience levels and less-than-ideal radiographic parameters. Training and experience are recommended to obtain the highest accuracy rates given forensic implications of erroneous personal identifications.

Anthropology; Positive Identification; X-Ray

A71 Investigating Variation in Trabecular and Cortical Bone Structure Due to Mobility Impairment: A Natural Experiment in Reduced Loading

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Learning Overview: The goal of this presentation is to discuss how mobility impairment is reflected in the trabecular and cortical bone of the upper and lower limb bones. Attendees will come away with an in-depth view of how bone responds to disuse due to reduced mobility, the utility of micro-Computed Tomography (micro-CT) imaging in such a context, and the implications this research has for identifying mobility impairment in the forensic context.

Impact Statement: This presentation will impact the forensic science community by demonstrating the utility of micro-CT imaging and bone microstructure analyses in assessing individualizing conditions such as mobility impairment. Since vulnerable populations, such as persons with physical disabilities, are often those most subject to violent crimes and thus more likely to show up in the forensic context, being able to recognize and diagnose mobility impairment can contribute toward identification of human remains.

Bone is a dynamic tissue that responds to changes in loading regime by increasing or decreasing in mass and adjusting the architecture. Since mechanical usage influences trabecular architecture and cortical density, reduced ambulatory ability should be reflected in the trabecular structure and cortical area of the limb bones. This research examines the effects of reduced loading on human bone by quantifying long bone trabecular and cortical bone structural variation associated with mobility impairment. Both left and right femora and humeri of 69 mobility-impaired individuals and one side of the sex- and age-matched fully mobile individuals were imaged using high-resolution computed tomography. Image stacks were processed to produce binary images and trabecular thickness and spacing maps. Trabecular architecture was quantified using multiple geometrically homologous spherical Volumes Of Interest (VOIs) positioned within each joint head for which Bone Volume fraction (BV/TV), Trabecular Thickness (Tb.Th), and Trabecular Spacing (Tb.Sp) were extracted. In addition, these VOIs were used to create maps of average Tb.Th and BV/TV values across the joint surface to view consistent patterns. Single spherical central VOIs were also extracted from each joint head for assessment of variation level captured within a single, more easily reproducible region. Results demonstrate that mobility-impaired individuals have significantly less Tb.Th and BV/TV and greater Tb.Sp. than the fully mobile individuals. Amount of time of impairment and impairment types were investigated with varied results, indicating an individualized response to bone loss due to impairment. Cortical bone area and geometry were quantified using BoneJ with results indicating that mobility-impaired individuals have less cortical area in all directions.

The results of this study demonstrate that the lack of biomechanical burden on mobility-impaired individual femora is reflected in their trabecular structure and cortical bone with more varied results in the humeri, depending on the impairment type. This research helps to improve our understanding of bone functional adaptation, especially as associated with disuse. Furthermore, the results of this research have implications for identifying and diagnosing mobility impairment in the forensic context for purposes of identification.

Bone; 3D Technology; Anthropology

A72 Facial Photo-Anthropometry for the Exclusion of Candidates Under Non-Restrictive Photographic Conditions

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Learning Overview: After attending this presentation, attendees will be informed about the real potential of photo-anthropometry in forensic facial comparison. This presentation shows that a sophisticated comparison method can reliably exclude a large number of candidates under realistic photographic scenarios, and even more so when augmented using artificial intelligence.

Impact Statement: This presentation will impact the forensic science community by changing the status of photo-anthropometry in the field of forensic facial comparison, turning a technique that was considered hardly applicable into a reliable and effective approach to the filtering of multiple candidates.

Photo-anthropometry is a facial image comparison technique where measurements of the face are taken from an image using facial landmarks. Both measurements and ratios between them, called "Dimensions and Proportionality Indices" (DPIs), are compared against DPIs from another facial image. Multiple studies concluded that simple photo-anthropometric facial comparison is suitable for elimination only in the unlikely circumstance that both images are acquired under the very same facial pose, expression, and camera parameters.[1,2] However, a recent study by Martos et al. demonstrated the usefulness of DPIs when using a more sophisticated approach.[3] The authors developed a series of formulas that, using the DPIs from a photo, allowed them to predict the corresponding DPIs of the 3D face of the subject of the picture. These predicted DPIs can then be compared against those of a candidate to determine compatibility. In their experimental study, the method was able to exclude 57% of the negative candidates while, critically, retaining all positive ones.

The study by Martos et al. provides proof of concept of their novel approach but suffers from important limitations. The number of subjects involved was only 30. With respect to the prediction of DPIs, the authors tested only one simple linear approach. This study is meant to address these limitations. We employed facial 3D scans from 122 subjects, from which we simulated 500 photographs each, for a total of 61,000 images. In addition to linear regression, we compared several modern regression techniques from the field of Machine Learning and Artificial Intelligence. Also, we studied the effect of several factors that were not included in the original study, such as the different photographic setup, availability of information about the image (e.g., metadata), and lack of visibility of certain landmarks due to occlusion.

Our experimental study validates the approach and measures its effectiveness in a variety of realistic scenarios. Positive cases are always retained as required, while the filtering of negative cases varies between 45% and 69%. This shows the technique is viable and leads to a substantial reduction of the number of candidates in the comparison.

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Facial Identification; Imaging; Artificial Intelligence

A73 Correlations of Facial Soft Tissue Thicknesses with Craniometric Dimensions to Improve Craniofacial Identification Estimates: Fact or Fiction?

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Learning Overview: After attending this presentation, attendees will understand that craniometric dimensions are only weakly correlated with Facial Soft Tissue Thicknesses (FSTTs) and not strongly enough for practically useful improvements in estimates of soft tissue thicknesses above arithmetic means in craniofacial identification casework.

Impact Statement: This presentation will impact the forensic science community by debunking the popularly held (but erroneous) notion that linear regression and craniometrics can be used to produce superior facial soft tissue estimates that outperform arithmetic means in casework.

Regression approaches that estimate the FSTTs from an individual's craniometric dimensions have been posited to boost the accuracy of craniofacial identification methods.[1] While alluring in principle, the method ultimately depends on strong correlations between craniometrics and FSTTs—but these have not been robustly observed or elucidated in prior research.[1,2] For example, Simpson and Henneberg's original concept paper employs approximations of craniometric values (rather than direct measurements) and uses statistical corrections in attempt to remove substantial noise from the data (and boost strength of associations).[1] Subsequently, the practical utility of a regression approach is presently unconfirmed and would benefit from a decisive test using medical imaging methods where craniometric and FSTT values can both be directly measured and assessed without statistical corrections.

This study uses Magnetic Resonance Imaging (MRI) to directly measure craniometrics and FSTT in 38 adult Australian cadavers (24 males and 14 females, aged 66 to 99 years) to examine their correlations and utility for regression. The measurement of cadavers was strategic to enable Simpson and Henneberg's protocols to be repeated in the very same heads for comparison to the MRI data.[1] Seven craniometric dimensions and 13 FSTT measurements (8 median and 5 bilateral) were taken for each individual using each method. MR measurements were taken using Osirix® v4.1.2 from T1-weighted MRIs taken at 0.8mm slice thickness, 256mm FoV, 6.2ms Repetition Time (TR), 2.3ms Echo Time (TE) with a 3T MRI (Magnetome Prisma^{fit}, Siemens). After MRI scanning, the very same heads were measured by the needle puncture method and craniometric dimensions approximated per Simpson and Henneberg's protocol whereby soft tissue over the craniometric landmarks was compressed by manual pressure exerted by spreading GPM® calipers.[1] Pearson Product Moment Correlation Coefficients (r) between FSTTs and craniometric dimensions were calculated for both approaches and standard errors of the estimates calculated. Simpson and Henneberg's linear regression models were applied to both MRI and manually acquired data.[1] Standard errors of the regression estimates were additionally compared to those obtained using data means for both this sample and the 2023 T-Table. All data analysis was performed in Statistical Program for Social Sciences (SPSS, version 28) and Microsoft® Excel® 2013.

Across both MRI and manually acquired data, craniometric dimensions exhibited weak correlations with FSTTs with r ranges from -0.41 to +0.31 (mean r across landmarks was $-0.1 < r < +0.1$). The application of Simpson and Henneberg's regression models to the MRI and manually acquired data of this study yielded higher FSTT estimation errors than those originally reported by Simpson and Henneberg's (Sest increased by a grand mean value of 3mm).[1] This study's means for MRI and manually acquired data, when used as FSTT estimators, consistently outperformed Simpson and Henneberg's linear regression models (MRI mean Sest = 3.3mm vs. regression model = 3.8mm and manual mean Sest = 3.5mm vs. regression model = 5.5mm). These findings contrast the common belief that craniometric-based regression models provide superior FSTT estimates, especially in comparison to arithmetic means. Craniometric measurements acquired by MRI were different to caliper compression methods (per Simpson and Henneberg's protocol) at statistically significant levels (Bonferroni adjusted, paired Student's t-test, $p < 0.005$).

This MRI study demonstrates that FSTTs are only weakly correlated with craniometric dimensions. The small r values observed in this study do not justify the calculation of regression models from craniometrics for FSTT estimation, not even when the craniometrics are directly measured from the MRI scans to remove any residual soft tissue components.

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Facial Reconstruction; MRI Scan; Accuracy

A74 **Facial Soft Tissue Thickness and the Positional Relationship of Hard and Soft Tissue in a United States Postmortem CT Sample, the New Mexico Decedent Image Database (NMDID)**

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Learning Overview: After attending this presentation, attendees will have learned about our newly developed semi-automated methodology for extraction of facial soft tissue depth and direction values. Using Computed Tomography (CT) medical images, we have trained algorithms to extract both facial and cranial 3D models through segmentation. Next, we have automated the placement of both cephalometric and craniometric landmarks, which results in a series of x, y, and z coordinates for each of the cephalometric and craniometric landmarks. We computed the statistical distribution of the depth and direction vector for each pair of cephalometric-craniometric landmarks. This semiautomated approach allows us to deal with unprecedented amounts of data.

Impact Statement: This presentation will impact the forensic science community by presenting research that changes the field of craniofacial identification by adding a new dimension: the study of the spatial relationship between bony and facial structures and its variability according to population, sex, Body Mass Index (BMI), and potentially other biological profile characteristics. Preliminary research results within our research group show radical improvement in the potential of positive identification using techniques such as craniofacial superimposition when incorporating this information.

In this work, we present a novel methodology developed to study the spatial relationship between cranial landmarks and their facial homologues. Most facial soft tissue depth studies have not addressed this spatial relationship between landmarks and its variability in different populations, sex, age, and BMI groups. However, prior research in the area seems to indicate that this information could potentially improve the results in the application of different craniofacial identification techniques, such as Facial Approximation and Craniofacial Superimposition.[1,2]

Our data consists of 800 anonymized Computed Tomography (CT) scans from the New Mexico Decedent Image Database, with an even distribution among male and female individuals and with an age ranging from 20 to 60 years old.[3] From the CTs, we extract two 3D models using a segmentation algorithm: one of the face using a given threshold of Hounsfield Units; another one of the skull with a higher threshold. At the same time, the algorithm developed performs a clipping of the area of interest. Afterward, a different algorithm, trained with the input of several anthropologists, automatically provides a number of cephalometric and craniometric landmarks for each model.[4] This results in a series of x, y, and z coordinates for each of the landmarks, allowing the assessment of their relationships and variation. With all the 3D models aligned using the Frankfurt plane, we computed the statistical distribution of the depth and direction vector for each pair of landmarks. Using this approach, we can observe how the thickness and positional relationship of soft tissue in relation to the underlying bone varies with different factors such as BMI, age, or sex.

This methodological approach allows us to deal with unprecedented amounts of data and can ultimately be used to develop regression models capable of estimating an individual set of 3D cephalometric landmarks for a given set of 3D craniometric ones.

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Facial Identification; Soft Tissue; 3D Technology

A75 The Latest Advancements in Craniofacial Superimposition Using Artificial Intelligence

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Learning Overview: After attending this presentation, attendees will better understand Craniofacial Superimposition (CFS), the challenges involved in its application, and the latest developments in the creation of accurate and robust automatic methods.

Impact Statement: This presentation will impact the forensic science community by detailing the design and validation of a novel skull-face overlay method that is a significant improvement over the current state of the art. This means performing CFS can be performed automatically, with increased precision and less subjectivity.

CFS is probably the most challenging skeleton-based identification method.[1,2] It requires superimposing a Postmortem (PM) skull onto facial Antemortem (AM) photographs, projecting the former onto the latter, in order to assess their anatomical consistency. Three stages can be distinguished in the CFS process: (1) acquisition and processing of the skull and facial images, together with the localization of anatomical landmarks that usually guide CFS; (2) Skull-Face Overlay (SFO), which focuses on achieving the best possible projection of the skull onto the AM facial image(s) of the subject; and (3) decision-making to determine whether the skull belongs to the person in the photograph(s) or not.

In the past decade, researchers developed several computer algorithms to carry out the skull-face overlay process automatically by leveraging advances in Computer Vision and Artificial Intelligence.[3] However, subjectivity over the position of the anatomical landmarks and lack of specific knowledge on the amount of the facial soft-tissue of the subject can lead to low-quality results. In this study, we aim to improve the SFO process by widening the information employed by the automatic method beyond the location of the landmarks. This includes a statistical model of the facial soft-tissue as well as information about the photograph, namely the focal distance, the subject-to-camera distance, and the overall facial pose. Moreover, the algorithm uses the shape and positional relationship of the chin profile, while the overall profile of the skull is matched to that of the face.

Our experimental study involved a sample of computed tomography scans of 27 subjects. From each scan, we obtained a 3D model of the skull, and we also simulated 10 frontal photographs and 10 lateral photographs, for a total of 540 images. Each photograph has been superimposed with all 27 skulls, for a grand total of 14,580 skull-face overlays. The results show a clear improvement over the current state of the art in terms of accuracy and robustness due to the additional information involved in the process.

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Facial Identification; Automation; Artificial Intelligence

A76　　Sex Estimation Methods in Forensic Anthropology: Current Practice and Trends

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Learning Overview: The goal of this presentation is to inform attendees about the current state of skeletal sex estimation in forensic anthropology, including practitioner preferences, reporting practices, and trends across time.

Impact Statement: This presentation will impact the forensic science community by reporting on the variation within the field for sex estimation from the human skeleton.

Estimation of sex is generally the first step when constructing the biological profile of an unidentified individual, primarily because many of the other biological profile parameter methods are sex-specific. However, method selection and reporting vary considerably across practitioners. An electronic research survey was distributed to forensic anthropology educators, practitioners, and students to understand more about the methods used by forensic anthropologists to estimate biological sex, as well as the factors that influence decisions regarding method selection. This survey (IRB# 2022-105) asked demographic questions about each participant's level of education and forensic anthropology casework experience and questions about sex estimation methods. A total of 150 survey responses were collected, with 119 participants completing some or all of the questions analyzed in this study.

Most respondents (93.3%) held a graduate degree in anthropology (MA, MS, or PhD), and 39.5% were American Board of Forensic Anthropology (ABFA) diplomates. Nearly all respondents preferred to combine metric and morphological methods to estimate sex. A rank system was used (1=most informative to 4=least informative) to rank skeletal regions by preference for sex estimation based on data type. For morphological methods, the pelvis was overwhelmingly preferred as the best indicator of sex (average rank 1.1), followed by the skull (2.1), long bones (2.9), and other regions (4.0). For metric methods, long bones were the most preferred skeletal region (1.7), followed by the skull (2.1), pelvis (2.2), and other regions (4.0). Participants were also provided with ~25 methods and were asked to indicate the likeliness of using those methods (1=extremely unlikely to 5=extremely likely). The most preferred sex estimation methods in order of average rating were FORDISC® (4.6), Klales et al. 2012 (4.4), MorphoPASSE (4.3), Walker 2008 (4.2), and Spradley and Jantz 2011 (4.0).[1-5] Respondents' decision-making process for selecting a particular method for estimating sex is frequently influenced by: (1) whether the method has been subjected to independent validation and error studies, (2) personal experience with the method, and (3) the type of equipment needed. When using multiple methods to estimate biological sex and the methods do not agree, most respondents indicated that they present the results from all methods utilized, but their final estimate is based upon their personal experience and general impression of the remains (37.1%) or they give preference to methods using pelvic traits or measurements (35.1%).

The results from this survey were compared to a previous survey on sex estimation conducted by Klales 2013, with surprisingly similar results.[6] FORDISC® and the three pubis traits (ventral arc, subpubic contour/concavity, and medial aspect of the ischio-pubic ramus) remained the most preferred methods/traits; however, the Klales et al. 2012 and MorphoPASSE revisions have replaced Phenice 1969 as the more commonly preferred method since the 2013 survey.[2,3,7] Since the 2013 survey, more respondents have indicated that they prefer to use *both* metric and morphological methods, rather than a preference being given to morphological methods when only one data type was used to estimate sex. Respondents also showed a high likelihood of using a method that combined data from multiple skeletal regions. The current paucity of methods that combine both metric and morphological data from multiple regions suggests opportunities for future method development.

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Sex Estimation; Practitioner Preferences; Methods

A77   **A Test of the Applicability of Decision Trees for Sex Estimation Utilizing an Expanded Suite of Morphological Traits**

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Learning Overview: This presentation will discuss the utility of decision tree models for estimating sex from an expanded suite of morphological traits of the skull. After attending this presentation, attendees will have a better understanding of the strengths and limitations of utilizing decision tree models for sex classification of the skull.

Impact Statement: This presentation will impact the forensic science community by building upon current research in forensic anthropology exploring the application of machine learning to improve sex estimation classifications for the skull.

Forensic anthropological casework incorporates morphological and metric assessment of the os coxae, skull, and long bones for sex estimation in cases of unknown individuals. Morphological sex assessment of the skull commonly utilizes the glabella, supraorbital margins, mastoid processes, nuchal crest, and mental eminence. However, morphological methods for the skull suffer from lower classification rates compared to the pelvis as well as an overreliance on the aforementioned traits. Recent studies have attempted to address these issues through the application of machine learning to produce sex classifications from morphological traits of the skull.[1] The present study builds on this research by exploring whether higher sex classification rates for the skull could be achieved utilizing decision trees and an expanded suite of traits. This research seeks to: (1) select an expanded suite of morphological traits and assess intra-observer error for each trait, (2) generate multiple decision trees, and (3) compare the classification performance of the generated trees to the performance of the tree developed by Langley and colleagues.[1]

Twenty-two morphological traits of the skull were chosen from frequently referenced sex estimation methods. A total of 215 individuals were sampled from the Hamann-Todd Osteological Collection and 147 individuals from the University of Tennessee Knoxville (UTK) Donated Skeletal Collection. Intra-observer error was assessed from 43 individuals from the UTK Collection. Statistical analyses were conducted utilizing R Statistical Software. Intra-observer error was assessed utilizing Krippendorff's Alpha to assess which traits to include when generating the decision trees. All analyses were conducted on the pooled sample (n = 362), European Americans (EA) (n = 209), and African Americans (AA) (n = 153). Each dataset was split into a training sample (80% of the data) and a testing sample (20%). The following decision trees were generated utilizing the training samples and the *party* package trees with traits from the entire skull, trees for cranial traits only, and trees for mandibular traits only.[2] The error of each tree was assessed using the testing sample. Finally, the performance of the Langley et al. tree for estimating sex from the present study's sample was tested.

Of the assessed traits, 12 achieved an acceptable level of intra-observer error. Overall, the highest performing trees identified incorporated traits from the cranium and mandible, with classification rates of 92.5% (pooled), 93.5% (EA), and 94.9% (AA). Trees that included cranial traits produced classification rates of 91.0% (pooled), 91.3% (EA), and 89.7% (AA), while trees that included mandibular traits produced the lowest rates of 79.1% (pooled), 84.8% (EA), and 84.6% (AA). When individuals from the present study were classified utilizing the Langley et al. tree, 76% of the pooled sample, 80% of the European American sample, and 70% of the African American sample were correctly sexed compared to their reported accuracy of 96%. The Langley et al. tree was built utilizing only European individuals, thus partially explaining why their tree resulted in an increased classification rate for the European American sample.

Overall, most decision trees achieved classification rates over 80%, with several over 90%. The population-specific trees often outperformed the pooled sample trees. Thus, it is recommended that future sex estimation research involving decision trees should develop and test methods for individual populations. However, trees that perform well for multiple groups would be beneficial for forensic cases in which an individual's population affinity is not estimated or unknown. Further, the comparison with the tree developed by Langley and colleagues indicates that tree classification performance decreases when applied to different skeletal samples. This research demonstrates the need for further testing of these methods and the importance of expanding beyond decision trees to more robust methods such as random forest modeling.

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Sex Estimation; Biological Profile; Machine Learning

A78   An Exploration of the Subadult Pelvic Sex Estimation Method Using Pelvic Metrics and Geometric Morphometrics

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Learning Overview: This presentation will provide attendees with a better understanding of the relationship between subadult pelvic size and shape and biological sex estimations, as well as a more complete ontogenetic analysis of skeletal growth.

Impact Statement: This presentation will impact the forensic science community by exploring two approaches to estimate the biological sex of subadult individuals. The current project will increase confidence in applying pelvic metrics and shape analyses for sex estimation in immature individuals, which substantiates previous subadult research using morphological pelvic traits.

The forensic anthropological field has posited that there should be a better understanding of the biological foundations that enable biological profile estimations of skeletal human remains. In alignment with this is a better understanding of the ontogenetic processes that influence variations in growth and subsequent trait expressions. Biological sex estimation represents a main component of the biological profile estimated by forensic anthropologists. Adult sex estimation methods using morphological and metric analyses of the pelvis consistently yield high classification accuracies. Subadult sex estimation methods have been less successful primarily because of a lack of large skeletal samples representing all of ontogeny. However, approaches can be reevaluated now that larger samples are available through virtual anthropology. Pelvic metrics have not been fully explored regarding their utility in sex estimation of subadults even though the innominate has potential at a young age because of its early age of fusion and mediation by hormones early in puberty.

A subset of 369 individuals aged between 8 and 20 years from the United States subset of the Subadult Virtual Anthropology Database (SVAD) were included in this study. The criterion of an actively fusing or completely fused ilio-ischial union at the triradiate cartilage was employed to limit the sample to individuals with complete innominates. A total of 34 pelvic landmarks were recorded for each individual; 16 landmarks were used to calculate 11 Interlandmark Distances (ILD) defined in the *Data Collection Procedures for Skeletal Material 2.0* with some minor definition modifications.[1] Seventeen landmarks were used in the geometric morphometric analyses. A General Procrustes Alignment (GPA) and Principal Components Analysis (PCA) was performed, and a Multivariate Analysis of Variance (MANOVA) was performed on all the Principal Components (PCs) as a multivariate test of significant shape differences between males and females. ANOVAs were then performed to test which specific PCs demonstrated significant sex differences. Linear Discriminant function Analyses (LDA) were used on a training sample for both the ILDs and on the PC scores to generate sex estimation models; a test set was used to evaluate the models.

The LDA using 11 ILDs was applied to a hold-out sample and correctly classified 97.14% of females and 92.73% of males for an overall classification accuracy of 94.44%. The LDA using PCs 1 through 5, which included all PCs with greater than 5% variance, correctly classified 80.00% of females and 90.57% of males. When all PCs with greater than 1% variance were included (PCs 1–22), 85.71% of females were correctly classified while 92.45% of males were correctly classified. The inclusion of centroid size in the LDA with PCs 1 through 5 improved the classification accuracies and reversed the sex bias; 91.43% of females and 90.57% of males correctly classified, resulting in an overall classification accuracy of 90.91%.

Results of this study suggest that both metric analyses and geometric morphometrics can correctly estimate sex of individuals with fusing or completely fused innominates with high accuracies. The current study relies on the biological age, or skeletal maturation of the individual, to inform the practitioner, which removes the need to estimate age prior to estimating sex. The geometric morphometric analyses highlight the importance of pelvic size as a meaningful component to accurate subadult sex estimation, which was seen with the sex bias when centroid size was not included. The success of this study expands the anthropological toolkit when faced with subadult remains and supports the use of pelvic metrics to improve biological profile estimations, including sex estimation, of subadult individuals.

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Anthropology; Sexual Dimorphism; Subadult

A79   Sex Estimation From Mandibular Morphometry Using a Discriminant Analysis–CT Scan-Based Retrospective Study

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Learning Overview: After attending this presentation, attendees will be able to appreciate the role of mandibular measurements taken from Computer Tomography (CT) scans for estimating the sex in a defined ethnic population.

Impact Statement: This presentation will impact the forensic science community because the importance of CT scan measurements of the mandible for estimating the sex in a defined ethnic population will be discussed, which may further be studied in other populations.

Partial identification of an individual can be established by criteria like age, sex, race, stature, etc. Sexual dimorphism is expressed in most human bones. However, at times only some body parts of the deceased may be available for forensic interpretation. The mandible is the largest and strongest bone of the face. Therefore, in comparison to other facial bones, it remains for a longer time and has a slower degradation rate. Sex dimorphic characteristics of the mandible have been reported from studies using either bone or CT scan images of the mandible.[1-3]

The sample size was estimated based on sample size estimation for area under the Receiver Operating Characteristic (ROC) curve. Under the alternative hypothesis, it was anticipated that the discriminatory ability of the discriminant function is 0.6 in estimating the sex with the CT measurements against the null hypothesis at 0.5 as the Area Under the Curve (AUC) value. Therefore, at a 5% level of significance and 90% power, accounting for the proportion of males and females in the sampling frame, the total sample size was estimated to be 280. The following parameters were measured: Bi-Condylar Breadth (BCB), Bi-Gonial Breadth (BGB), Bi-Antegonial Notch Breadth (BANB), Bi-Mental Foramina Breadth (BMFB), Maximum Ramus Height (MxRH), Maximum Ramus Breadth (MxRB), Maximum Mandibular Length (MML), Maximum Mandibular Body Length (MMBL), Minimum Ramus Breadth (MnRB), Coronoid HEIght (CrH), Condylar Height (CnH) using inbuilt measuring scale present in Syngo Via Software. Mandibular ANgle (MA) was measured using an inbuilt angle detector present in Syngo Via Software.

In our study cohort, we had a total of 150 (53.6%) males and 130 (46.4%) females. It was found that all the parameters except MA differed significantly between the two genders. We observed that among all the discriminant function that was built, function 2 (BGB) showed the least Wilk's lambda value ($\lambda = 0.343$), which indicated the best discriminatory ability of the model among all. MA gave the maximum Wilk's lambda value, suggesting it has the least value among all the discriminant functions for the discriminatory ability. The area under the ROC curve was plotted for each of the discriminant functions. Function 13 (combination of all parameters) had the best AUC (0.951; 95% CI = 0.920, 0.982) whereas Function 9 (MA) had the least AUC (0.498; 95% CI = 0.422, 0.573).

This CT scan-based observational study showed that all the measured mandibular parameters, except MA, were significantly able to estimate the sex of the individual with high accuracy.

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Sex Estimation; Mandibular Morphometry; Discriminant Analysis

A80   Increasing Transparency in Sex Estimation From the Skull by Monitoring Variation in Decision-Making Patterns

Nicole Mantl*, University College London, London, England; Sherry Nakhaeizadeh, Sherry Nakhaeizadeh, University College London, London, England; Ruth M. Morgan, University College London, London, England

Learning Overview: After attending this presentation, attendees will have a clear understanding of decision-making strategies and the impact of experience used in sex estimation of the skull.

Impact Statement: This presentation will impact the forensic science community by providing greater insight into decision-making strategies and how forensic anthropologists set about making inferences when estimating sex from the skull.

An increased focus on decision-making in forensic anthropology follows the growing demand for increased transparency in forensic reporting.[1,2] This has been particularly prominent in visual non-metric methods, such as sex estimation where the methods are inherently more subjective and appear to be more dependent on the experience of the observer.[3,4]

This presentation will show results from a study investigating intuitive and reflective decisions made in sex estimations from the skull. Students as well as academics and professionals participating in this study were asked to apply non-metric sex estimation to a sample of skulls.

At the initial stage of the study six archaeological crania (United Kingdom) were utilized. Participants were asked to provide an intuitive first impression based on a visual assessment of each skull under time-pressure (five seconds) followed by a full assessment scoring sexual traits on a five-point scale. Initial results showed that consistency, between first impressions and estimations drawn from method application, and confidence were lowest when the traits were most ambiguous.

Subsequently, the methodology was further developed where participants are instead asked to estimate sex from ten digitally rendered 3D skulls modelled on CT scans from a modern population from New Mexico.[5] Preliminary results show that participants are equally likely to correctly identify the sex of an unknown skull without contextual information. It is suggested that there is only a marginally significant difference between the consistency of intuitive and methodical sex estimations reported across the different experience groups. Overall, the results suggest that even when prompted to report a first impression, the more experienced participants based their intuitive estimation on overall appearance, three times more frequently than the less-experienced participants, followed by the recognition of multiple features of the skull. Furthermore, academics and/or practicing forensic anthropologists reported a moderate increase in confidence level compared to student participants.

Three key findings from these studies will be discussed: the impact of experience on decision-making; the significance of trait perception in intuitive first impressions; and the importance of characteristics affecting trait perception, such as population affinity, on the confidence levels reported by participants when it comes to self-perception of correctly estimating of sex from skulls.

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Sex Assessment; Skull; Psychological Behavior

A81   Combining Metric and Morphological Traits for Sex Estimation: A Pilot Study Using the Innominate

Kate M. Lesciotto*, University of North Texas Health Science Center, Fort Worth, TX; Alexandra R. Klales, Washburn University, Topeka, KS; Michael Price, Channel Cognition, Somewhere, AL

Learning Overview: The goal of this presentation is to inform attendees about the potential impact of combining metric and morphological data types into a single statistical model for sex estimation using pilot data from the innominate.

Impact Statement: This presentation will impact the forensic science community by presenting a new statistical model that combines metric and morphological data for sex estimation using the innominate and comparing this model to commonly used metric-only and morphological-only methods.

The estimation of osteological sex is a crucial component in the construction of the "biological profile" from a set of unidentified skeletal remains. Traditionally, sex estimation methods have focused exclusively on the use of either metric (e.g., FORDISC®, DSP2) or morphological (e.g., MorphoPASSE) data.[1-3] Surveys of forensic anthropologists have shown that the majority of practitioners use multiple sex estimation methods based on both metric and morphological data. However, there is significant divergence in how the results from different methods are combined into a final estimation of sex and expression of statistical confidence. If results of the methods do not agree, forensic anthropologists may give preference to one method over another, provide a final estimation based on personal experience, or take an average of all methods.[4]

While the long-term goal of this research is to develop a novel method for sex estimation that can accommodate metric and morphological data from multiple skeletal elements, this pilot study focuses on the integration of these data types using the innominate. The innominate is the most sexually dimorphic bone in the human skeleton; however, the most commonly used morphological features (e.g., ventral arc, subpubic concavity, etc.) are not captured by the most commonly used measurements (innominate height and breadth) and vice versa. Two experienced observers collected metric and morphological data from the innominate in a sample of 19 females and 16 males (n = 35) from the Southeast Texas Applied Forensic Science Facility Skeletal Collection. Metric data included the measurements defined by Brůžek et al. for DSP2, excluding greater sciatic notch height (IIMT).[2] Morphological data included the Ventral Arc (VA), Subpubic Concavity (SPC), and Medial aspect of the Ischiopubic Ramus (MIPR) from the MorphoPASSE program, as well as the shape of the greater sciatic notch, presence of a composite arc, pubis shape, and subpubic angle.[3,5-7]

Sex estimation was tested using four statistical models: DSP2 (using only metric data), MorphoPASSE (using only morphological data for the VA, SPC, MIPR), a Random Forest (RF) model (using all metric and morphological data), and a Feed-Forward Neural Network (FFNN) model (using all metric and morphological data). Using a decision threshold of PP > 0.95, the FFNN model had the optimal performance, combining high accuracy and low sex-bias, while remaining highly inclusive of the sample. MorphoPASSE and the RF model achieved perfect accuracy for both observers, while one female was misclassified by DSP for both observers. However, while these three models produced high accuracy levels, the implementation of a decision threshold reduced the sample from the initial full set of 35 individuals to as low as 23 individuals. Using the FFNN, Observer 1 achieved perfect accuracy for 32 individuals meeting the decision threshold (18F/14M), and Observer 2 achieved 97.2% accuracy with all 35 individuals meeting the decision threshold, with one female being misclassified as male.

While existing sex estimation methods using the innominate are highly accurate, this pilot study demonstrates the potential for achieving nearly perfect accuracy with high statistical confidence by combining metric and morphological traits using a FFNN model. This model will continue to be tested using larger samples and data collected from multiple skeletal elements with the long-term goal of creating a freely available program for sex estimation that can integrate metric and morphological data, incorporate traits from the entire skeleton, and accommodate missing data.

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Sex Estimation; Metric and Morphological Data; Neural Network Model

A82 The Impact of Chemical Maceration on Elemental Concentrations and [87]SR/[86]SR Values in Bone

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Learning Overview: After attending this presentation, attendees will be aware of how chemical maceration, the removal of soft tissue from bones, may affect elemental concentrations and strontium isotope ratios ([87]Sr/[86]Sr) in bone samples. Attendees will also learn which chemical elements are either contaminated in or removed from bone samples during maceration.

Impact Statement: This presentation will impact the forensic science community by demonstrating how chemical elements and [87]Sr/[86]Sr values are altered by maceration. Additionally, this presentation will suggest caution toward some maceration methods when [87]Sr/[86]Sr analysis may be used in both research and casework situations.

Chemical maceration is a common process to remove any remaining soft tissues from bone. This process is done both in anthropology research facilities and medical examiner labs. Previous research has evaluated chemical maceration for efficacy and preservation of DNA.[1,2] However, no previous studies have examined the preservation of elemental concentrations (used in determining postmortem alteration) or [87]Sr/[86]Sr (used in creating a geographic profile to assist with identification).

This study evaluated concentrations for major and trace elements as well as [87]Sr/[86]Sr values for five different maceration protocols. Five racks of pig ribs were obtained from a butcher. The pigs originated from a single farm and were raised and butchered in Oregon. The ribs of each rack were separated, and the bulk of soft tissues was mechanically removed. From each rack, two samples were set aside as controls and seven experimental samples were chemically macerated using modified parameters from published methods. All experimental ribs from a single rack underwent one maceration method and were compared to the controls from the same rack ensuring intra-individual comparison. A total of five maceration treatments were used: (1) Alconox® at 100°C for 24 hours, (2) Biz® laundry detergent with sodium carbonate at 90°C for four hours,[2] (3) Greased Lightning® at 100°C for 24 hours, (4) Adolph's® unseasoned meat tenderizer with Palmolive® dish soap at 90°C for 10 hours, and (5) Tergazyme® at 88°C for 24 hours.[1-4] Each reagent was dissolved in local tap water from Tempe, AZ.

Results indicate shifts in concentration for various elements. For sodium (Na), the Alconox®, Biz® with sodium carbonate, and Tergazyme® methods saw the largest increases in concentration (percent increase: 114%, 42 %, and 68 %, respectively), while the Adolph's® with Palmolive® method saw a more moderate shift (percent increase: 14%), and the Greased Lightning® method saw relatively no shift (percent increase: 2 %). These increases indicate treatments are inducing varying levels of Na contamination. For potassium (K), rubidium (Rb), and cesium (Cs), all maceration methods saw a decrease in concentrations (percent decrease in K: 70%, 42%, 75%, 43%, and 71%; Rb: 75%, 49%, 80%, 49%, and 76%; and Cs: 67%, 48%, 76%, 50%, and 71%). These decreases indicate all methods removed K, Rb, and Cs from bone. Commonly used elemental ratios such as Ca/P, Sr/Pb, and U/Ca were relatively unaltered by each maceration method.

For [87]Sr/[86]Sr, the Biz® with sodium carbonate and Adolph's® with Palmolive® methods had relatively small shifts (average shift for both methods: + 0.000006), while the Alconox®, Greased Lightning®, and Tergazyme® methods displayed shifts that are analytically significant (analytical error: 2SD for [87]Sr/[86]Sr = 0.000019) but not inferentially significant (average shifts: + 0.000032, + 0.000064, and + 0.000079, respectively). However, these average shifts are all enriched in [87]Sr compared to the control values and are trending toward the [87]Sr/[86]Sr value of the local tap water. Although the [87]Sr/[86]Sr values were shifted for some maceration methods, the Sr concentrations remained relatively consistent across all treated samples (largest average percent change in concentration was a decrease of 5% for the Tergazyme® method). Hence, the most likely factor in [87]Sr/[86]Sr value alteration is isotopic exchange with the maceration solution.

The elemental concentration data demonstrates that some elements are altered and should not be analyzed in macerated bone while the isotopic data suggest [87]Sr/[86]Sr analysis is acceptable for bones macerated within the parameters studied. However, variations of maceration methods should be evaluated independently since systematic shifts, although not inferentially significant, were measured.

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Bone Maceration; Elemental Concentration; Isotopes

A83   The Use of Multi-Isotopes for Geoprofiling Unidentified Decedents to Assist Law Enforcement Agencies in the United States

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Learning Overview: After attending this presentation, attendees will better understand how multi-isotopic analyses can improve human identification efforts. Isotope analysis of human teeth or skeletal material can illuminate the birth location, early childhood residence, and migration patterns based on specific elements embedded in the skeletal tissue of the individual.

Impact Statement: This presentation will impact the forensic science community by providing multi-isotopic reference data from a collection of skeletal materials with known origin.

Anthropologists continue to play a prominent role in the process of human identification, particularly in decomposing and skeletonized cases, as the parameters they provide about age, sex, and ancestry may provide investigative leads for unidentified decedent cases.[1] Forensic anthropologists have recently collaborated with geochemists to perform isotopic analyses of human tissues to estimate the geographic birthplace or migration patterns of unidentified decedents.[2-7]

Coupled with high domestic and international migration into Florida, the comparison of local missing persons and local unidentified persons cases is often insufficient for positive identifications.[8] Consequently, academic collaborations among the University of South Florida (USF) -Florida Institute of Forensic Anthropology and Applied Science (IFAAS), USF-Stable Isotope Lab, and the University of Florida Geological Sciences (UFGS) have utilized specific radiogenic isotopes that include strontium ($^{87/86}$Sr) and lead isotopes ($^{206/204}$Pb, $^{207/204}$Pb, $^{208/204}$Pb), along with stable isotopes oxygen (δ^{18}O) and carbon (δ^{13}C) for current and cold casework in the Tampa Bay area.

One of the major aims of these collaborations is to generate multi-isotopic data on modern human skeletal material with known origin to improve isotopic reference data.[9] To do this, donated teeth were collected from a local dentist (Dr. Amy Rotham, DDS) clinic in Tampa, FL, and by Dr. Caitlyn Menicucci DMD, (CPT, United States Army) from volunteers. Along with the donated teeth, very basic demographic information was collected from participants, and the identity of the participants was kept anonymous to the researchers. Stable isotopes (δ^{18}O, δ^{13}C) and radiogenic isotopes ($^{87/86}$Sr, $^{206/204}$Pb, $^{207/204}$Pb, $^{208/204}$Pb) were analyzed.

Results for enamel and/or tooth root (n=81) from donated teeth from individuals born in Florida and/or who lived in Florida through their early childhood, along with other individuals born in the United States and Central and South America as comparison samples. Individuals from Florida exhibit an average strontium isotope ratio of 0.70894 ± 0.00205. Lead isotope values fall into the expected United States ratios: $^{206/204}$Pb ~ (18.4 to 19.1), $^{207/204}$Pb ~ (15.6 to 15.7), and $^{208/204}$ Pb ~ (38.1 to 38.8). Oxygen isotope (δ^{18}O) values are more positive compared to other regions of the United States. The δ^{13}C values measured consistently indicate a typical diet in the United States. However, there were carbon values for individuals that were more positive than expected, possibly reflecting cultural practices and dietary choices. Overall, this data will be utilized to establish an isotopic reference data set for Florida and assist with local investigations of unidentified decedent cases. Additional research in this region and other geographic regions of the United States is critical for successful georeferencing of unidentified decedents and to improve the solvability of cold cases.

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Isotopes; Geoprofiling; Migration

A84   Origins and Isoscapes: A Reassessment of Oxygen and Strontium Isotopic Data for Human Identifications in North America

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Learning Overview: After attending this presentation, attendees will have an improved understanding of how the current oxygen and strontium isotope data available for geolocation and provenancing of unidentified persons in North America can be used, along with understanding areas of these isotopic studies that needs improvement.

Impact Statement: This presentation will impact the forensic science community by using data compiled from known and assumed origin human tissues throughout North America, reviewing how the data have been collected and types of data, analyzing spatial variation based on multiple variables, and offering suggestions for improvements for future isotopic research and applications to forensic identification efforts.

Isotopic geolocation methods rely on reference data documenting spatial isotopic variation in human tissues. Many studies report such data, and the use of isotopes for forensic applications and identification is becoming more frequent. The value and use of these data depend strongly on several factors such standardization of metadata, variation in analytical protocols, and the geographic coverage of data from any given tissue and isotope system.

After a large-scale review of oxygen and strontium isotopic literature on human tissues, a dataset of >3,000 isotopic data points were compiled from 25 studies. The broadest coverage in North America is for keratin. Tooth enamel isotopic values have the second broadest coverage, with isotopic values from 12 studies comprising >800 data points. We focus here on tooth enamel carbonate $\delta^{18}O$ data due to its excellent preservation and frequent use in human identification.

We compared the tooth $\delta^{18}O$ with isotopic maps (isoscapes) of precipitation $\delta^{18}O$ values, which are expected to provide a first-order estimate of the geographic pattern in tooth isotope values driven by variation in drinking water isotope values. Although there was a strong relationship ($R^2 = 0.4$), a substantial amount of variance in the tooth dataset was not explained by the water isoscape (residual standard error = 1.7‰). We investigate three sources of potential variability in the compiled data that may contribute to this residual variance. (1) While all enamel samples are reported as being of "known" origin, some studies have incomplete or unspecific data on residential life history or which teeth were sampled, which may compromise comparisons with the isoscape. (2) Each study reporting oxygen isotopic values used different sample pretreatment protocols and reaction times, which have been shown to affect resulting isotopic values.[1] (3) A variety of tooth types, representing different ages of mineralization, were analyzed.

We found that the variance of residuals for samples with and without high-quality life history information was similar, suggesting that incomplete information did not significantly degrade the comparison with the precipitation isoscapes. For the other two factors, we found substantial bias between studies using different lab methods and between teeth formed at different ages, suggesting that lack of standardization added noise to the comparison.

We further investigated these factors using the Quality Analysis (QA) tool in the assignR package to evaluate the expected improvement in the precision of isotope-based geographic assignments if we corrected for the mean residuals. For both factors (lab methodology and tooth mineralization age), we found that correcting for the regression offsets gave a 20% improvement in geographic specificity when assignments were conducted using confidence thresholds up to ~80%.

The gestalt of this dataset fits the expected patterns and expectations. As noted, several issues that were considered limitations have proven to not be problematic, such as assuming data origin. However, other issues confound or degrade the quality of the dataset, such as the use of different pretreatment protocols (affecting oxygen tooth enamel values specifically), analytical methods, and calibrations. The currently available data can still be used to generate models but requires certain variables and analytical aspects be taken into consideration. A large-scale collection of teeth from donors of known origin with controlled methods would benefit forensic anthropology and human identification efforts.

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Anthropology; Stable Isotope Analysis; Identification

A85 Isotopic Taphonomy: A Critical Review of the Effects of Diagenesis on Human Tissues Used for Stable Isotope Analysis

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Learning Overview: This presentation will provide a critical review of the taphonomic literature relating to the application of stable isotope analysis of human scalp hair and nail keratin for reconstructing the diet and provenance history of unidentified decedents. After attending this presentation, attendees will better understand the importance of taphonomic processes in altering stable isotope ratios of scalp hair and nail keratin, which is critical to proper interpretation of geolocation and diet from unidentified remains cases.

Impact Statement: This presentation will impact the forensic science community by providing a critical review of the literature surrounding the effects of taphonomic processes on scalp hair and nail keratin ratios. Further, the value of isotopic ratios present in scalp hair and nails in forensic investigations will be demonstrated through case examples.

The study of the influence of taphonomic processes on stable isotope ratios of unidentified individuals has become increasingly utilized in forensic investigations, as they affect whether accurate information can be provided about an individual's life history. Isotopic ratios reflect aspects of an individual's life such as diet and geolocation information. This analysis is especially pertinent to forensic investigations of unidentified individuals because it has the potential to narrow the pool of matching identifications as well as provide new leads where other methods have proven ineffective. Human scalp hair and nails provide snapshots in time that document the months leading up to an individual's death. Hair grows at an average rate of about 1cm per month and fingernails about 2-3mm per month, providing the potential for sequential sampling and the isolation of dietary and geolocational information from specific months prior to an individual's death.[1] This presentation will examine the literature documenting how the postmortem environment may influence reliable applications of stable isotope analyses of human scalp-hair and nail keratin.

Many studies have examined the interchangeability of isotopic ratios in human scalp-hair and nails, the provenancing of unidentified remains based on isotopic ratios in hair samples, the documentation of diet and geolocation in the isotopic composition of nails, the general reliability of isotopic ratios present in hair and nail samples, as well as many others to better understand the application of isotopic analyses to forensic investigations.[2,3] Many of these studies utilize samples taken from living individuals or recently deceased individuals, where isotopic ratios are expected to accurately represent aspects of the individual's life. However, there have been few studies that examine the effects of the taphonomic process on isotopic ratios of human hair and nails.

Saul's 2017 novel research examined the isotopic ratios of human hair from donated individuals at the Anthropological Research Facility (ARF) at the University of Tennessee, Knoxville, and the Forensic Anthropology Research Facility (FARF) at Texas State University.[4] Samples in this study were collected from individuals placed on the surface and placed in shallow burials at both facilities at various stages throughout the taphonomic process over a period of one year.[4] By examining buried and surface-placed individuals, Saul was able to examine two different placement scenarios within the same local environments.[4] The results of isotopic analysis showed that isotopic values in these environments retain accurate enough information to be considered reliable predictors of dietary and geolocation information.[4] Gordon et al. and Saul et al. further examined the preservation of hair samples during packaging in forensic contexts as well as summarized several considerations when examining the isotopic ratios of hair samples in the Tennessee postmortem environment.[5,6] This information is incredibly valuable to forensic investigations within Tennessee and Texas environments but is not representative of the taphonomic histories of other regions like high elevation and mountainous regions.

In forensic cases where individuals are not able to be identified, whether due to advanced decomposition, mutilation, or other taphonomic influences, isotopic analyses of scalp hair and nails have proven useful to provide information about geolocation and dietary information that can potentially narrow the focus of investigations.[7] Future applications of stable isotope analysis in forensic contexts should examine more geographic regions with more variable environments. The better we understand how the environment impacts isotopic values present in human scalp hair and nail tissues, the more applicable these results are to forensic contexts and investigations. Further, studies should also be held over a longer time period to continue to verify the reliability of the isotopic information provided by scalp hair and nail samples as the taphonomic and decomposition processes ensue.

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Isotopic Taphonomy; Hair and Nails; Stable Isotope Analysis

A86   The Impacts of Chronic Opioid Exposure on Bone Biomechanical Parameters

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Learning Overview: The goal of this presentation is to describe the impacts of chronic opioid exposure on bone fracture risk and patterns. Attendees will learn how Synchrotron Radiation-based micro-Computed Tomography (SRμCT) and biomechanical bend testing techniques allow for a novel multi-modal examination of the effects of opioid exposure on bone quality, cross-sectional geometric parameters, and fracture type.

Impact Statement: This presentation will impact the forensic science community by: (1) further elucidating the indirect effects of opioid use on the products of bone remodeling (e.g., resorption spaces, Haversian canals, secondary osteons) used in histological age-at-death estimation methods; and (2) describing the associated impacts on bone fracture risk, interpretation, and patterns relevant to trauma interpretation.

Prolonged opioid exposure has been demonstrated to disrupt bone remodeling by inhibiting osteoblast activity and decreasing trabecular bone connectivity, thus inevitably predisposing individuals to osteoporosis and increased bone fracture risk.[1-4]

Our study builds upon proof-of-principle data from a pre-clinical rabbit-opioid model system to explore the: (1) biomechanical impact of opioid exposure on bone fracture risk, (2) relationship between cross-sectional geometric values and fracture patterns, and (3) relevant microstructural parameters (e.g., cortical porosity and osteocyte lacunar density) that inform bone fracture risk as observed via high-resolution SRμCT. We hypothesized that: (1) a decrease in bone's cortical area and lacunar density, an increase in cortical porosity, and enlarged medullary cross-sectional area will be observed in opioid animals compared to controls, and (2) fracture risk will be increased in opioid animals, and less force will be required for bone failure.

New Zealand White (NZW) rabbits were selected for our work given their shortened remodeling period and recommendations from the Food and Drug Administration for bone research.[5-8] Male rabbits were divided into three groups of seven animals each: morphine sulphate, fentanyl, and controls for eight weeks of experimental treatment.[5]

High-resolution bone imaging data of left tibiae were collected using SRμCT at the Canadian Light Source Synchrotron Facility in Saskatoon, SK. Reconstructed images (1.5μm resolution) were segmented using a novel Deep Learning Model created in Dragonfly v.2020.2. Microstructural parameters of interest (e.g., pore morphometric variables including pore density, volume, connectivity, etc.) were extracted using a custom model in CTAnalyser v1.18.8.0.

Three-point bend tests were performed on the right tibiae from the same animals using an Instron® E3000 at a strain rate of 1mm/min until failure. Structural properties (e.g., flexural modulus and flexural strength) were collected from normalized biomechanical data and correlated with bone architectural properties (e.g., cortical area, orientation, moments of inertia, etc.) to associate structure with function.

Preliminary vascular pore morphometric data revealed that morphine animals have significantly greater (* = P < 0.05) porosity compared to the fentanyl group (morphine: 0.00004318 objects/voxel > fentanyl: 0.00001806 objects/voxel). Morphine animal pores are larger in size compared to the fentanyl group (Morphine: 16.67 pixels > Fentanyl: 11.16 pixels).

Preliminary biomechanical data demonstrated that all mean load (kN), displacement (mm), and test time (s) were significantly different between groups (**** = P < 0.0001). The morphine group demonstrated the greatest mean load (Fentanyl: 0.1105 kN < Control: 0.1193 kN < Morphine: 0.1233 kN). The fentanyl group demonstrated the greatest displacement (Morphine: 4.897mm < Control: 4.984 mm < Fentanyl: 5.89mm) and longest test duration (Control: 227s < Morphine: 227.4s < Fentanyl: 277.1s). Tissue distribution may help explain these findings, as distribution further form the neutral axis can increase strength and resistance to bending forces.[9] Additionally, opioids may have a dehydrating effect that increase sample stiffness, tensile strength, and hardness.[10-12]

The proposed work is culturally relevant as the misuse and addiction to prescription opioids (and illicit synthetic opioids) continues to be a serious public health crisis nationwide. Our work offers insight relating to: (1) the long-term impacts of opioid exposure on the products of bone remodeling used in histological age-at-death estimation, and (2) fracture pattern interpretation following drug exposure. Findings from our work further offer support to forensic practitioners by expanding understandings of bone biological parameters relevant to creating more precise biological profiles, especially in the case of age-at-death estimation.

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Bone Biomechanics; Fracture Patterns; Synchrotron Radiation-Based Micro-Computed Tomography

A87   **The Right to the Truth: A Multimodal Investigation of Mass Graves and an Analysis of Commingled Remains for Conflict Resolution in Humanitarian Forensics**

Xenia Paula Kyriakou*, University of West London, Fort Myers, FL; Nicholas V. Passalacqua, Western Carolina University, Sylva, NC; Matthieu Baudelet, University of Central Florida, Orlando, FL

Learning Overview: This presentation will review the current state of scientific and professional aspects in the investigation of mass graves and the analysis of commingled remains, and it will introduce the new multidisciplinary research consortium, CONFORM. During this presentation, professions, policies, processes, and technologies utilized in the investigation of mass graves and de-commingling will be highlighted; as well as the need for the operationalization of standards to ensure method efficiency, accuracy, and reproducibility as well as best practices for quality assurance.

Impact Statement: This presentation will impact the forensic scientific community by providing a review of the current methodological approaches to the investigation of mass graves and analysis of commingled remains. Attendees will learn about the different methodological approaches in locating and excavating mass graves, resolve commingling, and will be provided with a framework for future scientific developments that could enhance and support these processes.

Humanitarian forensics plays a central role in conflict resolution and peacebuilding. Forensic archaeology and forensic anthropology are key disciplines that support community transitions in post-conflict territories. Mass graves are a global phenomenon of humanitarian significance. They are characterized by diversity in the way they have been created and modified by human and environmental agents.[1] Commingled mass graves pose the highest level of challenge for archaeologists and anthropologists alike because the causes of commingling vary from one mass grave to another, whether primary, secondary, or tertiary burial contexts.[2] Therefore, from discovery to repatriation, archaeological and anthropological methods used in the investigation of mass graves differ depending on the context of their application and guiding mandates. Yet, the relationship between field processes and methods of anthropological analysis for de-commingling and identification is significant in ensuring efficiency in pair-matching, skeletal re-association, and the reconstruction of the Minimum Number of Individuals (MNI).

The aim of the symposium is to present original research and case studies pertaining to the search, recovery, and excavation of mass graves, and forensic methods for the analysis and evaluation of commingled, disarticulated, and fragmentary human remains. The symposium encourages the presentation of work that showcases multidisciplinary approaches, whether field-based, lab-based, or combined. The symposium presentations will discuss advantages/prospects, disadvantages/limitations, problems/solution, and most importantly highlight the role of forensic science in accounting for missing persons in a way that contributes to conflict resolution and peacebuilding.

The symposium presenters will also provide recommendations and suggestions stemming from their work experience as it pertains to the investigation of mass graves and processes of de-commingling. The objective of the symposium is to identify grounds for international and interdisciplinary research collaboration for the development of a standardized and operationalized set of guidelines for the investigation of mass graves that adhere to best practices, streamline the processes involved, but also provide flexibility to allow adaptation for different transitional justice mandates.

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Humanitarian Forensics; Mass Graves; Commingled Remains

A88   The Use of Computational Methods in Assessing Legacy Databases of Commingled Remains and Reconstructing Stratigraphic Relationships

Chris Mavromatis*, Dickson City, PA; Xenia Paula Kyriakou, University of West London, Fort Myers, FL

Learning Overview: This presentation will utilize archaeological stratigraphy, artifact, and human skeletal assemblages from a commingled context to demonstrate how these dataset types can provide valuable contextual information for reconstructing archaeological relationships with the use of computational methods. After attending this presentation, attendees will better grasp how engaging with stratigraphic, artifact, and human bone datasets can contribute to, and augment, their existing approaches to analyzing multiple commingled interments, using Python® and R-suite statistical applications.

Impact Statement: This presentation will impact the forensic anthropology community by showcasing how to create a flexible framework for extracting data from legacy and contemporary stratigraphic and assemblage datasets, using computational methods, to help contextualize commingled human remains recovered from forensic archaeological contexts. Attendees will gain insights into the project's techniques for cleaning datasets with the Python® programing language, combining stratigraphic layers into aggregated units, and analyzing these aggregated units and their assemblages with the Python® and R programming languages.

Commingled contexts present with complex stratigraphy must be excavated systematically and in detail. Archaeological data collected during excavation are by default legacy datasets in the sense that field recording methods, although universal in concept, their application varies significantly between agencies, companies, or country systems, but also between terrains, landscapes, and environments This is especially the case for the excavation of commingled contexts that may combine highly fragmentary skeletal remains and intermixing of bone with other material (e.g., artifacts, animal bones).

Field records are a valuable tool for reconstructing context, artifacts, and skeletal associations. Most archaeologists would agree that analyzing legacy archaeological datasets can provide new insights.[1,2] However, the evaluation and reanalysis of stratigraphic data for reconstructing stratigraphic sequences in relation to biological material (e.g., human remains) and artifacts can be problematic, and it presents various technical and methodological problems. The use of advanced computational approaches can be a useful tool in the assessment of legacy databases comprising of complex stratigraphic relationships, such as those encountered in commingled contexts.

Field records collected during the excavation of cist graves found at the archaeological cemetery of Kourion Gate Amathus (KAGC), in Cyprus, combined with an osteological inventory of 16,000 skeletal fragments, were evaluated using the Python® and R programming languages. The objective of the study was to establish new deposit profiles using a data-driven approach to consolidate recording discrepancies and assess the relationship between stratigraphy, artifacts, and skeletal remains. Data remediation (i.e., cleaning the project's datasets) was a critical first step in this process. The data-driven component relied heavily on the methods of the Python® class we developed specifically for stratigraphic analysis. A class provides the framework for defining and organizing an object's attributes and methods.[3] Objects in Python® (and other similar programming languages) provide a concise way to model complex systems by combining attributes (i.e., descriptive information) and methods (i.e., behaviors and actions) with data. Because each class instance contains the same methods and attributes, it provides a consistent way to compare multiple stratigraphic units and their assemblage profiles quickly. The combination of data-driven and descriptive information provided rich contextual information that helped explain the stratigraphic record and evaluate relationships between human skeletal remains and artifacts.

The analysis indicated that most cist tomb deposits exhibited a disturbance profile resulting from human and environmental agents, consistent with multiple uses of the grave over time, post-cemetery disturbance from salvage and reclamation activities, and hydroturbation and root action. Additionally, except for tombs that only contained Depositional Unit B501, the distribution of bone preservation suggested that higher levels consisted mainly of small fragments resulting from context intermixing over time, consistent with tertiary burial conditions. Despite in-depth analysis, artifacts could not be securely matched with skeletal remains.

In conclusion, the combination of Python® and R programming languages has shown great promise in cleaning, sorting, organizing, classifying, quantifying, and modeling excavation data statistically, as well as visually using Geographic Information Systems (GIS). Python® allowed for combining different sets of data quickly and when paired with R programming language, it aided in the efficient evaluation of complex statistical relationships. Python's® computational capabilities have remarkable potential for streamlining data in forensic archaeology and therefore can be beneficial in a range of ways during post-excavation assessment of field records.

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Python®; Mass Graves; Commingled Remains

A89   A Case from "EL TUBO": An Analysis of Commingled and Highly Fragmented Skeletal Remains Recovered From an Abandoned Water Exploration Well in Northeastern Mexico

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Learning Overview: After attending this presentation, attendees will have an understanding of two new protocols for inventorying and describing commingled human remains and appreciate both the advantages and the limitations of using these standardized procedures when dealing with commingled human remains in forensic contexts.

Impact Statement: This presentation will impact the forensic science community by showing that a systematic and standardized approach to recording commingled human remains provides a wealth of information to the analysis. Furthermore, using such an approach results in additional statistical data for use in anthropological reports that allows for comparative studies and for the recording of skeletal completeness in a standardized and objective manner.

The complexities associated with recording and analyzing commingled remains have been particularly highlighted in the forensic field following the investigations of mass graves and investigations of human rights violations. Mass graves in the former Yugoslavia found in secondary contexts, commingled cases in Chile, and in the Republic of Kiribati (Tarawa Project), etc., are just a few examples of complex cases of commingling.[1-4] While DNA is often cited as the most reliable method for resolving commingling, for challenging cases where preservation is poor and identification hypotheses are limited (i.e., open context assemblages), its use is not fail-safe, and primarily anthropological techniques are being recommended.[3,5]

In light of this premise, this presentation shares findings from a case in Nuevo Leon, Mexico, known as "EL TUBO." The human remains from El TUBO were recovered in a highly commingled and fragmented state from an abandoned water well, over several months. This presentation describes the process from the recovery of the remains to the anthropological-anthropometric analysis including the assessment of relationship between disarticulated and commingled body parts. The implementation of a standardized protocol for the inventory of the fragmented commingled material provided a systematic and time-efficient recording process. This resulted in the inventory of a total of 23,828 bones and bone fragments, from which it was possible to individualize 17 distinct individuals. Not surprisingly, the state of preservation influenced the success rate of the DNA analysis and the attribution of bones to specific individuals. However, the detailed recording and analysis resulted in the positive human identification of 13 of the 17 individuals. Furthermore, the application of this methodology has allowed for the recording of a quantitative description of the individualized human remains in a standardized and objective manner based on well-established formulas in physical anthropology to calculate the percentage completeness, bone representation index, and fragmentation analysis for each set of remains.

The complexity of commingled cases is challenging, but nonetheless, the management of such collections needs to be discussed and standardized given the difficulties they present. In summary, this case study is an attempt to outline and present a specific standardized methodology within the framework of forensic protocols for the anthropological management of commingled remains.

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Forensic Anthropology; Commingling; MNI

A90   The Reassociation of Disparate Elements at a Human Decomposition Facility

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Learning Overview: After attending this presentation, attendees will have a better understanding of the difficulties of reassociating disparate skeletal elements from a human decomposition facility. The various organizational systems undertaken to manage this process will be reviewed.

Impact Statement: This presentation will impact the forensic science community by demonstrating the real-world impacts of skeletal commingling, pair matching, and inventories from observing how an organizational system was developed for reassociation at a human decomposition facility.

The Forensic Osteology Research Station (FOREST) and associated Western Carolina Human Identification Laboratory (WCHIL) at Western Carolina University in Cullowhee, NC, are home to an active willed body donation program that creates the foundation for the John A. Williams Human Skeletal (JAW) Collection. Since FOREST's opening in 2008, most donors have been placed on the surface to decompose naturally; this allows for scavengers, mainly turkey vultures (*Cathartes aura*), black vultures (*Coragyps atratus*), and opossums (*Didelphis virginiana*), to interact with the donor remains.[1] Prior to PVC cages being regularly used in the summer of 2023, donors were either left uncovered or were covered with black plastic tarping at various points throughout their decomposition process at FOREST. This led to skeletal elements being scattered throughout the facility and not always immediately reassociated with the correct donor, creating commingling situations. Elements that could not be reassociated out at FOREST had been brought into WCHIL. Often, though not always, notes were taken as to where these disparate elements were located at FOREST, what donors they were found by, and what date they were located, as well as any other pertinent information; the elements were not always processed of any remaining dirt or tissue.

During the fall 2021 semester, a concerted effort was undertaken to begin systematically inventorying and organizing the disparate elements. At this point, there were at least three boxes with notes of varying quality on sheets of paper identifying the elements within each box and which bags they were in; it was discovered that these notes were not often accurate. Senior undergraduate students had been assigned that semester to create a digital inventory in Excel® of what bones were present. Following completion of the master inventory sheet, students worked to reassociate elements with their donor. As many bags of elements lacked appropriate notes, this effort was not overly successful; less than 20 disparate elements were reassociated that semester.

For the following year, this method continued, though with more advanced students who had stronger osteology skills, making them more adept at identifying pair matches and recognizing the degrees of human variation within the JAW Collection. It became apparent that the lack of consistent skeletal inventories was also an issue for this project, so students were directed to create skeletal inventories whenever they encountered a donor without one. This step was critical to realizing what was present or absent for each donor and often revealed other problems with the collection, such as the commingling of elements that was not caught in the initial curatorial process. The presenting author overhauled the master inventory sheet and overall collection of the disparate elements in December 2022. Elements were inventoried by bodily region, with boxes being numbered and bags within those boxes being labeled to match these regions. Notes were also added to the sheet that included sex and age indicators, as well as which donors could be missing these elements based on when they were found. Additionally, sex and age for donors within the JAW Collection were added to the master inventory sheet so students working on this project could use that contextual information to better pair the elements to the appropriate donor. With this new organizational system, the authors were able to reassociate at least 55 elements in just three and a half months. This mode of reassociation will be augmented by the addition of a searchable database for all donor inventories, while the use of PVC cages at FOREST has thus far limited the amount of scavenging and commingling of donor remains from occurring.

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Commingling; Skeletal Remains; Bone

A91   De-Commingling the Past—When an Attempt to Resolve Misidentifications Results in Setbacks: The Tihovici Case

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Learning Overview: The goal of this presentation is to outline an ongoing attempt to resolve individual presumptive misidentifications of human remains from one commingled mass grave, exhumed in 1996 following the 1992–1995 Bosnian war. This case study will show how coordination and due diligence should never be discounted in governmental intra-institutional efforts to address complex, non-routine cases such as decades-old misidentifications.

Impact Statement: This presentation will impact the forensic science community by providing a vivid narrative of tackling presumptive misidentifications as a complex project with intertwined actors and stakeholders and by offering a lesson on ethics: on unintended, avoidable, and serious consequences.

Following the 1992–1995 Bosnian war, the mass post-war identification process in Bosnia and Herzegovina (BiH) has had two phases: the presumptive identification phase of around 8,000 missing, starting even during the war with war-time exchanges, and the DNA-based identification phase, starting in 2001, when the International Commission on Missing Persons (ICMP) established a human identification laboratory in Sarajevo, tasked with high-throughput matching of postmortem samples from skeletal remains with blood reference samples of surviving relatives.[1,2]

A significant number of human remains that were presumptively identified originated from mass grave and mass deposition contexts, such as caves and pits; however, there were cases of human remains from the same contexts which remained unidentified, even after the advent of DNA analysis, indicating that their existence was a consequence of previous non-systematic presumptive misidentifications.[3] Subsequent DNA analysis of a limited number of presumptive identifications indicated an error rate of 30-38%, but for specific commingled contexts that were excavated by inexpert personnel, and which saw no adherence to scientific standards of forensic archaeology and anthropology, the error rate may be much higher.[4]

Misidentifications can impact at least two, if not more families: if the previous presumptive misidentification is not uncovered with DNA testing, then the family of the victim buried under the wrong name will never find their missing family member. On the other hand, addressing a potential misidentification can have serious negative consequences for the family that already had closure —psychological and socioeconomic—on the matter decades ago.

We will outline a case study of a mass grave exhumed in 1996 in Tihovici, near Sarajevo, from which all but two sets of human remains were identified presumptively and repatriated to their families. Decades later, DNA analysis of the remaining unidentified human remains and a targeted blood reference sample collection project exposed a current error rate of 100% for the previous presumptive identifications from this mass grave and triggered a domino effect of reexhumations. We will discuss the right and wrong ways to approach such a situation through the lenses of ethics, rule-of-law mechanisms, and psychosocial impact.

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Identification; Mass Graves; DNA

A92 Matching Antimeres: Prospects and Limitations of Statistical Methods for the Reassociation of Commingled Left and Right Humeri

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Learning Overview: This presentation will provide support for the use of statistical methods when reassociating commingled remains. After attending this presentation, attendees will better understand the prospects of employing statistical analysis in conjunction with visual techniques valid for sorting commingled remains.

Impact Statement: This presentation will impact the forensic science community by informing attendees that traditional methods of reassociation related to commingled remains rely on subjective analyses, such as visual matching of traits (for example, texture, coloration, and density).[1] The addition of statistical metrics in this process provides a framework for the quantification of pair-matching and, thus, a more objective approach that can add validity to pairing determinations.

For this study, the sample included humerus measurements from 119 individuals of Greek-Cypriot ancestry from the Cyprus Research Reference Collection (CRCC) (1975–2015). These measurements were used to evaluate the use of two statistical techniques to pair antimeres: m-statistic and linear regression. Recent research has shown the benefits of discriminant function analysis in pairing antimeres and of linear regression in pairing bones in the lower and upper limbs.[1-3] Additional research has suggested the use of m-statistics to aid in the exclusion of non-matching pairs.[4]

M-statistics compare the difference between two measurements by creating a proportion of the average value. M-statistics were calculated for all individuals in the sample and the distributions for each measurement location were found to be non-normal for males, females, and the total sample. Given this result, previous metrics were not optimal for use as an exclusionary method.[4] Therefore, a log transformation of the data was conducted, and one-tail 95% confidence intervals were created using a modified Cox method.[5] This method did not achieve the desired results, with 167 of the 899 total measurements (18.59%) falling outside the confidence interval. For females, 73 of 467 measurements fell outside the confidence interval (15.63%), and for males 81 of 432 measurements fell outside the confidence interval (18.75%). However, counting the number of matched measurement pairs that are excluded per individual, only 8 of 115 (6.96%) had five or more measurement pairs excluded. For females, only 4 of 60 (6.67%) had five or more measurement pairs excluded. For males, only 3 of 55 (5.45%) had five or more measurement pairs excluded.

Two linear regression equations were developed for each measurement location. The first equation was a simple linear regression predicting the left-side measurement from the right-side measurement. For the second equation, sex was added as a potential predictor and was found to be a significant predictor for half of the measurement locations. The resulting linear regression equations showed strong coefficients of determination (r^2 = 0.583–0.910) and low residual standard error (RSE = 0.787–6.528). All analyses were evaluated using R Studio 4.2.1.

The results indicated that linear regression outperformed m-statistic in this case, as it provided excellent predictions with high levels of accuracy. In comparison, m-statistics do not provide as much predictive power, as by definition, they are measures of exclusion, rather than inclusion. Still, m-statistics may add some benefit in aggregate, as approximately 95% of individuals had fewer than half of the measurement locations excluded based on 95% confidence intervals. Therefore, evaluating m-statistics for the entire bone, rather than per measurement location, may prove useful as a method of exclusion. In sum, statistical techniques, especially linear regression, will provide added benefit to researchers already using visual or other techniques when reassociating commingled remains.

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Statistical Analysis; Commingled Remains; Humerus

A93   The Chemical Reassociation of Commingled Remains Via Laser-Induced Breakdown Spectroscopy

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Learning Overview: This presentation describes the utility of Laser-Induced Breakdown Spectroscopy (LIBS) in the sorting of commingled remains. After attending this presentation, attendees will understand how chemical profiles obtained via LIBS provide valuable information for differentiating individuals'   remains.

Impact Statement: This presentation will impact the forensic science community by explaining the feasibility of reassociating skeletal assemblages based on chemical information. This study shows how chemical analysis may supplement existing physical methods for sorting commingled remains and thus promotes interdisciplinary collaboration.

A major challenge in forensic anthropology is the development of efficient and effective methodologies for sorting skeletal remains in mixed assemblages.[1] Reassociating bones to their respective individual using physical and morphological traits is a tedious process, especially if the bones are fragmented or have undergone taphonomic changes. Examining the chemical composition of bone may provide valuable information to support the sorting process. Several studies have shown that chemical variation exists between individuals' bones as a result of various factors, such as diet, health, and living environment.[2,3] This study explores how the chemical profile of bones may be incorporated into a method for classifying commingled remains. The chemical information from bones is acquired using LIBS. LIBS is a visually non-destructive analytical technique used to obtain chemical information from the surface of a sample in seconds. Furthermore, the commercial availability of field-deployable, portable LIBS instruments contributes to efficient analysis of skeletal samples in the field.

In this study, LIBS profiles were collected from 1,284 bones across 45 individuals from the John A. Williams documented skeletal collection. In total, over 8,000 chemical signatures from the skeletal remains were acquired for statistical testing. A newly developed feature selection algorithm based on principal component analysis was combined with machine learning models such as linear discriminant analysis. The approach revealed which chemical elements varied most between individuals and classified bones to their corresponding individuals with an accuracy ranging from 85% to 100%. These findings demonstrate how chemical information of bone may be key for expediting reassociation of large skeletal assemblages recovered in a forensic context. Ultimately, this research study provides evidence that LIBS is a promising technique to complement the reassociation methods currently available to forensic anthropologists.

Note: This project is supported by NIJ Award #15PNIJ-21-GG-04151-SLFO, "Pre-grouping of commingled skeletal remains by elemental analysis."

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Spectroscopy; Laser; Skeletal Remains

A94 Don't Forget About ME: How Material Evidence Can Inform (or Complicate) Commingling From the Battle of Tarawa

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Learning Overview: This presentation will combine historical records, modern archaeological excavation, and modern-day forensic identification and accounting practices to demonstrate the complications that can arise from both the use, and misuse, of incorporating Material Evidence (ME) or recovered personal effects into the resolution of commingled skeletal assemblages. After attending this presentation, attendees will better understand the nuances and potential pitfalls associated with historic and modern archaeological recovery as they relate to the use of material evidence in positive identification.

Impact Statement: This presentation will impact the forensic science community by showing the contributions that material evidence and personal effects can make to the resolution of commingled skeletal assemblages and the identification of skeletal remains, but also highlight where caution should be applied when associating such items with a single individual within a commingled assemblage.

During World War II, from November 20–23, 1943, United States forces invaded and fought for control of the Japanese-occupied Betio Island as part of an island-hopping campaign in the Pacific. The battle (known as the Battle of Tarawa) resulted in the loss of over 1,000 United States service members with approximately 375 individuals still unaccounted-for. The Defense POW/MIA Accounting Agency (DPAA) is responsible for the location, recovery, identification, and repatriation of the remains of unaccounted-for service members and, since 1979, the DPAA (and its predecessor organizations) along with History Flight, Inc., a DPAA strategic partner, have conducted excavations to recover the remains from the Battle of Tarawa. Complications such as battlefield burial practices, the relocation of grave markers, and repeated disinterment and reinterment of remains have affected modern-day accounting practices and contribute to struggles surrounding the resolution of the commingled remains. Many of the individuals who were buried following the battle were buried with associated personal effects, which, historically, were used to aid in identification. These items can also assist in the resolution of commingled assemblages during present-day analysis and can provide a unique insight into battle history and the prioritization of equipment and personal effects during military campaigns.

However, the inaccurate association of material evidence in the past due to the inexperienced and incomplete recovery of remains can contribute to pitfalls with modern-day accounting practices. The value placed on material evidence in the historic record, and how we approach the presence of personal effects within commingled assemblages in forensic investigations today, has far-reaching implications for identification within these contexts. While the presence of material evidence can reconstruct both past events and the relationship an individual may have had with the people and places around them, its association and utilization in positive identification of skeletal remains should be done carefully and with a strong understanding of the environment and context in which both the remains and evidence was found.

Archaeology; Commingling; Anthropology

A95   **Preliminary Longitudinal GPR Data on an Experimental Secondary Mass Grave at the ARISTA Forensic Taphonomy Facility**

Coen Nienaber*, Netherlands Forensic Institute, Den Haag, Zuid-Holland, Netherlands; Xenia Paula Kyriakou, University of West London, Fort Myers, FL

WITHDRAWN

A96   The Dirt on DNA: Utilizing Soil as Evidence of Clandestine Burials and Mass Graves

Meradeth H. Snow*, University of Montana, Missoula, MT; Kaylee Hinds, MA, University of Montana, Missoula, MT; Atlantis Baron, BA, University of Montana, Missoula, MT

Learning Overview: The use of DNA in mass grave scenarios is often limited to the testing of individuals and the attempt to reassociate comingled remains. However, as attendees at this presentation will learn, there are several ways in which DNA from the surrounding soil can be applicable toward mass grave scenarios. These include: aiding with identifying primary, secondary, and tertiary mass grave location; managing non-destructive analysis of the remains themselves; and the use of the microbiome for identification purposes.

Impact Statement: This presentation will impact the forensic science community by addressing ways in which soil DNA can be obtained and analyzed, allowing for a larger toolkit for identification and management of mass graves. The operationalization of these methods into a practical set of guidelines or best practices can serve those working in humanitarian forensics and help facilitate the extraction and analysis of DNA from soil.

DNA evidence from soil is typically not the first choice of forensic professionals working at any kind of crime scene, especially that of mass graves. Soil is often seen as the material that must be removed to facilitate identifications. However, the soil within a burial context houses a great deal of information regarding the individuals interned within it or on its surface. Through analyses completed, as well as work within the ancient DNA field, DNA from individuals will leach into the surrounding soil, thus creating a potentially rich microbiome that contains human DNA and differs from the surrounding soil.[1-3] This DNA can be detectible at the surface level (influenced primarily by the soil type and environment), allowing for soil DNA testing to facilitate unknown burial location detection with a relatively low-cost and low-labor method.

Soil also has the benefit of being tested for DNA from individuals without requiring destruction of the remains. As there are many cultures who prefer to retain their relation's remains as intact as possible, the ability to sample soil surrounding the remains affords the ability to avoid drilling or hazardous chemical exposure of the remains—as well as potentially avoiding disruption of the burials at all. An example of where this would be a key addition to the best practices would be the boarding school burials of Native American children that are scattered across North America.[4]

The development of this process whereby we create standard methods that can be implemented toward the sampling, transportation, DNA analysis, and then individual identification is ongoing. Preliminary analysis utilizing pig limbs to test the movement of DNA in the soil in the Montana forests throughout the summer and fall has demonstrated that DNA lingers in the soil both below the surface and on the surface above the burial, even after the removal of the limbs, for an extended length of time.[1] Additionally, contaminants in the soil (arsenic, cadmium, etc. from mining activities) influence the movement and longevity of pig DNA within the soil, ostensibly as they also influence microbial activity.[5] Additional work in this area will continue to aid in the ability to identify and restore individuals to their families, which in turn promotes justice for the victims.

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Soil Analysis; DNA; Mass Graves

A97　The Multidisciplinary Resolution of American Military Casualties From the Korean War

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Learning Overview: This presentation will demonstrate the multidisciplinary, complex, and systematic analytical process used in the identification of deceased United States military personnel from the Korean War through multiple case studies. After attending this presentation, attendees will have a better understanding of the complexity involved in the processing of commingled remains, including the triage, segregation, and name association workflows that support recommendations for identification.

Impact Statement: This presentation will impact the forensic science community by sharing the successful, multifaceted approach utilized by the Defense POW/MIA Accounting Agency (DPAA) to process, analyze, and identify commingled remains from the Korean War. This promotes and highlights the necessary collaboration from multiple disciplines to achieve the identification of individuals from commingled contexts.

The DPAA's mission is to provide the fullest possible accounting of missing United States service members from past conflicts to their families and the nation. The DPAA's Scientific Analysis (SA) directorate is the largest forensic skeletal identification laboratory in the world, employing a multidisciplinary team of anthropologists, odontologists, DNA and stable isotope scientists, and war historians, among other specialists.[1] One of the many conflicts investigated by the DPAA is the Korean War, which claimed the lives of approximately 36,500 United States service members in its three years, of which nearly 7,500 are still unaccounted for.

The Korean War Identification Project (KWIP) was established in 2011 to support the analysis and identification of United States causalities recovered from the Korean War. The impetus for the project's formation was the recognition that the remains being received and recovered were often highly commingled. For example, many boxes from unilateral turnovers were initially thought to contain single individuals, but DNA analysis produced multiple mitochondrial DNA (mtDNA) sequences per accession. The high degree and complexity of commingling observed in the Korean War remains necessitated the development of a systematic workflow that focuses on the segregation of remains into individuals by aggregating data from multiple lines of evidence. As such, KWIP's mandate is primarily the segregation and name association of these commingled remains. KWIP cases are approached in two ways: (1) mtDNA sequence-based or (2) accession-based. Case examples of each approach and the challenges they involve will be presented. This process has been formalized within DPAA-SA by the Commingled Human Remains analysis (CHR) standard operating procedure. The CHR process originally developed by KWIP has now been adopted by all other special projects involving commingled remains within the DPAA. The marked success of using a multidisciplinary approach to resolving commingling is highlighted by several complex case studies. These cases will demonstrate the synthesis of DNA, stable isotope, historical, archaeological, anthropological, odontological, and radiological evidence that is used to resolve commingling and support identification while also working with family members towards resolution. Overall, KWIP represents a unique assemblage involving highly commingled human remains associated with thousands of unresolved service members with multiple proveniences. As of July 2023, KWIP has identified a total of 664 United States service members and repatriated more than 300 foreign allies to their home countries, illustrating the strength of this approach.

Reference:

1. Go, Matthew C., Veronica A. Keyes, Jessamy H. Doman, Kristen M. Grow, Amanda R. Hale, Elizabeth Nagengast-Stevens, Briana T. New, Devin N. Williams, and Jennie JH Jin. The Korean War Identification Project: 30 years of expanding scope and complexity in the accounting of American war dead. *Wiley Interdisciplinary Reviews: Forensic Science* (2023): e1485.

DPAA; Commingling; Humanitarian

A98   The Role of Starvation in the Underestimation of Living Stature from Skeletal Remains

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Learning Overview: After attending this presentation, attendees will understand the potential role that severe dietary deficiency plays in the underestimation of living stature from human skeletal remains.

Impact Statement: This presentation will impact the forensic science community by bringing awareness to the potential impact that severe dietary deficiency has on stature and living stature estimation and how to mitigate the risk of underestimating stature in affected populations.

The estimation of stature is a key component in the forensic identification of unknowns. In commingled assemblages, stature is often used as one line of evidence, capable of assisting in the segregation of remains, as well as helping to strengthen or rule out potential identification matches. Previous research has shown that changes to stature are not only a function of developmental stage, but that bone growth and stature are particularly sensitive to nutrition and dietary wellness. Despite this, dietary deficiencies and other nutritional considerations are rarely incorporated into stature analyses or interpretations, leaving the potential for an underestimation of living stature based on skeletal measurements.

This study compares the recorded living statures to the forensically estimated postmortem statures of two temporally consistent populations to determine if known dietary deficiency impacted stature estimations during the identification process. The first sample population consists of individuals who died during internment in the Cabanatuan Prisoner of War Camp during World War II; the second sample population consists of individuals who were killed during the World War II Battle of Tarawa. The individual records from Cabanatuan had documented a pattern of extensive dietary deficiencies preceding burial at the Camp.

The study consisted of 141 individuals from Tarawa and 24 individuals from Cabanatuan (n=165). All individuals were between the ages of 18–48, with an average age of 23 years. While the correct stature was captured within the 95% Confidence Interval (CI) for all individuals identified and living stature was consistently underestimated from the Probability of Identity (PI) across the board, the degree to which stature was underestimated for those individuals from Cabanatuan was demonstrably more significant than those individuals from Tarawa (mean difference Cabanatuan 1.26 inches, mean difference Tarawa 0.40 inches; p=0.003). The extent to which the forensically ascribed stature and living stature vary between those individuals who had documented cases of severe nutritional arrest and dietary deficiency and those who do not reaffirms that stature is sensitive to outside pressures and that health status at time of death may affect stature estimation.

This research highlights the role that nutrition and diet, particularly around the time of death, play in forensic and living stature estimation. While it is widely accepted that nutritional stress may interrupt skeletal development in the skeletally immature, correction factors for stature decrease are rarely employed for adults under 45 years of age. It is proposed that in order to mitigate the risk of underestimating stature and preemptively or falsely excluding a potential identification match, researchers take into consideration additional contributing factors—such as severe dietary deficiencies—when making exclusions based on stature, regardless of age.

Stature; Diet; Mass Graves

A99   Mass Graves: An Analysis of Injuries and Causes of Death

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Learning Overview: The goal of this presentation is to focus on the determination of cause and manner of death for human remains exhumed from mass graves in Bosnia and Herzegovina and the distinction between antemortem and perimortem trauma, as well as postmortem breakage in these. This presentation will discuss the challenges encountered in the identification of trauma on human remains in different degrees of decomposition and how these challenges are mitigated.

Impact Statement: This presentation will impact the forensic scientific community by showcasing the application of forensic science in overcoming challenges in the identification of trauma on human remains and present examples from casework conducted in a humanitarian setting. Attendees will learn how the work of the local forensic team supports the Transitional Justice framework in Bosnia and Herzegovina.

The modern history of the Balkans is painted in blood. The Yugoslav wars, a series of conflicts between different Balkan nations in part of what is today former Yugoslavia, had devastating consequences for the people of the region, and mass atrocities are seen in the discovery of mass graves. Bosnia and Herzegovina were affected by a war that lasted four years between 1992 and 1995, which has left more than 30,000 missing persons. Mass graves in Bosnia and Herzegovina are characterized by varied burial conditions. To this day, a total of 23,000 sets of human remains have been exhumed from numerous mass graves ranging from burials in the ground, pits, wells, septic tanks, and caverns. Human remains were occasionally also found exposed and unburied above ground, placed in open fields, meadows, and forests. While the majority of the remains were found completely skeletonized, occasionally, in some instances, individuals were found saponified or mummified depending on the burial conditions they were found in.

During forensic postmortem autopsy of remains exhumed from these mass graves, it has been noted that these remains often exhibit morphologies and defects associated with postmortem modification to the bones, especially postmortem breakage. Similarly, injury to preserved internal organs under postmortem conditions may also pose a problem. It is deduced that this is in part due to the variable burial conditions and mode of deposition of the remains in primary mass contexts; the transfer of the remains from primary to secondary, or even tertiary, mass graves while undergoing decomposition and/or have been skeletonized; processes of decomposition themselves such as the effect of saponification; and also in part due to the different forensic archaeological techniques used during the exhumation process.

The Transitional Justice model established for Bosnia and Herzegovina revolves around the accounting and repatriation of these missing persons and has justice for the victims at its core. As a result, the identification and interpretation of trauma, as it relates to cause and manner of death, is central to forensic analysis as it is paramount in securing the conviction of perpetrators. Similarly, the assessment of antemortem injuries is not only important in the legal process in order to determine the cause of death, but it is also helpful in the identification process when considering antemortem information obtained from family members or witnesses about injuries sustained. Therefore, the assessment of antemortem injuries for individuals retrieved from such mass grave, commingled, contexts in Bosnia and Herzegovina is a challenging task. It requires a multidisciplinary approach and a good understanding of the interplay between the everchanging field conditions, forensic taphonomy, and how these may affect skeletal structures and subsequent determination of the cause of death.

Mass Graves; Cause of Death; Forensic Pathology

A100   Substance Misuse, Stress, and Sternal Rib End Morphology: Implications for Forensic Age Estimation

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Learning Overview: This presentation demonstrates the effects of chronic drug misuse on age-at-death estimation using the morphology of the fourth sternal rib end. Attendees of this presentation will have a better understanding of how chronic environmental stress affects morphological indicators of age in the skeleton.

Impact Statement: This presentation will impact the forensic science community by informing attendees that in the context of a drug overdose epidemic, forensic anthropologists should consider the potential effects of chronic substance misuse on morphological, or phenotypic, indicators of biological age in the skeleton. These data indicate that secular change is occurring in the morphology of the fourth sternal rib end.

One of the basic tenants of method development and application in forensic anthropology is that the reference sample should be an accurate and unbiased representation of the subject under analysis. Since 2014, drug-related mortality has risen precipitously, and synthetic opioids have been the primary driver of this trend. Synthetic and non-synthetic opioids are known to have variable effects on bone metabolism and healing.[1] In a more global sense, substance misuse is accompanied by a variety of environmental stressors, including housing insecurity, food insecurity, risk of infectious disease, and risk of accidental injury. This study investigates whether an individual experiencing significant prolonged stress due to drug misuse behaviors will exhibit premature skeletal aging. This preliminary study compares phase scores of the fourth sternal rib end in decedents with a reported history of substance misuse and those with no drug-misuse history. Bone Marrow Adipose Tissue (BMAT) volume is presented as a potential biomarker of stress.

From December 2022 through June 2023, the fourth sternal rib end was sampled from decedents receiving a full autopsy at the Western Michigan University Homer Stryker M.D. School of Medicine (WMed). The Department of Pathology and Office of the Medical Examiner are co-located at WMed. Over seven months, 202 ribs (n=135 substance users; n=67 no substance misuse) were sampled. The ribs were scored following Hartnett's system.[2] Males scored Phase 4, 5, 6, and 7 have a sufficient balanced sample to evaluate differences of mean age for a given phase by drug misuse history. In all four phases, the mean age of decedents with a drug misuse history is younger than the decedents with no history of drug misuse. The difference in mean age was tested using a Wilcoxon Rank Sum test with significant differences in Phase 6 and Phase 7 males (p=0.0500 and p=0.0166, respectively). All four phases exhibited significant differences in mean age at $\alpha \leq 0.10$. These preliminary data suggest that forensic anthropologists will systematically over-age males with a history of drug misuse.

Although the synthetic opioid fentanyl is detected in the postmortem toxicology of most drug overdose deaths, the norm is for individuals to use multiple substances that include both Central Nervous System (CNS) depressants and stimulants. This study does not consider decedents' preference for CNS depressant versus stimulant drugs. Different substances undoubtedly affect bone metabolism diversely; however, this study argues that the overall stressful environment of people with substance use disorders is more important in the expression of fourth sternal rib end morphology than the specific substances used.

BMAT was recently identified as a unique type of adipose tissue with osteoclastogenic and insulin resistance properties. The relative volume of BMAT is an indicator of stress where individuals under higher stress tend to have more BMAT.[3] All the decedents at WMed are imaged by Computed Tomography (CT) upon intake. The relative volume of BMAT to bone marrow in the manubrium, sternum, and xiphoid process was measured from CT data. Initial results from a small subset (n=9; n=5 substance misuse, n=4 no substance misuse) indicate that regardless of sex, decedents with a drug misuse history have more BMAT relative to bone marrow. The pattern suggests that BMAT is a sensitive biomarker of stress, and that decedents with a drug misuse history are subject to more stress than those with no drug misuse history.

These study findings demonstrate significant secular change occurring in the United States population with respect to skeletal age indicators. Reference samples that are the basis for rib aging methods are in need of updating in an environment of increased drug-related morbidity and mortality.

References:
1. Coluzzi, F., Scerpa, M. S. & Centanni, M. The Effect of Opiates on Bone Formation and Bone Healing. *Current Osteoporosis Reports* vol. 18 325–335 Preprint at https://doi.org/10.1007/s11914-020-00585-4 (2020).
2. Hartnett, K. M. Analysis of age-at-death estimation using data from a new, modern autopsy sample - Part II: Sternal end of the fourth rib. *J Forensic Sci* 55, 1152–1156 (2010).
3. Muruganandan, S., Govindarajan, R. & Sinal, C. J. Bone Marrow Adipose Tissue and Skeletal Health. *Current Osteoporosis Reports* vol. 16 434–442 Preprint at https://doi.org/10.1007/s11914-018-0451-y (2018).

Age Estimation; Drug Abuse; Biomarkers

A101   Aging Increases Regional Variation in the Pore Morphometry of Human Ribs

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Learning Overview: After attending this presentation, attendees will understand the importance of accounting for cross-sectional variation, age, and sex in quantifying porosity.

Impact Statement: This presentation will impact the forensic science community by improving understanding of sources of intraskeletal variation in porosity, a key determinant of bone quality and fragility.

Porosity is an independent predictor of fracture risk, particularly at common sites of osteoporotic fracture. Pores concentrate mechanical stress, allowing microcracks to initiate and propagate into fracture even under low nominal stress. Localized mechanical strain also regulates the frequency and extent of bone remodeling, influencing how pore systems are shaped and modified over the lifespan. Defining relationships between this strain patterning and the resulting pore structure should improve predictions about pore morphometry in a tissue region, strengthening inferences about fragility. In the human midthoracic rib, simplified bending places the cutaneous and pleural cortices into opposing tension and compression. Histological studies have identified a significant elevation of percent porosity in the cutaneous cortex, compared to the pleural cortex. Porosity also increases with age, especially as pores expand and coalesce from small, rounded "cortical" pores into large, irregular "trabecularized" pores. The objective of this research was to investigate whether pore morphometry also displays regional patterning, and whether these patterns are altered by pore type, age, and sex.

Human midthoracic ribs (n = 124, [males = 76, females = 48], 22–97 years old, mean = 57.4 years) were cross-sectioned at the midshaft, histologically prepared, and imaged at 100X magnification. Pore morphometry was quantified with a custom ImageJ macro, Pore Extractor 2D for each pore type (all pores, cortical pores only, trabecularized pores only) within each rib region (cutaneous cortex, pleural cortex).[1] For each pore type, linear mixed models compared pore morphometry between the cutaneous cortex and pleural cortex, co-varying and interacting with age and sex.

Percent porosity was significantly higher in the cutaneous cortex for all pores, without age interaction ($p < 0.001$). Cortical percent porosity was initially also higher in the cutaneous cortex, but the pleural cortex increased more with age, and surpassed the cutaneous cortex around the sixth decade ($p = 0.002$). Trabecularized percent porosity remained significantly higher over the lifespan and increased more with age in the cutaneous cortex ($p = 0.022$).

Pore density was initially higher in the cutaneous cortex for all pores ($p < 0.001$) and cortical pores ($p < 0.001$) but was surpassed by a steep increase in the pleural cortex in middle age. Trabecularized pore density was significantly higher in the cutaneous cortex, without age interaction ($p < 0.001$).

Pore size (mean pore maximum diameter) and pore elongation (mean pore aspect ratio) were both significantly higher in the cutaneous cortex for cortical pores ($p < 0.001$). Males maintained larger, more elongated trabecularized pores in the cutaneous cortex over the lifespan. Females initially formed especially large, elongated trabecularized pores in the pleural cortex, which decreased with age. Increases in cutaneous cortex size and elongation overtook the pleural cortex around the sixth decade ($p = 0.002$).

Regional differences in pore morphometry accelerated with age, shifting the cutaneous cortex toward trabecularized porosity and the pleural cortex towards cortical porosity. Trabecularized porosity, trabecularized pore density, and overall pore size remained highest in the cutaneous cortex across the lifespan. Cortical porosity and cortical pore density were initially also highest in the cutaneous cortex but were overtaken by the pleural cortex in middle to older adulthood. Regional strain mode appears to influence not only the extent of porous bone loss, but also the preferred mechanism of either trabecularization or cortical pore formation.

Reference:
1.   Cole ME, Stout SD, Dominguez VM, Agnew AM. 2022. Pore Extractor 2D: An ImageJ toolkit for quantifying cortical pore morphometry on histological bone images with application to intraskeletal and regional patterning. *American Journal of Biological Anthropology* 179(3): 365-385. https://doi.org/10.1002/ajpa.24618.

Cortical Porosity; Bone Histology; Age-Associated Bone Loss

A102   Testing a Novel Method for Estimating Adult Age at Death From the First Rib in Forensic Casework

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Learning Overview: This presentation utilizes characteristics of the first rib to test a novel method of estimating the ages at death of unknown decedents in forensic casework. After attending this presentation, attendees will better understand that scoring the ribs using the current descriptions and photographic references is difficult, yielding age-at-death estimates with extended ranges.

Impact Statement: This presentation will impact the forensic science community by demonstrating that novel methods of estimating adult age at death need to be tested to be validated. The proposed method of estimating age at death using the first rib is difficult to use but may be improved with further modifications.

Age at death is one of the first traits estimated when developing a biological profile of an unknown decedent. The first rib, which is more robust than the traditionally assessed fourth rib, more commonly recovered in skeletal cases, and more easily identifiable, has been proposed to yield reliable age-at-death estimates using a probabilistic approach.[1] However, *Daubert* criteria requires that the applicability of this method in forensic casework be validated.

The aim of this study is to test the method proposed by Luna and Aranda for estimating adult age-at-death from the first rib.[1] The sample consists of 32 first ribs from decedents at the Maricopa County Office of the Medical Examiner and the Forensic Anthropology and Bioarchaeology Laboratory at Arizona State University. The articular surfaces and periarticular margins of the costal surfaces and heads of these ribs were scored following guidelines by Luna and Aranda.[1] Twenty ribs were scored independently by each author to determine inter-observer repeatability, and ten ribs were scored twice by the same author two months apart to calculate intra-observer error. Rib scores were used to estimate the age at death of each decedent, and these estimated ages were compared to actual ages or mean estimated ages using the pubic symphyses and fourth ribs.[2,3] The Tau B coefficient (τ) showed that inter-observer repeatability was moderate to good (τ = 0.53-0.92), and intra-observer repeatability was good (τ = 0.81-1.00). Age estimation accuracies were 9.4% for within 1 year of the actual age at death, 12.5% for within 2.5 years, 31.3% for within 5 years, and 43.8% for within 10 years. These accuracies are much lower than those reported by the original authors (43.5%–94.7%), and the method, at present, does not improve on current methods for use in forensic casework. Each author found the ribs difficult to score by using the current category descriptions and photographs as a guideline. As a result, ribs may have been scored incorrectly, resulting in inaccurate age-at-death estimates. More detailed descriptions and clearer photographs of each category may aid in increasing the accuracy and inter-observer repeatability of this method, and thus its applicability in forensic casework. This method may be used with caution if traditional elements are unavailable to assess.

References:
1. Luna, L. H., and Aranda, C. M. (2022). Adult age-at-death estimation using the first rib: A simple probabilistic approach. *Journal of Forensic Sciences,* 67, 2173-2191. https://doi.org/10.1111/1556-4029.15119.
2. Hartnett, K.M. (2010). Analysis of age-at-death estimation using data from a new, modern autopsy sample—Part I: Pubic bone. *Journal of Forensic Sciences,* 55(5), 1145-1151. https://doi.org/10.1111/j.1556-4029.2010.01399.x.
3. Hartnett, K.M. (2010). Analysis of age-at-death estimation using data from a new, modern autopsy sample—Part II: Sternal end of the fourth rib. *Journal of Forensic Sciences*, 55(5), 1152-1156. https://doi.org/10.1111/j.1556-4029.2010.01415.x.

Age Estimation; Rib; Method Validation

A103 The Utility of Deciduous Dental Microstructures in Forensic Anthropology

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Learning Overview: After attending this presentation, attendees will better understand how the Neonatal Line (NNL) can be used in forensic anthropological analysis to estimate age and identify a live birth.

Impact Statement: This presentation will impact the forensic community by demonstrating the utility of the NNL in medicolegal death investigations and will aid in the development of more precise and statistically accurate age estimates within a forensic context. It also serves to identify the utility of accentuated striae as indicators of general stress events.

The dental enamel serves as a record of growth and development *in utero* through to late adolescence. Enamel cross striations (i.e., daily secretions of enamel) can be used to count days of enamel deposition to create age estimation methods and to understand dental development. The enamel can also record episodes of non-specific stress in the form of accentuated striae. These striae can be identified in histological cross-section of the enamel under low magnification. The first identifiable accentuated stria in the enamel is considered to be the NNL, an artifact found in the enamel microstructure of teeth associated with an individual leaving the intrauterine space. As such, the NNL can be used to identify a live birth, is a means to calculate age since birth, and can be used as a marker of events pre- and post-birth visualized in the dental structure. However, not much is known about the NNL, including how often it occurs, why it occurs, which teeth best illustrate the NNL, nor how long a tooth needs to develop before and after the development of the NNL for it to be visualized. This preliminary study aims to fill this gap through an investigation of microanatomy of the NNL (and accentuated enamel striae) of deciduous teeth and associated structures.

Dental histological slide preparation was undertaken on a sample of two individuals with known prenatal and postnatal health, gestational age, and sex (with permission of both parents and children in the United States). All available naturally exfoliated deciduous teeth were sectioned for analysis (n=22), which included each tooth type in the dental arcade for the upper and lower jaws. The NNL was recorded, and its width documented. Data were recorded on the number of cross-striations visible prior to and after birth. Accentuated striae in the enamel were also recorded and correlated to known health events of the two individuals.

Preliminary results indicate that the NNL could be visualized in deciduous teeth that had completely formed. Further, accentuated striae did not always correspond to known times of stress in these deciduous teeth. Future research will continue to count cross-striations of dental development to understand how long teeth develop and to create robust age estimation methods for infants and children. Additional developed teeth will be included as well as the dentition of fetal and infant remains of developing teeth to test and validate age estimation methods. This pilot study demonstrates the potential utility of the NNL in deciduous teeth and how dental microstructure can be used to understand life events and aid in forensic anthropological analysis.

Age Assessment; Anthropology; Histological

A104   Dental Age Estimator Performance Relative to Weighted Kappa: A Simulation Study

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Learning Overview: After attending this presentation, attendees will understand how weighted kappa measures of observer agreement correspond to dental age estimation precision, accuracy, and bias. This presentation will demonstrate potential consequences of reduced agreement in scoring.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of observer agreement in controlling downstream estimation error.

Cohen's weighted kappa is a common measure of observer agreement for ordinal data such as dental development scores, with values above 0.8 generally considered good. How weighted kappa values correspond to downstream error in age estimates is not known. Here we test the relationship between weighted kappa values and dental age estimation error by estimating ages from a single sample with additional simulated observers, each scoring at a fixed weighted kappa value relative to the original observer.

The dataset consisted of Moorrees et al. scores of left permanent mandibular molars from 1,040 London children 5–18 years old, distributed uniformly by year of age.[1] Multivariate cumulative probit models were fit to the original observer data with age on a log scale. Ages were estimated with simulated observers of validation data and ten-fold cross-validation. To simulate observer error, the original scores were jittered via a normal distribution, then rounded to whole number scores. Error was balanced, over scored, and under scored, each with kappa values of 0.80, 0.90, and 0.95. Ages were also estimated from the original scores (kappa = 1.0) for a total of ten combinations of error direction and weighted kappa. Performance metrics were age interval width relative to true age, the success rate of the age interval at capturing the true age, and residual error.

Within each type of scoring error (balanced, over, under), lower kappa values were consistently associated with worse performance across all metrics. Notably, the overall success rate from the original scores was 0.93 (expected value 0.95). The next highest was 0.85 from over scoring at kappa = 0.95, and the lowest was 0.55 from under scoring at kappa = 0.80. Over scoring produced positive residuals and under scoring negative residuals. Success rates were better from over scoring than under scoring or balanced scoring at equal kappa values. This is potentially related to larger age intervals from over scoring, particularly at younger ages. Age interval width was narrower and less variable across the age range from under scoring than from over scoring or balanced scoring at equal kappa values.

In conclusion, age estimation performance testing on live multi-observer dental development data is warranted. A cutoff for weighted kappa of 0.80 may be insufficient to control estimation error introduced through observer discrepancies.

Reference:
1.   Moorrees, C. F., Fanning, E. A., & Hunt Jr, E. E. (1963). Age variation of formation stages for ten permanent teeth. *Journal of dental research*, *42*(6), 1490-1502.

Age Assessment; Method Development; Machine Learning

A105 Estimating Age in Adults Using Measurements of Dental Radiographs: A Validation of the Kvaal Method

Katherine Skorpinski*, Defense POW/MIA Accounting Agency, Omaha, NE; Laura E. Cirillo, SNA International in Support of the Defense POW/MIA Accounting Agency, San Francisco, CA

Learning Overview: After attending this presentation, attendees will better understand the potential use of dental radiographs for estimating age in adults.

Impact Statement: This presentation will impact the forensic community by providing a performance assessment of a non-invasive method of adult age estimation, discussing current limitations and challenges of this method, and proposing future directions to improve method performance.

Age estimation in adult remains is limited by several factors. Specifically, adult skeletal age estimation involves few reliable indicators and focuses on markers of degeneration. These challenges are further complicated by damaged or absent elements in forensic and historical contexts. However, the durability of enamel results in greater tooth preservation and retention in a variety of taphonomic conditions. Although dental age estimation methods primarily focus on development, secondary dentin deposits throughout adulthood have been used to estimate age beyond young adults.

The Kvaal et al. method of measuring pulp cavity size to estimate age was developed on a small clinical sample ranging in ages from 20 to 87 years, and measurements on three maxillary teeth (central and lateral incisors, and second premolars), and three mandibular teeth (lateral incisor, canine, and first premolar).[1] In order to examine the applicability of this method to other forensic contexts, a sample of dental radiographs of identified male United States servicemembers from World War II and the Korean War (n = 107, ages ranging from 17 to 52 years) was tested.

Inter-observer error was first tested on a subsample of ten cases with complete dentition for all measurements and ratios used in the Kvaal et al. method. Although some individual measurements demonstrated a significant difference between observers, the mean difference in measurement was consistently under 0.5mm and did not significantly alter the age estimate. T-test results indicated that there was no significant difference in the ages estimated between observers for all teeth.

The method was applied to a total of 272 teeth with an overall accuracy of 83.5% within one Standard Error of Estimate (SEE). Accuracy by tooth type ranged from 78.6% (mandibular first premolars) to 93.1% (maxillary central incisors). Individuals with a known age that fell outside of the 1 SEE interval were underestimated in 65.9% of cases. Results using the three maxillary or mandibular teeth equations were accurate in the majority of cases but did not achieve as high of a percent accuracy as the single tooth equations. The method performed with a higher percent accuracy for ages under 30 years, including those younger than the original sample (i.e., under 20 years of age).

Despite the high accuracy overall in this application, several limitations excluded a large portion of the test sample, including dental restorations and pathological conditions. The method also relies on measurements of single-rooted teeth, but the high frequency of multiple roots in teeth used in the method significantly impacted the ability to test the multi-tooth equations. Further complications, including root morphology, observer error, and radiograph quality and orientation, were identified and adjustments and future considerations are proposed.

Reference:
1. Kvaal, S. I., Kolltveit, K. M., Thomsen, I. B., & Solheim, T. (1995). Age estimation of adults from dental radiographs. *Forensic Science International*, 74, 175-185. https://doi.org/10.1016/0379-0738(95)01760-G.

Adult Age Estimation; Dental Radiographs; Method Validation

A106   Innominate Weight Over the Lifespan: A Method for Estimating Age From the Pelvis

Andrea M. Zurek-Ost*, University of North Carolina at Chapel Hill, Durham, NC; Mark Sorensen, University of North Carolina at Chapel Hill, Chapel Hill, NC

Learning Overview: After attending this presentation, attendees will gain a better understanding of how innominate bone weight changes throughout the lifespan.

Impact Statement: This presentation will impact the forensic science community by demonstrating the utility of innominate dry bone weight as a trait that can be employed in age-at-death estimations.

The innominate is frequently used to estimate age at death in both forensic and paleodemographic contexts. Age changes in bone can be difficult to quantify and score consistently, particularly between males and females, due to changes in bone mineral density, calcium intake and absorption, endocrine status, nutrition, hormones, and biological sex.

This research investigated the relationships between bone weight, chronological age, and biological sex. This study examined 383 innominates from the Hamann-Todd Collection from individuals aged 16-93 years to investigate how bone weight (here a proxy for bone mineral density) was patterned throughout the lifespan. Standard osteology laboratory equipment as well as a scale were used to measure innominate height and dry bone weight. While dry bone weight is an imperfect proxy for bone mineral density, the phenomenon of decreases in bone weight with age was observed and quantified in this dry bone sample. While both males and females are affected by a slow reduction in bone mineral density over the course of their lifespans, this effect is exaggerated in women and was reflected in the study results.

To quantify these changes, the authors created a predictive Bayesian linear model to estimate age at death from dry bone weight and height. Both sex-specific and sex-unknown tests were conducted using JASP 0.17.1.[1] In Bayesian linear regressions, parameter estimates are drawn from the posterior probability distribution of the observed data, which is proportional to the product of the likelihood of the data and the prior probability of the parameters. In this study, the posterior predictive distribution was drawn with Markov Chain Monte Carlo sampling using the Beetle Antennae Search (BAS) algorithm. A model was then fitted to the simulated data, and these new parameter estimates were compared to the final model. A 95% credible interval was estimated on the final model to predict age given known innominate weight and length. The model R^2 was 0.32, with an average inaccuracy of 14.02 years. Estimated age was within ±10 years of known age for 42.7% of the sample, and 75.2% of estimated ages at death were within ±20 years of known age

In conclusion, when used alongside other age-at-death estimation methods, this pilot study demonstrates that dry bone weights may be used to support estimations of chronological age. These analyses should be utilized in concert with well-validated age-at-death estimation methods.

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Biological Profile; Age Estimation; Innominate

A107   An Age-at-Death Estimation From Pubic Bone Mineral Density on Postmortem CTs

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Learning Overview: The objective of our study is to estimate age at death through a simple measurement of the pubic Bone Mineral Density (BMD) on Postmortem Computed Tomography (PMCT). PMCT cannot be analyzed in the same way as clinical CTs; it is crucial to consider the structural changes in tissue caused by putrefaction.

Impact Statement: This study will have a significant impact on the forensic community by enabling quick and straightforward estimates of age at death through a simple measurement of pubic bone mineral density on PMCTs. Our results are particularly accurate when accounting for the degree of putrefaction

Age-at-death determination is an important issue in forensic medicine and anthropology. The use of imaging in the forensic field has facilitated the development of new techniques that provide age estimation while preserving remains. These techniques include morphological studies using 3D scanographic reconstructions as well as methods using bone mineral density. However, most of these studies primarily focus on CT scans of living individuals.[1-7] In this study, the authors investigated the correlation between pubic bone mineral density and age at death on PMCTs.

We conducted a study on postmortem CTs of cadavers autopsied in the Forensic Institute of Tours (France) to determine whether there was a correlation between the BMD of the pubic symphysis measured on PMCT and age at death. We excluded individuals under the age of 15 years, unidentified bodies, or those with trauma that hindered the measurement of Radiological Alteration Index (RAI) or BMD. Postmortem delay was not a determining factor for inclusion, as our study specifically sought bodies with varying degrees of decomposition.

CT measurements of BMD are an estimation (in Hounsfield units) and are potentially modified because of putrefactive gases into the cancellous bone tissue. This phenomenon could falsely decrease bone density and thus overestimate age at death. In the specific postmortem context, we therefore used a radiological degradation score (RAD) to quantify the degree of putrefaction and establish whether putrefaction interferes with the determination of age at death according to our method.[8]

Results*: Our sample consisted of 402 PMCTs, comprising 148 females and 254 males. The average RAI was approximately 25, indicating that the majority of bodies were only slightly decomposed. Our study showed a correlation between pubic BMD and age at death. To use this method, it is necessary to quantify the degree of putrefaction by calculating the RAI index. Indeed, in order to increase the accuracy of the results, the equations used to calculate age at death are different according to RAI subgroups (0, 1 to 24, 25 to 49, 50 to 74, 75 to 100). However, our technique cannot be used for highly putrefied bodies (RAI >75) as there is no correlation between age at death and pubic BMD in this group due to significant structural changes associated with the putrefactive gas infiltration of the cancellous bone.

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Age Assessment; Radiology; Bone

A108   Decoding Age-at-Death With DNA Methylomics: A Cutting-Edge Approach for Forensic Analysis of Skeletal Remains

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Learning Overview: After attending this presentation, attendees will be aware of a novel application of genome-wide DNA methylomics to forensic anthropology. The learning objectives here are to gain an appreciation of how advances in whole-genome sequencing offer potential to increase precision in Age-At-Death (AAD) estimation in medicolegal investigations.

Impact Statement: This presentation will impact the forensic community by providing an innovative tool for the estimation of the AAD of human skeletal remains using a genome-wide approach. The DNA methylation-based epigenetic age prediction model presented here leverages a large *in silico* dataset focused specifically on bone material and will allow for the future development of targeted forensically relevant epigenetic approaches for improved AAD estimations.

The estimation of AAD from skeletal remains using routine anatomical approaches lacks precision and may be hampered by missing skeletal elements or environmental weathering. Many reports have described forensically applicable assays that use DNA methylation patterns at specific CpG loci to predict biological age; however, these approaches were developed using readily obtainable DNA sources such as blood, saliva, and teeth.[1,2] The use of biomolecular markers to estimate AAD from skeletal remains offers potential for greater precision, but their application has been hampered by poor DNA quality and quantity.

CpG locus signatures applicable to bone samples have been identified using the standard molecular clocks; these signatures were developed in both skeletal and non-skeletal tissue as age-associated markers.[3,4] However, robust, bone-specific CpG loci of aging that are compatible with DNA derivatives from decomposed skeletal remains are yet to be described.

In order to address this question, two approaches were conducted. First, an *in silico* study focused on identifying a forensically applicable CpG aging signature for bone was developed. An ideal forensic assay to predict age would use a small number of loci (i.e., ≤20 loci), enabling its assessment with Polymerase Chain Reaction (PCR) -based approaches. We gathered six publicly available genome-wide bone methylation datasets with reported donor age (total n=173; average age 65 years [range 0.25-92, spanning the whole human lifespan]). This dataset assayed CpG methylation at >450,000 loci. We tested previously published biological age-prediction signatures and, using cross-validation, employed six different machine learning algorithms to develop age-prediction signatures. The best performing algorithm was GLMNet, where a 20 CpG signature for predicting AAD had a Root-Mean-Square Error of 6.2 years, Mean Absolute Error of 4.7 years, and R^2 coefficient of 0.89.

The second investigation addresses the difficulty of obtaining DNA of sufficient quantity and quality from skeletal remains. We employed an optimized ancient DNA extraction protocol to maximize DNA recovery. To detect methylation using sequencing or hybridization, sodium bisulfite conversion is typically used to induce a methylation-dependent change to the primary DNA sequence. Bisulfite conversion is a destructive process (>90% of DNA is lost). In order to preserve DNA, we used a gentler enzymatic methylation conversion kit that is suitable for low DNA inputs. These methods were tested on a pilot cohort of donor human skeletal tissue samples (28–93 years age; 0–2,236 days Postmortem Interval [PMI]) collected from Southeast Texas Applied Forensic Science (STAFS) facility and Forensic Anthropology Center at Texas State (FACTS).

The methylation conversion rate for five pilot samples was >99% and all were run on the TWIST Biosciences Human Methylome Panel. The range of loci with >5 reads ranged from ~470,000 to 3,750,000 CpG loci; >90% of the CpG aging signature loci were measurable using the TWIST panel, enabling cross-platform integration. Ongoing analysis of the rest of the bone DNA cohort (n >45) is proceeding and will be presented to the attendees at the conference, where we will address the accuracy of AAD estimation using the TWIST panel and the *in silico* cohort.

This study represents one of the largest collections of genome-wide methylation status in bone. Fundamentally, the TWIST assay offers potential for methylome capture from skeletal bones recovered from burial sites. The applicability of this methodology will be discussed for future applications on forensically relevant human skeletal remains.

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Methylation; Aging; Epigenetics

A109   Intra-Skeletal Variation in Lower Limb Microstructure for Histological Age Estimation

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Learning Overview: After attending this presentation, attendees will understand the importance of bone microstructural variance and sampling location for developing and applying methods of histological age-at-death estimation.

Impact Statement: This presentation will impact the forensic science community by establishing the need for research on intra-skeletal variation in bone microstructure to inform method development and accuracy in histological aging.

Age-at-death estimation is a major and challenging component of the biological profile. The most commonly applied methods after macroscopic approaches are histological. Although various methods exist, almost all are exclusively based on one of two skeletal elements: the femur or rib. Furthermore, previous research demonstrates that sampling location and Region Of Interest (ROI) size also influence the reliability of age estimates, demonstrating that intra-skeletal variation plays a significant role in the success of these methods.[1,2] So long as this remains the case, the use of histological methods for estimating age at death will be limited.

One of the only papers to consider other bones in histological aging is Kerley's foundational work using remodeling events (i.e., secondary osteons) counted in the femur, tibia, and fibula to estimate age.[3] Kerley found that fragmentary counts of osteons in the fibula demonstrated the best relationship with age of the entire sample, but this finding has largely been overlooked in subsequent years. As such, the current paper analyzes remodeling and relative cortical area in the fibula and femur relative to each other and to age as a preliminary exploration of the fibula's potential for age estimation.

The sample consisted of right fibulae and femora from ten individuals of known age (15-105 years, sd=9.71 years) and sex (5F, 5M). Samples were ethically obtained via the body donation program at The Ohio State University and prepared following standard histological protocols. Images captured at 100x magnification were used to calculate Relative Cortical Area (Rel.Ct.Ar) for each cross-section in mm^2. Live microscopy using a 20x objective was utilized to count intact and fragmentary osteons across the entire cross-section. These data were then used to calculate three variables: Osteon Population Density (OPD), intact OPD (iOPD), and fragmentary OPD (fOPD).

Paired samples *t*-tests found no significant differences in Rel.Ct.Ar between the fibulae and femora, though a strong correlation between the elements may reflect allometry. For combined OPD, there were no significant differences either, but when split, both iOPD ($p= 0.008$) and fOPD ($p= 0.002$) were significantly different. Summary data indicates fibulae have higher fragment counts, whereas femora possess more intact osteons. This may result from the femur's larger cortex requiring more vasculature, combined with earlier and/or increased remodeling of the more constrained number of intact osteons in the smaller fibula.

Rel.Ct.Ar showed no significant relationship with age. A negative trend observed in the femur, possibly resulting from cortical trabecularization, was not seen for the fibula. All OPD relationships demonstrated significant, positive relationships with age for both elements. In both elements, the strongest relationship with age was for OPD (Fib $R^2=0.83$, Fem $R^2=0.84$), then fOPD (Fib $R^2=0.81$, Fem $R^2=0.69$), and finally iOPD (Fib $R^2=0.58$, Fem $R^2=0.58$). Again, fragments in the fibula appear to comprise a more substantial part of the microstructural variance than in the femur.

These findings suggest that other long bones, such as the fibula, merit development as sites for histological age-at-death estimation based on their similarities with the more commonly utilized femur and due to its own unique relationship between fragmentary and intact remodeling events.

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Age Estimation; OPD; Cortical Area

A110   Introducing OA-TA: A New Tool for Adult Age-at-Death Estimation Using Osteoarthritis of the Synovial Joints

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Learning Overview: After attending this presentation, attendees will have a better understanding of how to evaluate Osteoarthritis (OA) and utilize its presence or absence in synovial joints to estimate age from human skeletal remains.

Impact Statement: This presentation will impact the forensic science community by introducing OA-TA, a new Graphical User Interface (GUI) for use by forensic anthropology practitioners, to generate an adult age-at-death estimation from osteoarthritis presence/absence scores.

Age-at-death estimation is a crucial facet of the biological profile that forensic anthropologists generate from human skeletal remains. Adult age estimation often relies on particular indicators, such as the pubic symphyses or sternal ends of the fourth ribs, which are regularly taphonomically damaged or altogether missing from incompletely recovered cases. To circumvent this reliance and bolster forensic anthropologists' adult age-estimation toolkit, recent research has demonstrated the correlation between age and osteoarthritis in multiple synovial joint complexes among diverse groups of modern United States individuals.[1,2]

Here we present OA-TA, a GUI for generating adult age estimates based on the presence or absence of OA in synovial joints. Users may select to utilize as few as one or as many as nine appendicular joint complexes and create an estimate based on pooled sex, female-, or male-sex-assigned-at-birth reference samples from the University of Tennessee Knoxville (UTK) Donated Skeletal Collection (database N=408 individuals). For each joint, all bones are examined for OA and scored on a binary presence/absence scale; this score is then entered into the respective joint's field. For example, when evaluating the elbow, the presence of OA anywhere on the distal humerus, radial head, or proximal ulna would yield a score of 1 (present) for this joint. Based on the work of Winburn and Stock, OA-TA uses transition analysis—binomial generalized linear models with the probit link function—to generate age estimates based on the age at which 90% or 95% of the population transitions from "no OA present" to "OA present" in the given joints.[1] An age estimate interval, or floor or ceiling age estimate, is then provided for this individual.

OA-TA Version 1 is available for download at http://www.apwinburn.com/oata. If peer feedback is received prior to presentation, this will be incorporated into OA-TA Version 2 and shared using the same link.

We performed a test of the OA-TA GUI using a sample of 100 unique UTK donors (distinct from those in the database sample) using all available joints as well as testing each of the nine joints independently (i.e., ten iterations per individual, where applicable [joints previously demonstrated to have weak age correlations, such as the ankle in female individuals, were not assessed]).[1,2] Each iteration was performed using the sex-specific and pooled-sex equations and assessed for accuracy at both the 90% and 95% thresholds, for a total of 3,400 test cases. The OA-TA age-estimation interval was correct in 68.4% of these cases (2327/3400). The most accurate joints (i.e., where the age interval included the individual's known age-at-death) were the hip and shoulder; the least accurate joints were the TMJ and male ankle, with accuracy rates generally decreasing distally along the limb. When all available joints were utilized, accuracy rates for the test sample ranged from 82% (at the 90% threshold) to 78.5% (at the 95% threshold). These results corroborate findings from a previous study noting the proximal-to-distal accuracy pattern in age estimation using OA, with the low accuracy of the ankle—even among males—also recommending against the ankle joint's use for OA-based aging.[2]

The intervals yielded by OA-TA are often broad, and we are not advocating that OA replace primary age estimation methods in forensic anthropological practice. However, since OA has demonstrated a significant and reliable relationship with age, its presence/absence provides a tenable method to estimate age in incomplete or fragmentary cases and enhance age estimation during complete skeletal analyses. The binary scoring of OA in the synovial joints is already a straightforward process; with the OA-TA GUI, we offer a free, easy-to-use, openly accessible tool that further streamlines and standardizes the process of interpreting OA for purposes of forensic anthropological age estimation.

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Forensic Anthropology; Osteoarthritis; Age Estimation

A111   Validating Population Affinity Method Platforms for Macromorphoscopic Traits in Specific 20th-Century Contexts

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Learning Overview: After attending this presentation, attendees will better understand the relative performance of Macromorphoscopic (MMS) method platforms for populations consistent with those from the Defense POW/MIA Accounting Agency (DPAA).

Impact Statement: This presentation will impact the forensic science community by validating multiple MMS method platforms for forensic casework and demonstrate their relative accuracy.

MMS traits are used in non-metric analysis for assessing population affinity. However, it is necessary to establish validity of the statistical platforms for MMS traits applied to specific contexts. This ensures their applicability to context-specific casework and adherence to laboratory accreditation requirements. One method platform more recently introduced for these methods, the Osteomics' hefneR GUI, also requires validation per laboratory accreditation for inclusion in DPAA laboratory casework.[1] Therefore, the purpose of this study was: (1) to validate the method platforms for MMS traits in accordance with laboratory accreditation, and (2) to understand the relative efficacy of each platform for DPAA casework.

Data was aggregated from 347 forensic anthropology case reports from the DPAA. Per these reports, traits were scored following Hefner and Hefner and Linde.[2,3] Traits were then evaluated with four MMS method platforms, namely: Optimized Summed Score Attributes (OSSA); the Hefner and Ousley decision tree; the discriminant functions for 3-, 5-, and 7-traits; and the hefneR GUI for 3-, 5-, 7-, and 11-traits.[1,4,5] Sample sizes for each test are as follows: OSSA ($n=219$), decision tree ($n=309$), 3-traits ($n=253$), 5-traits ($n=83$), 7-traits ($n=75$), and all traits ($n=47$).

Trait scores were used to classify individuals into population groups for each statistical platform. The OSSA method results in White or Black classifications. The decision tree and Hefner methods can result in White, Black, or Hispanic classifications, while the hefneR GUI also includes Asian and Native American classifications.[1,4,5] The recorded perceived demographics from the antemortem records of the 347 identified individuals include: White ($n=326$), Black ($n=15$), Mexican ($n=2$), Native American ($n=1$), and Asian (e.g., Korean; $n=3$).

For the OSSA and decision tree methods, the correct classification rates were 94.5% and 90.5%, respectively. Using the discriminant function equations from Hefner, the 3-trait equation had a correct classification rate of 93.6%, 91.5% for the 5-trait equation, and 0% for the 7-trait equation.[5] Using the hefneR GUI, there was a correct classification rate of 77.3% for 3-traits, 73.2% for 5-traits, 87.8% for 7-traits, and 85.1% for 11-traits.[1]

Both the OSSA and decision tree methods performed best based on correct classification rates, but neither of these methods could account for individuals with Asian or Hispanic population affinity. The 3-trait discriminant function performed better than the 5- and 7-trait equations overall, but the opposite pattern was observed for the hefneR GUI results, with the 7-trait performing better than all other trait suites.[1] Notably, the 7-trait discriminant function analysis had a correct classification rate of 0% for this sample compared to the reported cross-validated correct classification rate of 83.4% in Hefner.[5] While a Hispanic estimation does not preclude consistency with a White antemortem record, all cases but three resulted in a Hispanic classification. This suggests a potential bias within the reported formula as this pattern was not observed for the 3- and 5-trait equations. Excluding the 7-trait discriminant function analysis, accurate classifications were for individuals having White, or European, recorded in their antemortem records, while incorrect classifications tended to occur for individuals with "non-White" antemortem records. In conclusion, these results suggest that all platforms, excluding the 7-trait discriminant function, perform relatively well for this context.

Traits scores were aggregated from 347 forensic anthropology case reports and associated notes and do not reflect analyst interpretations. Reported perceived race refers to the category of race in Individual Deceased Personnel Files for all deceased servicemembers relevant to DPAA investigations. For more detail, see Hale and colleagues.[6]

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Macromorphoscopic Traits; Population Affinity; Validation

A112   A Comparison of Ancestry-Specific Versus Ancestry-Independent Stature Estimation

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Learning Overview: After attending this presentation, attendees will understand the differences in error between ancestry-specific and ancestry-independent stature calculations.

Impact Statement: This presentation will impact the forensic science community by identifying where there are areas of significant difference between ancestry-specific and ancestry-independent equations for stature estimation.

Ancestry assessment in a forensic context is a widely discussed topic as the field weighs the potential harms and benefits of its application.[1,2] One area that proponents of the continued use of ancestry assessment in the biological profile point to is its application in estimating other aspects of the biological profile such as sex and stature. The aim of this study is to evaluate the impact of a prior ancestry assessment in estimating stature through a comparison of error between the point estimates based on ancestry-specific and ancestry-independent calculations and known statures. For prior ancestry assessment to be considered impactful, the results of ancestry-specific calculations should significantly differ from ancestry-independent calculations, and individuals from a reported ancestry group should have the least error between their known stature and the stature estimate from the corresponding ancestry-specific equation.

The significance of ancestry-specific stature estimation was tested on a skeletal sample of 270 identified White male service members from the USS *Oklahoma* incident of World War II. Individuals were selected based on the following criteria: consistent reported height throughout their antemortem records (as defined by a deviation of ≤0.5 inches across all documents), known ancestry (reported as "race" in the antemortem military records), and a maximum length measurement of at least one applicable skeletal element (humerus, radius, ulna, femur, tibia, or fibula). In total, 158 left and right humeri, 80 left and right radii, 89 left and 77 right ulnae, 207 left and 200 right femora, 180 left and 172 right tibiae, and 111 left and 113 right fibulae were used to calculate point estimates across the three sets of regression equations available in FORDISC® 3 19th-Century FStats and Trotter MStats).[3] For all equations, the Black male (BM), White male (WM), and Any male comparative groups were used, with the addition of Hispanic male (HM) for 20th-Century FStats.

When the stature estimates from the 19th-Century CStats equations were compared, WM consistently produced a point estimate closest to known stature (error of 0.94 to 1.42 inches between elements). The Any male equation results were significantly different from the WM estimates ($p < 0.01$, overall error of 1.21–2.24 inches, average difference of 0.5 inches), but consistently performed better than BM equation.

When the stature estimates from the 20th-Century FStats equations were compared, WM produced a point estimate closest to the known stature in 6/12 of element (error of 0.89–1.32 inches). The Any male equation produced a point estimate closer to the known stature in 4/12 elements (error of 1.04–1.46 inches), but all results were not significantly different from the WM estimates (< 0.25-inch difference). HM produced the point estimate closest to the known stature in 4/12 elements.

When the stature estimates from the Trotter MStats equations were compared, WM consistently produced a point estimate closest to known stature (error of 0.90–1.31 inches between elements). The Any male equation results were not significantly different from the WM point estimates (error of 0.92–2.07 inches). The Any male equation consistently performed better than BM equation (error of 1.64–2.89 inches).

The Any male equations produced values that did not significantly differ from the WM point estimates for White males using the 20th-Century FStats and Trotter MStats equations. Further, the Any male values often matched the error of the WM estimates in the FStats and MStats samples, and even outperformed the WM estimates for multiple elements in the FStats sample.

Future directions should evaluate more diverse populations, as the current study's impact is limited by the homogenous reported sex and ancestry of the test sample.

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Ancestry; Stature Estimation; Method Validation

A113   Evaluating the Prevalence of Torus Mandibularis and Torus Palatinus in Relation to Ancestry, Sex, and Age in a Modern Skeletal Sample

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Learning Overview: This presentation aims to inform attendees about the prevalence of Torus Mandibularis (TM) and Torus Palatinus (TP) in a modern skeletal sample and how such traits may covary to ancestry, sex, age at death, and their interactions. In addition, this study should fill some of the gaps in our current knowledge of TM and TP by examining a contemporary sample neglected in bioanthropological literature.

Impact Statement: This presentation will impact the forensic science community by helping attendees gain a better understanding of the prevalence of TP and TM and how such traits are linked to ancestry, sex, age at death, and their interactions. This quantitatively justifies the use of these skeletal traits in formulating biological profiles in forensic anthropological analysis.

Few features of the human skull have attracted the attention of anthropologists more than TP and TM. Historically, these distinctive oral bony outgrowths have been classified as useful "racial biological markers" because of their varying frequencies in specific populations and ethnic groups.[1,2] As a result, these traits have been adopted in forensic anthropology to estimate ancestry of unknown skeletal remains. When present, they are considered indicative of East Asian and American Indian ancestry.[3,4] However, their connection to sex and age at death remains unclear, and the effects of all independent demographic variables on their expression are weak.[4,5] Therefore, the validity of each trait in biological profile analysis must be carefully evaluated.

A contemporary sample of 307 individuals (148 females, 159 males), aged 21–107 years old, were analyzed from the osteological collection at the University of Hawai'i. The donors were classified into one of four subgroups: (1) African, (2) Asian, (3) European, and (4) Pacific Islander. TP and TM were scored based on: (1) presence or absence, (2) shape, and (3) size. The following multivariate model was used to express the possible relationships between the variables:

$$\text{torus expression} = \text{sex} + \text{ancestry} + \text{sex*ancestry} + \text{age} + \text{error}$$

"Torus expression" is the dependent variable, "sex*ancestry" is the interaction between those two categorical independent variables, and "age" is a continuous covariate. Ordinal and binary logistic regression were then employed to assess the effects of each independent variable.

A total of 65.1% (200 of 307 individuals) exhibited TP, while 34.2% (105 of 307 individuals) exhibited TM. TP was observed in 94 females (63.5%) and in 106 males (66.7%), while TM was identified in 50 females (33.7%) and 55 males (34.5%). However, statistical tests indicate that the effect of sex on the presence of TP or TM was not significant ($p > 0.05$). With respect to ancestry, TP was recorded in 50% (7/14) of Africans, 80.5% (66/82) of Asians, 55.2% (101/183) of Europeans, and 92.9% (26/28) of Pacific Islanders. These differences indicate a significant effect of ancestry ($p < 0.05$), with Europeans being far less likely to express TP than other subgroups. In contrast, TM was observed in 14.3% (2/14) of Africans, 32.9% (27/82) of Asians, 36.6% (67/183) of Europeans, and 32.1% (9/28) of Pacific Islanders. This indicates ancestry does not have the same impact on TM expression. Last, the two-way interaction between sex and ancestry was not significant for either torus, nor was the effect of age at death. Given these results, this study serves to contextualize the significance of these bony oral traits as indicators for ancestry, sex, and age at death of unknown skeletal remains in a forensic context.

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Human Biological Variation; Non-Metric Cranial Traits; Tori

A114   How Do Agencies Use Biological Profile Data? A Pilot Study Investigating Stakeholder Engagement With Forensic Anthropological Estimates

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Learning Overview: After attending this presentation, attendees will understand how medicolegal personnel view the various aspects of the biological profile estimated by forensic anthropologists as well as where opportunities for miscommunication can arise.

Impact Statement: This presentation will impact the forensic science community by highlighting areas where forensic anthropologists' findings may be differentially interpreted by medicolegal personnel, indicating a need for more direct engagement between forensic anthropologists and client personnel as well as more clarity in communicating findings.

Over the past several years, the field of forensic anthropology has begun reevaluating how biological profile estimates are generated, reported, and potentially affect case outcomes. Special attention has been given to estimation of ancestry or population affinity and consideration of gender in addition to skeletal sex. Notable points of debate often center on the terminology used to report classifications as well as how terminology or a category of the biological profile itself could affect decedent identification.[1,2] Despite extensive engagement on what is considered best practice in anthropological casework, no research has directly engaged with the mode of communication between anthropology producers and their clients who act on the information.[3-5] In this research, we present preliminary data from a Qualtrics® survey aimed at understanding how forensic anthropological biological profile data are used by consulting agencies, and if differences arise in the identification process when a forensic anthropologist's reported findings are translated into missing persons profiles by non-anthropologist stakeholders.

Medicolegal personnel were recruited via the *AAFS Newsfeed*, listservs, and social media to participate in an online, anonymous, Institutional Review Board (IRB) -approved Qualtrics® survey. This survey asked participants to review three mock forensic anthropology case summaries involving an unknown individual with no Believed-To-Be (BTB) match and report the demographics for that decedent according to their typical protocols (i.e., by creating a NamUs profile or other standard procedure). Overall trends in reported demographics were analyzed across cases, as well as responses to questions about the value of particular biological profile aspects, and forensic anthropology consultations in general, to making identifications.

In total, 41 individuals answered at least a portion of the survey (17 individuals completed the survey). These individuals consisted of medical examiners or pathologists, operations directors, death investigators, forensic anthropologists, forensic artists, and other medicolegal personnel from the Midwest, Southeast, Southwest, and Northeast United States.

Most respondents (73.17%, or 30 individuals) utilized NamUs, but around a quarter (26.83%, or 11 individuals) did not. In general, sex, age ranges, and stature ranges were entered verbatim as stated in the forensic anthropology mock case summaries (regardless of whether NamUs or another standard operating procedure was utilized). However, discrepancies did arise when ancestry was entered, with respondents frequently utilizing different ethnic classifications (Hispanic/Latino, Hispanic/Latino and Black/African American, Uncertain, Other) based on the same description. Additionally, when a mock case reported both skeletal sex and indication of gender (i.e., pelvic morphology consistent with male individuals observed in a decedent wearing women's clothing and exhibiting indications of facial feminization surgery), respondents again listed different classifications (Male, Female, Unsure, Other, or combinations thereof) for screening missing persons' lists. When asked to rank the importance of the components of the biological profile in making identifications, sex was listed as the most important, followed by age, then stature, and finally ancestry/population affinity. Notably, just under half of respondents (46.15%) stated that they would exclude a missing person from consideration if one or more of the forensic anthropologist's biological profile estimates did not match a potential candidate.

While preliminary, this research contributes to a better understanding of how biological profile data are applied in processes of decedent identification and furthers a dialog aimed at bettering communication and facilitating the more accurate use of forensic anthropological reports. Furthermore, it elucidates areas where disjuncture can occur between forensic anthropologists' phrasing and medicolegal personnel's interpretations of their reports and highlights opportunities for collaboration to improve decedent identification processes on both ends.

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Forensic Anthropology; Biological Profile; Ancestry Estimation

A115   Assessing the Reliability of the DSP2 Software for Skeletal Sex Assessment in a Modern Italian Reference Collection From Syracuse, Sicily

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Learning Overview: After attending this presentation, attendees will understand the potential of the DSP2 sexing tool, as it is shown that it is highly accurate on a modern Italian skeletal collection.

Impact Statement: This presentation will impact the forensic science community by illustrating the applicability of the DSP2 sexing tool on a modern skeletal collection. This further validates DSP2 as an invaluable tool with universal application.

Sex assessment is an essential step in forensic as well as archaeological settings when examining human skeletal remains. The os coxae has been deemed the most reliable skeletal element to assess biological sex as it is sexually dimorphic. A variety of methods exist for attributing sex, some relying on the differential morphology between the male and female pelvis while others employing osteometry. DSP (Diagnose Sexuelle Probabiliste) is a free online software that relies on ten measurements of the os coxae that are then compared to an extensive database in order to statistically estimate sex. In the original publication, it is stated that the software can confidently be used on any anatomically modern human populations, thus making it suitable for forensic as well as archaeological applications. When building the reference dataset, 12 populations were included. The more recent version of the software (DSP2) utilized two additional collections. Both the original and second versions claim an accuracy rate of over 99%. Somewhat lower but highly significant results were obtained when the method was validated on different populations. DSP2 was tested on 133 individuals from Crete, Greece, showing an accuracy rate of 85.6%, while an 88.34% accuracy rate was shown on a mixed Brazilian population. The software has also been successfully validated in a virtual environment as well as on Pre-Colombian mummies.

The aim of the current study was to test the accuracy of the software on modern Italian remains as this population was not included in the original reference dataset nor has it ever been tested. A total of 51 individuals were included, 35 males and 16 females, with recorded sex, age at death, and cause of death. The remains were recovered from a cemetery in Syracuse, Sicily, in 1909 and are currently housed at the Museum of Anthropology and Ethnology in Florence, Italy. When employing DSP2, an overall accuracy of 88.2% was recorded with 100% accuracy for females and 85.71% for males. However, it is important to note that no individuals were misclassified, but not all males were classified due to the software's 0.95 posterior probability of correct group assessment. When taking into consideration only the individuals who were classified by DSP2, the accuracy rate reaches 100% for both sexes.

Further statistical analyses were conducted to calculate inter- and intra-observer error using the Technical Error of Measurement (TEM), relative Technical Error of Measurement (rTEM) as well as the coefficient of Reliability (R). All ten measurements were taken three times per individual, which served as the basis for the assessment of intra-observer reliability. Significant results were obtained with the highest rTEM being 1.72% and the lowest R being 0.97. To determine inter-observer reliability, eight ossa coxae were measured by a second observer using the same TEM, rTEM, and R. As expected, higher error rates were recorded than for intra-observer reliability, with the highest rTEM being 2.15%, but R was equal or higher to 0.94 in every case. For both inter- and intra-observer error, the measurement showing the highest error rates was SPU.

Overall, the study concludes that DSP2 is an excellent and reliable method for sex determination in an Italian population and its use is highly recommended. This further solidifies the software's claim to non-population specificity.

Biological Profile; Forensic Anthropology; Osteometry

A116   Sex Estimation of Modern Greek-Cypriots Utilizing Measurements of the Calcaneus and Talus

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Learning Overview: This presentation utilizes a forensic approach to osteometrics from the calcaneus and talus to establish sex estimation models for modern Greek-Cypriots. After attending this presentation, attendees will better understand the applications of binary logistic regression in forensic anthropology for purposes of sex estimation and the benefit of considering a stepwise backwards approach to develop models for individual and combined skeletal elements.

Impact Statement: The research conducted will impact the forensic science community by showcasing the application of statistical methods in the development of population-specific standards for human identification. It will inform the forensic science community on the validity of sex estimation models for modern Greek-Cypriots.

Population-specific standards are very important in human identification due to the biological variation that characterizes human populations. In humanitarian forensics, the application of osteometry provides an objective level of validation for sex estimation. Proxy populations serve geographical regions where religious guidelines prevent access to human skeletal remains for research or war prevents research from being conducted. Metric sex estimation models on small bones such as those in the ankle are not as prominent as cranial and long bones studies. This research aimed to use measurements of the calcaneus and talus to estimate biological sex of modern Greek-Cypriot individuals to create population-specific reference data. Metric assessments were performed on a total of 270 calcanei (left = 134, right = 136) from 144 individuals (males = 70; females = 74) and on a total of 208 tali (left = 103, right = 105) from 118 individuals (males = 61; females = 57). A total of 27 measurements were taken, 13 for the calcaneus and 14 for the talus. The measurements were adopted from a combination of publications and names were adapted for database recording purposes.[1-8]

Binary logistic regression analysis using k-fold cross validation was utilized to determine if the measurements taken could predict biological sex. The independent-samples *t*-tests showed that all measurements conducted on the calcanei and tali are significantly smaller for females than males ($p < 0.05$). Additionally, approximately half of the measurements for both the calcanei and tali were significantly different than their antimeres ($p < 0.05$). The calcaneus showed classification accuracy of 75.83% and 79.97% for the right and left side, respectively. The talus showed a classification accuracy of 75.54% and 77.62% for the right and left side, respectively. Last, stepwise regression was utilized to exclude measurements not beneficial to the models. The reduced models showed increased accuracies for the calcaneus (right = 84.62%; left = 84.62%) and talus (right = 85.71%; left = 86.52%). Overall, the results indicate a good but not great validity for the classification of sex utilizing osteometric data, with the talus having higher classification accuracy than the calcaneus. Although sex estimation using these skeletal elements may be possible, results should be considered with caution and utilize reduced, more effective measurements.

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Sex Estimation; Forensic Anthropology; Osteometrics

A117   Sexual Dimorphism in the Human Mandible: The Application of 3D Statistical Shape Modeling

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Learning Overview: This presentation demonstrates the ability to distinguish sex through the human mandible using 3D statistical shape modeling. After attending this presentation, attendees will understand what 3D statistical shape modeling is and how it serves as a useful method in estimating sexes or age groups through bone morphology. Furthermore, attendees will gain insight into the morphological differences in the mandible between males and females in a 3D aspect, which is the main agenda of this presentation.

Impact Statement: This presentation will impact the forensic science community by illustrating that sex estimation through bone morphology could be made in quantitative method: the application of 3D statistical shape modeling. Moreover, this presentation fosters interdisciplinary collaboration between forensic anthropology and medical engineering in pursuit of accurate and precise identification methodologies.

Statistical shape modeling allows for the creation of an average 3D model specific to groups (such as by sex or age) and also produces various models through statistical shape analysis of a group. Therefore, the application of statistical shape modeling could facilitate a more quantitative and accurate method for sex or age estimation in forensic anthropological context. This presentation will illustrate sex-specific differences in the human mandible, based on statistical shape models, and evaluate their utility in sex classification.

The analysis utilized 3D mandible data, derived from Computed Tomography (CT) scans of 44 cadavers each from Korean adult male and female subjects. To concentrate on the morphology of the mandible, teeth and the alveolar bone were excised, followed by a necessary post-processing procedure. The processed mandible data was analyzed with *ShapeWorks*, an open-source software specialized in statistical shape analysis. Morphological differences between average mandible models of each sex were visualized using vector data and statistical significance was tested.

In comparison of the average shape models, there were no significant differences in the overall size between males and females. However, certain regions exhibited disparity. Notably, the mandibular condyle and coronoid process were more medially developed in males, resulting in a narrower overall width compared to females. In addition, males demonstrated lateral development at the gonial angle and superior development at the extramolar sulcus. The female mandible body itself appeared more anteriorly developed, suggesting a longer anterior-posterior length in females compared to males. Of these, the gonial angle region and extramolar sulcus demonstrated statistical significance ($p<0.05$). The classification learner in MATLAB® was trained based on the Principle Component Analysis (PCA) scores from the statistical shape analysis, and the Efficient Linear Support Vector Machine (SVM) yielded the highest classification accuracy of 76.1%. In this training scheme, Analysis of Variance (ANOVA) and Kruskal Wallis tests revealed that Principal Components (PC) 49 and 12 reflect important features in classifying sex. PC 49 represents morphological changes in the coronoid process, while PC 12 reflects changes in the mandibular condyle and gonial angle.

The present research suggests substantial insights of the sexual dimorphism in human mandible morphology and emphasizes the capability of statistical shape modeling in forensic anthropology. Moreover, this study could lay a groundwork for the development of a classification model based on mandible morphology.

Mandible; Sexual Dimorphism; Statistical Shape Analysis

A118　The Ontogenetic Development of the Preauricular Sulcus and Its Utility in Estimating Sex in Children and Young Adults With Considerations for Pregnancy and Parturition

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Learning Overview: After attending this presentation, attendees will have increased knowledge regarding the ontogenetic development of the preauricular sulcus.

Impact Statement: This presentation will impact the forensic science community by providing information regarding the utility of the preauricular sulcus as a sex indicator in children and young adults and whether pregnancy or parturition are likely to impact trait expression.

The utility of the preauricular sulcus in sex estimation has long been debated, and it has been suggested that this trait may be indicative of pregnancy and parturition. Recent studies have investigated these questions using adult samples and have found that the preauricular sulcus is sexually dimorphic and parity status is not likely to significantly impact trait expression.[1] To better understand the applicability of the preauricular sulcus in estimating sex in children and young adults, the trait's ontogenetic trajectory was examined using a large sample of postmortem computed tomography scans of known individuals (n = 583, m = 358, f = 225) aged 8 to 20 years from the Subadult Virtual Anthropology Database (SVAD). Eight years was chosen as the lower limit to match the age that females from the United States typically enter puberty, since hormone-mediated changes occurring in females at puberty lead to the differences in pelvic morphology that allow for differentiation between the sexes.[2] Trait expression was assessed following the three-stage scoring procedure outlined in *The Ontogenetic Subadult Sex Estimation System (OnSEt) Manual*, where a score of 1 represents pronounced expression, a score of 2 represents minor expression, and a score of 3 indicates the trait is absent.[3]

Two different approaches were used to explore the ontogenetic trajectory of the preauricular sulcus. The first was to determine when the sexes diverge in trait expression. Ridge plots of trait score frequencies for each sex and annual age cohort were generated to visualize when score frequencies first become disparate. Differences in trait score frequencies between subadult males and females were also evaluated using Chi-square or, for comparisons using small samples (n < 5), Fisher's exact test of independence with Monte Carlo simulation. The second approach was to determine the age at which full expression was reached in each sex. Full expression was considered attained when there was no further change in score frequencies with advancing age. Kruskal-Wallis with a Dunn's test and Holm's stepwise adjustment were used to determine the age at which score frequencies stabilized for each sex. The SVAD is comprised of cross-sectional data; thus, the preauricular sulcus of each individual in the sample was only evaluated once, and the onset of sexual dimorphism between the sexes and the attainment of full expression within the sexes was inferred based on changes occurring across chronological age cohorts.

Significant sex differences in trait score frequencies were observed by 13 years of age. Females 13 years of age and older were most frequently assigned a score of 1 (48.7%), followed by scores of 2 (36.1%) and 3 (15.2%). Conversely, males 13 years of age and older were most often assigned a score of 3 (66.3%), followed by a score of 2 (33.0%), with scores of 1 being rare (0.7%). Full expression was attained between 17 and 19 years for females. No significant differences in trait score frequencies were observed for males across any age cohorts, meaning full expression was already attained by 8 years.

The results indicate the preauricular sulcus is a useful trait for estimating sex in the young, with a score of 1 being strongly suggestive of female classification, and a score of 3 being fairly suggestive of male classification after 13 years. Although pregnancy and parturition information are not available for the sample used in this research, the high frequency of scores of 1 for many adolescent females strongly suggests that pronounced expression of the preauricular sulcus cannot be associated with parity alone, which is in agreement with studies that have investigated this question using adult samples.

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Juvenile; Puberty; Sex Assessment

A119   The Utility of the Maxillary Sinus in Estimating Biological Sex in a Modern American Population

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Learning Overview: After attending this presentation, attendees will: (1) be familiar with the maxillary sinus as a predictive element for estimating biological sex in forensic anthropology, and (2) be informed about automatic 3D model-based methods for measuring the maxillary sinus.

Impact Statement: This presentation will impact the forensic science community by: (1) providing recommendations on the potential of the maxillary sinus for predicting biological sex, and (2) addressing the current drawbacks and limitations in research on sexual dimorphism in the maxillary sinus.

Forensic anthropologists develop biological profile estimates (biological sex, ancestry, stature, and age) from skeletal remains to provide an investigative lead to law enforcement and, in turn, the public about the decedent. The maxillary sinus has recently been examined as a potential tool for estimating biological sex within forensic anthropology.[1,2] This current research examines biological sex variation of the maxillary sinus's volume and dimensions using a 3D model constructed from Computerized Tomography (CT) scans within a contemporary United States population. The research study aims to: (1) determine whether there are differences between the right and left maxillary sinuses, and (2) examine whether the maxillary sinus exhibits sexual dimorphism.

The reference sample (*n*=90) used for this research study included 45 identified males and 45 identified females. The sample was drawn from the New Mexico Decedent Image Database.[3]

The dependent variables of this study (maximum height, length, width, volume, and surface area) were examined using 3D modeling of CT scans through 3D Slicer® and MeshLab® following Robles et al. protocol and measured automatically in a uniform manner in Meshmixer®.[4]

Data were analyzed using JMP Pro16.1.0. Univariate descriptive statistics for the male and female samples were calculated for the linear dimensions (maximum length, width, and height), volume, and surface area. A paired t-test was used to test for significant differences between the right and left sides of the maxillary sinus. To test for sexual dimorphism between males and females, paired two-tailed t-tests were conducted using the measurements of the left maxillary sinus (given that there was no significant difference between the sides of the maxillary sinus).

Results of these t-tests reveal no significant differences between the right and left sides of the maxillary sinus or between the biological sexes ($p > 0.05$). The statistically insignificant results between biological sexes contradict international studies stating that the maxillary sinus is a tool that can be utilized for biological sex estimation.[5-7] However, these results could either indicate: (1) that sexual dimorphism within a United States population is lower than in international samples, (2) sample sizes need to be increased to account for more population variation and to better interpret results, and (3) automated measurements from 3D models differ from measurements taken from 2D measuring techniques.

The reliability of the Robles et al. method to construct and segment, and isolate the model, as well as the methods used to measure the maxillary sinus, were assessed through intra-observer error rates. Considering $p > 0.05$ for intra-observer error, intra-observer error was insignificant, indicating high reliability.

These results indicate that the maxillary sinus does not exhibit significant sexual dimorphism and, as such, may not be applicable in forensic anthropological casework. However, further validation studies of previous research are recommended regarding the: (1) collection of 2D versus 3D maxillary sinus measurements, and (2) automation of maxillary sinus measurements versus manual measurements. Such studies will aid in determining the validity of using maxillary sinus as a sex estimation method for forensic anthropological casework.

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Anthropology; Sex Assessment; Sexual Dimorphism

A120   Translating Results: Accessing Accuracy Within Sex Estimation Methods From the Biological Profile

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Learning Overview: This presentation will utilize the Forensic Anthropology Database for Assessing Method Accuracy (FADAMA), focusing specifically on sex estimation methods used within the United States to demonstrate the importance of assessing method accuracy utilizing a repository where the decedent-identified sex is known. After attending this presentation, attendees will better understand that individual sex estimation methods that are used in high frequency have high accuracy rates and are comparable to the case-report level accuracy found in earlier studies.

Impact Statement: This presentation will impact the forensic science community by indicating to forensic anthropologists that the most common sex estimation methods appear to have consistently high accuracy. However, when considering overall method validation, both reproducibility and reliability may vary for sex estimation methods. Although methods such as "generalized morphology" are consistently used in casework and provide high accuracy rates for the FADAMA study sample, forensic anthropologists should be cautious when utilizing this method due to its highly subjective approach. If generalized morphology continues to be used in casework, it should undergo reproducibility and reliability assessment using an appropriate study model (e.g., black box study) to establish its validity.

Forensic practitioners are able to utilize a variety of methods to draw conclusions for the estimation of the biological profile, with recent studies indicating accuracy for sex estimation to be around 98% at the *case report level*.[1,2] However, the accuracy for the sex estimation at the *method level* should also be assessed in order to compare to the collective outcomes as reported at the *case report level*. The present study explored method-level accuracy for some of the most frequently used sex estimation methods, based on a robust sample of United States forensic anthropology casework from FADAMA. FADAMA cases (n = 641; cases submitted to FADAMA at the time of download on July 9, 2023) were analyzed to infer the methods that had the highest frequency of application, and the top six methods were selected for further analysis. Accuracy (whether the practitioner's sex estimation matched the decedent's identified sex) was assessed for the six methods and yielded rates of accuracy between 97% to 100%, which is comparable to the 98% accuracy of case-report level sex estimation accuracy in a FADAMA sample.[3]

Generalized Morphology (GM) was one of the top six most frequently used sex estimation methods for the FADAMA dataset. GM is an approach where the forensic anthropologist chooses to focus on whatever aspect of the skeletal remains indicate sexual dimorphism to them, which may or may not incorporate non-metric traits associated with other methods and is not grounded in statistical analysis nor applies/considers the same features for each case. GM in particular stood out because, unlike other methods, it is not validated nor grounded in statistical inference, yet provided accuracy rates comparable to the other five studied methods, compelling further analysis to assess its validity. Thus, it was next assessed whether GM's accuracy rate remained high when being used in isolation (i.e., only method used to estimate for sex for given case, n = 52) as compared to when it is being used in conjunction with other methods (n = 150). Out of the 150 cases, accuracy was 99% when GM was utilized in conjunction with other methods and 96% when utilized in isolation. While the GM's accuracy appears to be consistently robust, accuracy is not the only component when establishing a method's reliability. GM continues to be utilized in practice, given that over 50 cases use it as the sole method for estimating sex and its application remains consistent even within the past decade of casework. As such, GM should undergo reproducibility and repeatability testing comparable to the "black box study" approach recommended for subjective methods in the 2016 President's Council of Advisors on Science and Technology Report.[4]

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Anthropology; Accuracy; Sex Assessment

A121   Creating Continuity: The Proper Handling of Unprovenanced, and Unidentified, Legacy Forensic Cases and Prevention of Future Mishandling

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Learning Overview: This presentation will provide a workflow for dealing with unprovenanced and unidentified forensic material encountered as a result of retirements, job vacancies, and mishandling. After attending this presentation, attendees will better understand how to deal with unprovenanced unidentified skeletal material of forensic significance in a laboratory or university setting.

Impact Statement: This presentation will impact the forensic community by highlighting the problem of mishandled forensic legacy skeletal materials, providing achievable solutions for handling such collections and preventing their existence in the first place.

Although increasingly more rare, human skeletal material from forensic cases may be retained in forensic anthropology laboratories when cases remain unidentified. In some instances, cases may remain unidentified, stored in labs for years while undergoing yearly reevaluations with new techniques. During periods of transition, such as retirements, changing of jobs, closing down, or consolidation of labs or departments, such "legacy" forensic cases can get lost, only to be found again by someone that has no knowledge of their existence. Currently, we are at an inflection point in the discipline, since for the first time in the disciplines' history, we have large populations of forensic anthropologists of retirement age that are beginning to give up their labs and step away from their practice. When continuity is lost, provenance information, report data, and even general knowledge of the remains existence may be lost.

Recently the Fresno State Forensic Anthropology Laboratory encountered a large forensic legacy skeletal collection lacking in any documentation. In the section below, we discuss: (1) encountering a skeletal collection and what first steps should be taken, (2) how to ensure that there is continuity while on the job, and (3) what to do at the time one departs a position when a replacement is uncertain.

If you encounter undocumented human remains, the first step is to review all cases to assess whether it is: (1) forensic, (2) archaeological, or (3) anatomical. Forensic significance can be established by the presence of modern skeletal or dental alterations, possible connections to historic/prehistoric populations, associated grave goods, or remaining documentation. One should prepare new peer-reviewed case reports on all material. C^{14} bomb testing is recommended for any remains in which the time of death is uncertain and samples for DNA testing should be submitted to the appropriate authority. Reestablish chain of custody by contacting all potential law enforcement entities and sharing an electronic drive containing an inventory, biological profiles, and photographs of the remains.

Creating continuity requires access to information by individuals outside of the laboratory space. Create a lab electronic repository (like Google® drive shared with others who are in a position to step in if needed). Make sure you have an accounting of all human skeletal material associated with your laboratory, both forensic and non-forensic, where it is located. This inventory should be placed in the electronic repository. Consider an outside audit to review standard operating procedures, handling and storage of material, equipment needs, etc.

When one leaves a position, whether by retirement or subsequent employment, this often means that both lab and secured storage is lost. If another qualified person or agency is assuming the responsibilities of casework, then the material can be transferred to their care. However, if that is not the case, the departing anthropologist must secure their cases so nothing is lost if needed in the future.

If the anthropologist is a consultant to a number of agencies, steps must be taken. (1) Skeletal material and other items, such as radiographs, should be returned to the consulting agency. (2) Case files should be digitized and copies sent to the consulting agency. Unless there is secured storage available, hard copies of the case files are often best returned to the agency as well. (3) Digitized copies of cases should be retained by the anthropologist in case their testimony is needed at a future date. (4) A "death plan" should be established so heirs know what should be done with any case material when the anthropologist dies.

Unprovenanced; Skeletal Remains; Continuity

A122   Identification Trends in Forensic Anthropology Cases at the New Mexico Office of the Medical Investigator

Nicollette S. Appel*, University of New Mexico, Glen Head, NY; Cris E. Hughes, University of Illinois at Urbana-Champaign, Urbana, IL; Paige Lynch, Environmental Archaeology Laboratory, Albuquerque, NM

Learning Overview: This presentation examines identification trends in forensic anthropology casework at the Office of the Medical Investigator (OMI) in New Mexico, building on recent analyses of United States forensic casework that examined how decedent demographics related to identification success by Hughes and colleagues.[1] After attending this presentation, attendees will understand that OMI decedent demographics and biological profile estimations do relate to identification rates and identification method for decedent race, but not sex and age.

Impact Statement: This presentation will impact the forensic science community by highlighting how agency-specific analyses provide insight into how decedent demographics and biological profile estimations are related to investigations. The identification trends presented also more broadly show how societal contexts may play a part in how identifications are made.

There are continuing dialogs among practicing forensic anthropologists as to whether decedent demographics and biological profile estimations, in particular population affinity, should be provided in formal reports to death investigators. Before broad changes are made to how forensic anthropologists contribute to the investigative process, extensive scientific analyses must be done to prove or disprove whether forensic anthropological estimates foster biased investigative outcomes.

Previous research examined forensic anthropology case data of more than 1,200 identified and unidentified persons broadly representing United States forensic anthropology casework.[2] The authors emphasized that trends are likely agency or region-specific, and thus more research should be performed at local agencies to compare the trends found in the broader United States study. The present study addressed this need by focusing on trends for the OMI. Based on the most recent annual report of OMI cases with known race/ethnicity information, 49% are a race/ethnicity other than White, Non-Hispanic. Within this group, 71% are Hispanic, 22% are Native American, 6% are Black, and 2% are Asian/Pacific Islander.[3] This large proportion of Black, Indigenous, and People Of Color (BIPOC) decedents provides an appropriate dataset to expand the questions of whether decedent demographics are related to identification success.

To infer whether differences in identification rate correspond with decedent demographics, comparisons using Fisher's Exact test were made between identification status and decedent race/ethnicity, sex, or age. The sample included individuals with a biological profile estimated by a forensic anthropologist from 2017–2023 at the New Mexico OMI (n=68). Further analysis with Fisher's Exact test examined the relationship between positive identification methods (i.e., DNA, dental records, fingerprint comparisons) and decedent race/ethnicity, sex, or age. This sample included all individuals that had a forensic anthropological analysis performed, regardless of whether a biological profile was estimated (n=182).

There is a significant relationship between identification status and decedent race/ethnicity (two-sided test, p value = 0.0055), with identification rates greatest for Native American (86% identified) and White (67% identified) decedents compared with lower rates for Black (28% identified) and Hispanic (25% identified) decedents. Identification methods also differed significantly among decedent races (two-sided test, p value = 0.003), with DNA identifications more common for Black and Hispanic decedents, and dental identification more common in Native American and White decedents. Fingerprint identifications were most common in Hispanic and Native American decedents. Decedent sex and age had no significant relationship with identification method or status. These findings indicate that decedent race has a significant relationship with both identification method and identification status for the OMI sample, further highlighting the agency-specific trends relating to investigative outcomes. Contextual factors potentially contributing to the observed trends will be addressed in this presentation.

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Anthropology; Casework; Identify

A123 Forensic Anthropology Casework and Outreach: 50 Years of Operational Trends for Chico State's Human Identification Laboratory

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Learning Overview: This presentation provides a historical perspective regarding Chico State's Human Identification Laboratory's (HIL) impact to California's medicolegal and first responder community through both casework and outreach. An emphasis on training, especially during the past two decades, has produced a substantial increase in cases submitted to the HIL. After attending this presentation, attendees will understand the pivotal role that outreach has on increasing casework and involvement in mass fatality responses.

Impact Statement: This presentation will impact the forensic anthropology community by demonstrating that outreach in the form of education and training has a dramatic effect on increasing casework and improving statewide disaster responses.

A recently published study finds several factors that affect forensic anthropologists' annual caseload.[1] Those most notable factors include the number of search and recovery reports produced annually, the publication record, and the institution where the doctoral degree was received. However, the study failed to identify one of the most important factors: outreach training and education.

Although beginning casework 50 years ago, Chico State's HIL initially received few cases. Some early years saw little to no casework. Over the decades, the annual caseload increased dramatically, especially in the past two decades. The first three decades of lab operations saw an average caseload of ten cases per year. The past two decades saw an average caseload of 70 cases per year. That rise in caseload is correlated with an expansion in outreach and training, especially since 2012. The past decade of lab operations has seen an increased average caseload of 112 cases annually. The largest single factor for the HIL's increasing caseload can be tied to medicolegal outreach and training. Between 1974 and 2011, the HIL worked with 52 agencies, steadily adding new partners over that period. By 2012, HIL faculty and staff significantly increased yearly training courses and workshops, most notably becoming instructors in homicide courses within California's Peace Officer Standards and Training system (POST). Between 2012 and 2022, the HIL added an additional 91 new agencies. Of the current agencies the HIL works with on cases and recoveries, 64% of the agencies were added after 2012.

California is currently seeing an increase in the frequency and size of mass fatality events. While wildfires are the largest disaster threat each year, flash floods, mudslides, and earthquakes have also contributed to California's mass fatality events. Chico State's increased partnership with agencies across the state is valuable for another reason: mass fatality response. The deadliest and most destructive wildfire on state record was the 2018 Camp Fire. Through California's mutual aid system, nearly every county in California sent resources to that event, including their search and rescue personnel. Local and state agencies' familiarity with the HIL allowed the lab to play a centralized role in victim recovery and identification from that event. That led to the HIL communicating with state officials on improving future disaster responses and again increased the resulting yearly trainings that the HIL provides. Since 2018, the HIL has trained more than 3,000 medicolegal professionals through annual continuing education courses and workshops and through "Just in Time" training on disaster sites. The HIL conducts annual outreach training for an array of medicolegal professionals and first responders, including: law enforcement agents (POST); death investigators and coroners (California State Coroners' Association); search and rescue personnel (Search and Rescue Workshops); state emergency managers (California Office of Emergency Services); cold case investigators (California Department of Justice's Missing and Unidentified Persons); and fire investigators. By regularly and frequently presenting to these groups, providing lectures, and conducting workshops, authorities remain aware of the laboratory's presence and expertise as well as its role as a statewide deployable resource.

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Training; Outreach; Casework

A124 **The Evolution of Forensic Anthropological Casework: Exemplifying the Importance of Routine Reanalysis of Long-Term Unidentified Remains at the Forensic Anthropology Center at Texas State (FACTS)**

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Learning Overview: After attending this presentation, attendees will better understand the necessity of systematically reviewing long-term unidentified human remains cases with new methodologies through a summary of casework reanalysis conducted at the FACTS. The assessment of updates to biological profile estimations with the use of new methods will be illustrated via a quantitative analysis of past and present case work.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of continued commitment and advocacy for the long-term dead and the significance of reviewing open cases in forensic humanitarian casework.

Forensic anthropologists in academic settings will sometimes store and maintain unidentified human remains following analysis until positive identification is assessed by the proper jurisdictional authority, enabling return to the next-of-kin. When these individuals remain unidentified despite forensic analytic efforts, they can be relegated to curation for an extended period pending updates, sometimes spanning decades. In these situations, proper stewardship and continued commitment to identification is paramount to resolving these cases and continuing to advocate for the long-term dead.[1]

When requested by jurisdictional authorities, FACTS consults on forensic cases and often maintains custody at the request of authorities until identifications are made. As of this writing, 58 forensic cases have been held at FACTS for more than five years with no further updates, with original dates of analysis for 22% of those cases occurring prior to 2005 and the earliest dating back to 1967. Several punctuated efforts have been made since then to reanalyze these cases and update all case reports, resulting in the majority of cases being examined at least twice. A select few were also included in a third round of analysis as part of an initiative started at FACTS in 2021 to systematically review all casework. This presentation summarizes the changes in resultant biological profiles over the course of all documented analyses. All assessments used the most current and accepted forensic anthropological methods and statistical programs available at that time.

Of the 58 forensic cases still in FACTS custody, 32 have been reexamined thus far, resulting in updates to all 32 biological profiles and one count of Minimum Number of Individuals (MNI). Changes made to all biological profile components and MNI estimations following each round of analysis were recorded. Any increase or decrease from the original range for stature (+/- 1 inch) and age (+/- 5 years) were noted as changed from the original estimate, and any modification from indeterminate to more specific (or vice versa), as well as complete change to a different category, were noted for both sex and population affinity.

After the first round of reanalysis in the 32 cases, approximately 28% of sex estimations, 75% of age estimations, 44% of population affinity estimations, and 37.5% stature estimations changed from their original results, and one case required a change to MNI. Following the second round of FACTS analysis, which included only six cases, approximately 17% of sex estimations, 83% of age estimations, 33% of population affinity estimations, and 17% of stature estimations required adjustments.

These updates have resulted in 16 of the 19 cases currently entered in the National Missing and Unidentified Persons System (NamUs) requiring official changes to their entries. The cases with recorded methodologies showed diverse changes in cited literature over time, with certain methods trending in similar years. However, changes to biological profile estimations notably evolved with different versions of software applied, exemplifying the impact of more inclusive samples and robust statistics.

These results emphasize the importance of revisiting all long-term unidentified casework with updated methodologies at regularly spaced intervals. Renewed efforts can result in more specific and/or accurate estimations due to new or improved methodologies, updated reference data, and increased practitioner knowledge and experience over time. As stewards of individuals who are often marginalized in life and are still vulnerable in death, forensic anthropologists can serve as advocates for the unidentified by implementing regular reanalysis of open cases.[2] By highlighting this ongoing reanalysis from an academic forensic anthropology laboratory, our hope is to continue the conversation of how to hone our strategies in identification efforts to best serve unidentified individuals in these settings.

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Forensic Analysis; Human Remains; Cold Case

A125   A Comparison of Forensic Anthropology Casework Across Two Urban, Midwest Medical Examiner Offices, 2013–2021

Erin B. Waxenbaum*, Northwestern University, Evanston, IL; Lindsay H. Trammell, St. Louis County Office of the Medical Examiner, Berkeley, MO; Anne Grauer, Loyola University Chicago, Chicago, IL

Learning Overview: After attending this session, attendees will have an appreciation for the similarities and differences in forensic anthropology casework between two urban, Midwest medical examiner jurisdictions: the Cook County Illinois Medical Examiner's Office and the St. Louis County Office of the Medical Examiner between 2013–2021.

Impact Statement: This presentation will impact the forensic science community by helping attendees appreciate comparative forensic anthropology casework between two geographically similar urban medical examiner offices and be able to more broadly compare data to better understand how forensic anthropology practitioners are utilized across jurisdictions.

Forensic anthropology has garnered greater attention in the academic and medicolegal community with more practitioners embedded in Medical Examiners and Coroners' (ME/C) offices and increased coursework and forensic-oriented graduate programs offered at colleges and universities across the country. However, practitioners situated within the medicolegal system often have varied roles and responsibilities, with few opportunities to compare the nature, breadth, and outcomes of their casework with others. Sharing data will provide foundations upon which resource distribution, planning, and needs assessment can be based, and provide essential information for training the next generation of professionals in the field.

The present analysis compares work conducted by three forensic anthropology practitioners across two jurisdictions: two working as part-time consultants and one employed full-time within a Medical Examiner's Office (MEO). In the Cook County MEO (CCMEO) of Illinois, two anthropologists (EBW and ALG) consult on a part time, case-by-case basis. The St. Louis County, Missouri MEO (SLMEO) employs one full-time forensic anthropologist (LHT) who worked concurrently as a medicolegal death investigator during the period under analysis.

With the objective of comparing the composition of forensic anthropology casework at the CCMEO and the SLMEO between 2013–2021, the following variables were recorded and compared: month of discovery, decedent depositional environment, condition of remains upon initial discovery, type of anthropology consult requested, time from discovery to consultation request, time from consultation request to analysis, and time from analysis to report submission.

Between 2013–2021, forensic anthropologists working at the CCMEO completed a total of 119 cases (13.2 cases per year) with SLMEO completing 339 cases (37.6 cases per year). The greatest number of cases occurred at the CCMEO in 2021 (n=19) and the fewest occurred in 2020 (n=8). For SLMEO, the greatest number of cases occurred in 2019 (n=61) and the fewest in 2013 (n=19). A majority of cases at the CCMEO were discovered in June (n=18) and the fewest in December (n =1), while at the SLMEO, the majority of cases occurred in April (n=35), with the fewest cases discovered in January (n=19). Most cases were outdoor, surface recoveries for both jurisdictions. Upon initial discovery, a majority of cases for the CCMEO were in a stage of decomposition (52.1%), while for SLMEO, a majority of cases were fully skeletonized (50.4%). While consult requests may include multiple components, for the CCMEO, 68.9% of requests included biological profile estimation, while for SLMEO, 32.7% included radiographic identification—a task assigned to full-time radiology technicians at the CCMEO. On average for the CCMEO, 77.8 days elapsed from discovery to anthropology consult, 60.8 days from consultation request to anthropology visit, and 13.2 days from anthropology analysis in person to report submission. For the SLMEO, 4.3 days elapsed from discovery to anthropology consult, 2.2 days from consultation request to anthropology analysis, and 7.6 days from anthropological analysis to report submission. This is a direct result of the difference between employment within vs. outside the MEO context and the type of consult request.

The role and breadth of forensic anthropology is expanding both academically and within the medicolegal community. Greater discussion and comparison of the range of services, support, and expertise that forensic anthropologists provide ME/C offices broadens the understanding of the field, allows for the formulation of best practices, and serves as data upon which decisions regarding protocol, funding, resources, and need can be based. With additional practitioners collecting and sharing their data, a clearer window into the scope and utility of the field will be appreciated by both our colleagues and the greater forensic scientific community.

Anthropology; Casework; Consultation

A126 Introducing the Forensic Science Research and Training Laboratory (FSRTL), a New Forensic Anthropology Research Facility at George Mason University

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Learning Overview: After attending this presentation, attendees will have a better understanding of the history, ongoing research efforts, and expected results as well as opportunities for collaboration afforded by the FSRTL at George Mason University (GMU).

Impact Statement: This presentation will impact the forensic science community by highlighting the ongoing processes and research at the FSRTL at GMU and offering opportunities to collaborate and advance knowledge in forensic taphonomy.

This presentation introduces the FSRTL, a human decomposition research facility at GMU. The human decomposition facility was first proposed in 2018, in recognition of the need for a forensic anthropology research facility in the mid-Atlantic region that contributes to a deeper understanding of the geographic variation in human taphonomy as well as develops practical forensic applications that aid federal and local agencies in the Washington metropolitan area. Approval from GMU was granted in 2020 and the facility was opened in 2021 on the Science and Technology Campus of GMU in Manassas, VA. The five-acre facility is located in a flat, wooded area and divided into an inner one-acre area dedicated to human decomposition research and an outer four-acre area for training and animal decomposition projects. Research to establish baseline environmental data has been completed to date.

A survey of the literature showed gaps in research that the FSRTL will fulfill. Using "forensic taphonomy" as the search keyword, we identified 107 entries in the *Journal of Forensic Sciences* between 1981 and 2023 on the Web of Science database. Of these, 23 (21.5%) were research articles or case studies conducted in human decomposition research facilities in the United States. The majority of the studies (12 studies, 52.2%) focused on decomposition rates, progression, or estimation of postmortem interval, while 30.4% (seven) of the studies reported scavenging patterns. Ongoing research at FSRTL will focus on the location and identification of human remains while supplementing research efforts with baseline data on geographic variation in scavenger activities and decomposition rates and progression.

At present, donors at FSRTL are accepted through the Virginia State Anatomical Program (VSAP), an agency within the Virginia Department of Health specializing in processing and receiving donations of human bodies for scientific study. The VSAP processes and transports the donors to the facility to be placed and provides documentation such as informed consent of donation, basic demographic information (sex, age, self-identified race, self-reported height and body weight), basic life and medical histories, as well as results of additional serology exams to GMU. The first donor is expected to be placed in the second half of 2023, with a second donor following before the end of 2023.

Plans are in place to collect more information regarding lifestyle, life, and medical histories, all of which will be made available in the future for research. Biological samples from donors will also be collected and stored for further DNA analysis. Current ongoing research efforts include establishing regional data regarding decomposition rates, progression, and scavenger activity, methods to identify clandestine graves, and methods to improve DNA recovery.

Finally, this presentation also provides information regarding the infrastructure that is in place to facilitate collaboration requests from interested researchers and future plans to develop data-sharing mechanisms.

Forensic Anthropology; Human Decomposition; Taphonomy

A127　Stakeholder Perspectives and End of Life Planning: The Future for Body Farms, Donation Programs, and Student Development

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Learning Overview: The University of South Florida (USF) Donated Skeletal Collection is a critical resource, ethically sourced, for the development and validation of methods, student research, and education. The aim of this presentation is to investigate why people pre-register, choosing a forensic program over other final resting options, to ensure the policies and practices positively impact research opportunities.

Impact Statement: This presentation will impact the medicolegal community who contribute to end-of-life care, illustrating the needs and concerns of families and donors while highlighting the positive role donated collections have on research opportunities and student development.

The USF Human Donation Program was created in 2016 and is among 12 such programs worldwide. The research facility consists of a living donor program in which people pre-register to become donors (n=300+); the outdoor training facility where individuals are buried or placed for decomposition (the Facility for Outdoor Research and Training [FORT], a.k.a. body farm); and the USF Donated Skeletal Collection, the permanent skeletal collection and repository of the remains available for research and classroom instruction (n=128). The objective of this research paper is to investigate why people pre-register, choosing a forensic program over other final resting options and how the policies and practices of the 12 forensic facilities compare to one another in terms of who is admitted into the program and the impact that has on the types of research opportunities available.

Data comes from two sources and was analyzed using quantitative summary statistics and qualitative method analysis based on participant responses. Life history data about economic status, occupation, and the reasoning for choosing a forensic donation program was collected from the USF donor packets (n=300). Many donors added written letters to the packet explaining their interest and motivation for choosing the program and these were included. Second, a questionnaire was completed by each of the program directors (n=12) regarding types of research, funding sources, access to collections, and applications of research to criminal investigations within their programs.

Interestingly, of the pre-donor packets that indicate a reason for choosing this program, the top three reasons (84%) include its high educational value, the opportunity for multiple family members to be part of the same collection, and the long-term commitment of the program. Further, most respondents self-identified as middle- to upper-class socioeconomic backgrounds (70%). Anatomical collections have been widely misinterpreted as consisting of individuals of only lower socioeconomic status with false assumptions made about their motivations (i.e., financial necessity). One of the first donors into the USF program was a local principal who dedicated his life to teaching. Upon his unexpected death, the wife donated his remains stating, "There's so much bittersweet in all of this. [He] wanted to continue teaching after his death. It would be my last gift to education, he'd say. This couldn't be more perfect." Another pre-donor said, "Being a retired homicide detective, I'm still very much intrigued with forensic anthropology," indicating an understanding of the direct value that donation programs and forensic anthropology have on homicide investigations.

Multiple members of numerous families are also opting into the program, showing that such donation programs are an integral part of the preparation that goes into end-of-life planning, with donors viewing the collection as their eternal family resting place. The growth of similar forensic donation and outdoor facility programs is shown to be imperative not only for the advancement of forensic research, but also for individuals who have made their wishes of donation clear in their end-of-life plans. One-hundred percent of the pre-donors who came into the program over the past year called us as they were entering hospice.

Similar trends are seen across most of the other forensic facilities surveyed. When policies of collections were compared, program variation was mostly around whether unclaimed human remains are accepted. Three facilities do accept unclaimed remains. This practice has been subject to ethical debates about research on individuals who have not granted consent and the limited potential of research applications without known life and medical histories. Programs also varied in terms of research practices, with only a few programs allowing destructive analysis such as experimental trauma research (33%) or collaborations with outside government labs for DNA research (50%).

Skeletal Remains; Body Donation; Decision Making

A128   The Forensic Taphonomy Education and Research Facility: An Interdisciplinary Educational and Professional Resource

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Learning Overview: After attending this presentation, attendees will have a better understanding of how a local forensic taphonomy facility has impacted forensic anthropological casework and professional development.

Impact Statement: This presentation will impact the forensic community by demonstrating practical interdisciplinary uses for forensic taphonomy facilities and provide baseline decompositional data for southeastern Louisiana.

Forensic taphonomy facilities primarily have focused on observing variation in rates of human decomposition to establish more accurate Postmortem Interval (PMI) estimations. Through body donation programs, forensic taphonomy facilities have explicated the progression of human decomposition, augmented donated skeletal collections, and created spaces for medicolegal professional development and interdisciplinary research. In 2019, the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Laboratory broke ground on a human decomposition research facility, and the associated body donation program was fully developed by the fall of 2022. Both the FACES Laboratory and the Forensic Taphonomy Education and Research Facility (FTERF) operate within the Department of Geography and Anthropology at LSU.

As of July 2023, the Human Body Donation Program has resulted in 21 registered living donors and 16 next-of-kin donations. Of the remains placed at the FTERF, 11 are male and 5 are female, with ages spanning from 26 to 91 years ($\bar{x}$ = 61 years). Individual weights ranged from 125 to 339lbs. ($\bar{x}$ = 191 lbs.) and statures ranged from 5'2" to 6'2" ($\bar{x}$ = 5'9"). Currently, the longest interval of placement at the FTERF is 167 days. Preliminary taphonomic observations include a color sequence with a high presence of orange and red discoloration, extended persistence of marbling into advanced decomposition, and cycling dehydration and rehydration of tissue from intermittent rainfall.

The FTERF is located in the Cfa climate zone (temperate climate with a hot summer and no dry season) according to the Köppen-Geiger climate classification system—similar to multiple other forensic taphonomy facilities.[1] However, climate zones do not account for all environmental variability (e.g., elevation or ecoregions). Uniquely, the FTERF is situated in eastern temperate forests (Level I ecoregion) on the Mississippi Alluvial Plain and Mississippi Valley Loess Plains (Level III ecoregions).[2-4] Additionally, being positioned on the Mississippi River floodplain, specifically the Hammond alloformation, soil texture at the FTERF extends from silty clay to medium sand.[5] Regional decompositional differences are likely present between forensic taphonomy facilities in the same climate zone; accordingly, local taphonomic data are a vital resource for PMI estimation in forensic anthropological casework.

While the FTERF does work toward the traditional goals of forensic taphonomy facilities, being situated in a dual-discipline department also directs the goals of the FTERF toward interdisciplinary education. Consequently, the FTERF provides instructional space for students in physical geography, paleoanthropology, archaeology, and forensic anthropology. Training is available for various geophysical methodologies, recognizing taphonomic agents for both paleoanthropological and forensic contexts, and human remains recovery techniques. The FTERF also produces data relevant to climatology, soil science, DNA and isotopic analysis, and forensic imaging.

Regarding professional development, 82 individuals received training at the FTERF (representing 17 undergraduates, 13 graduate students and undergraduate student workers, and 52 medicolegal professionals). Of the student workers, 10/13 subsequently found positions in graduate programs or employment in forensics or archaeology. By extending the resources of taphonomy facilities to other disciplines, students gained a more robust education that has resulted in diverse academic and professional opportunities.

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Taphonomy; Interdisciplinary; Education

A129   A Retrospective Review of the Skeletal Trauma Consultations at Western Michigan University Homer Stryker M.D. School of Medicine From 2018 to 2022

Ginelle K. Köcher, Reno, NV*

WITHDRAWN

A130   Considerations for Age Estimation Accuracy: Method-Derived Outcomes and Practitioner Interpretations

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Learning Overview: This presentation utilizes forensic anthropology case data to assess the performance (accuracy) of the most commonly applied age estimation methods in United States casework. After attending this presentation, attendees will better understand the accuracy of different aging methods and the importance of studying practitioner-based interpretations with the "black-box study" model.

Impact Statement: This presentation will impact the forensic science community by providing accuracy rates for methods and by highlighting the discrepancies between method-derived outcomes and the practitioner modifications/interpretations of these outcomes. Validation studies to date tend to focus on the explicit method outcomes, but practitioner adjustments can play a key role in improving the accuracy of the final age estimation outcomes on case reports and must be incorporated into validity studies.

Recently, Hughes and colleagues observed that case-report level accuracy for age-at-death estimations by United States forensic anthropologists was at 92%.[1] The present study compares this case report level accuracy to method level accuracy for the most commonly used methods in United States casework, based on the Forensic Anthropology Database for Assessing Methods Accuracy (FADAMA).[2] Method application frequency was analyzed for n = 641 cases and found only 15 methods with an application rate greater than 45 cases (based on cases submitted to FADAMA at the time of data download on June 6, 2023). Accuracy (whether the known decedent age fell within the age range yielded by a given method) was assessed for each method. Of the 15 methods, only four yielded accuracies greater than or equal to the 92% documented for case-report level accuracy. The other 11 methods produced accuracy rates ranging from 54% to 91%, with six of these below 70% accuracy, indicating that caution should be used in the application of these methods' specific outcomes to casework. In fact, this disconnect between highly accurate age estimations at the case report level compared to the poor performance at method level suggests that practitioner interpretation of the methods is a critical step for increasing the accuracy rates of the combined age estimations as reported on the final case report. Additional findings further support this hypothesis—the study next examined whether case report-level accuracy was positively correlated with the number of aging methods used in a case.

For the cases that used at least one of the most common 15 methods, the median number of methods used in a single case is three (100% range is one to seven methods). Cases using a single method as well as cases using up to six methods all have comparable rates of accuracy for age estimations at the *case report level* (n =525, Fisher's Exact 2-sided test p = 0.93; 80-90% accuracy). The findings of the present study clearly suggest that practitioners are interpreting and modifying specific method outcomes in ways that improve the overall accuracy of age estimations on their final reports. The implications for these findings greatly alter how forensic anthropologists should proceed with validation studies of age estimation methods and should be evaluated using "black-box studies" comparable to those outlined in the 2016 President's Council of Advisors on Science and Technology Report.[3] Such approaches will render a more representative picture of the validity of the methods that have substantial practitioner interpretation.

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Anthropology; Age Assessment; Accuracy

A131   A Regression-Based Machine Learning Framework for Adult Skeletal Age Estimation

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Learning Overview: After attending this presentation, attendees will understand and be aware of the potential of regression-based approaches for skeletal age estimation, both in the construction of point-estimate and predictive interval models.

Impact Statement: This presentation will impact the forensic science community by increasing awareness of novel computational approaches and open source tools.

Age-at-death estimation of adult human remains poses significant challenges in forensic anthropology and medicine, in particular for the elderly. The intricate nature of adult age estimation arises from the interplay of biological and methodological complexities. Current anthropological approaches to age estimation suffer from limitations, including sparse array of skeletal traits, overemphasis on specific traits like the pubic symphysis, and the absence of a formal procedure to combine information from multiple skeletal parts, thus failing to reflect individual variation.

To address these prevalent methodological issues in adult skeletal age-at-death estimation, a novel scoring protocol for age-related skeletal traits and a machine learning regression-based framework have been developed. The regression-based approach uses deep random neural networks for point estimates and conformal prediction theory to construct predictive intervals for age-at-death estimates. Deep random neural networks use randomization techniques and multiple layers to improve feature extraction while balancing generalization and computational efficiency. Conformal prediction is a machine learning technique that quantifies prediction uncertainty, aiding the assessment of confidence and predictive interval construction.

Through results based on a rigorous cross-validation analysis, this presentation will demonstrate that age estimation from skeletal remains can be accurately inferred across the entire adult age span, yielding a mean absolute error of approximately six years. Moreover, this approach facilitates the generation of informative estimates and prediction intervals specifically tailored for the elderly population. Attendees will have access to open-source software tools developed using R and C++ programming languages, which they can apply to their own datasets to produce accurate models based on machine learning techniques.

This report will also present the Deep Random Neural Networks for Adult Skeletal Age-At-Death Estimation (DRNNAGE) tool, a software that operationalizes the proposed regression-based framework and provides a graphical user interface to a computationally intensive approach to adult skeletal age estimation. The comprehensive inclusion of multiple skeletal traits and the consideration of individual variation through statistical modeling of uncertainty for predictive intervals provide a robust and reliable tool for forensic anthropologists and medical professionals. DRNNAGE serves as a pivotal advancement in accurately determining the age at death of adult human remains, thereby enhancing the accuracy and precision of biological profiling in forensic investigations.

Age Assessment; Machine Learning; Identify

A132   Cranial Masculinization: How Age Progression Affects the Accuracy of Sex Estimations in Adults

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Learning Overview: This presentation will look at primary methodologies used for sex estimation in forensic anthropological casework by examining both metric and non-metric methods. After this presentation, attendees will better understand how various methods are impacted by cranial masculinization caused by aging processes and the ideal method to use depending on an individual's age. Additionally, this research will look at which specific cranial landmarks used for metric and non-metric sex estimation methods are significantly affected by cranial masculinization.

Impact Statement: This research will impact how forensic anthropologists estimate sex in future casework, especially for older individuals. These findings will aid in making biological profiles more accurate and increase positive identifications, promoting equal justice for victims of all ages.

While there have been studies on sexual dimorphism in human crania, there have been few studies that take post-puberty age-related changes into consideration. A majority of age-related studies conducted tend to focus on the physiological changes that occur from childhood to early adulthood. Thus, different sex estimation methods have been developed based on physical developmental milestones. Regardless of multiple studies showing there are also age-related changes that occur throughout adulthood, methods post-puberty are the same for the remainder of an individual's life. Some of these post-pubertal changes are referred to as cranial masculinization, an aging process caused by a variety of factors leading to increased thickness and robusticity of different parts of the cranium in both males and females that minimizes sexually dimorphic features.[1] This research aims to further evaluate the changes caused by cranial masculinization in adults and how these changes impact the accuracy of commonly used sex estimation methods in forensic anthropology.

The accuracy of metric and non-metric cranial sex estimation methods was tested using a portion of the Florida Institute for Forensic Anthropology & Applied Sciences' donor collection (n= 84) at the University of South Florida. Donors were divided into three age cohorts: 36–50, 51–70, and 71+. Metric measurements were taken following the Data Collection Procedures for Forensic Skeletal Material 2.0 guidelines before being input into FORDISC® 3.1 to estimate sex.[2,3] All measurements and non-metric scoring using the mandible were excluded due to the significant inter-observer error that has been previously reported.[4] The Walker and Langley et al. non-metric sex estimation methods were also tested.[5,6] Current literature regarding cranial masculinization suggests the accuracy of all three sex estimation methods were expected to be impacted, especially for older age cohorts. Research also suggests predicting sex will be more accurate when using metric compared to non-metric methodologies.[7]

The sex of each donor was unknown prior to testing each methodology. The accuracy of each method was then analyzed using McNemar's test, with a few statistically significant results. Lastly, each measurement and trait were analyzed using a Multivariate Analysis of Variance (MANOVA) to better understand the interactions between age and sex. The MANOVA test showed that multiple measurements were significantly impacted, especially when age was a factor. Landmarks located on the hard palate and cranial base were more affected by age than any other region. Both the Walker and Langley et al. methods were less accurate in the 51–70 and 71+ age cohorts as compared to the 36-–0 cohort.[5,6] Overall, the Langley et al. method was far more accurate in estimating an individual's sex than the Walker and metric methods.[5,6] Based on this study's results, the methods and landmarks used when estimating sex should be altered to increase the accuracy of their biological profile, especially when an individual is assumed to be older than 50 years of age.

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Sex Estimation; Cranial Masculinization; Craniometrics and Non-Metric Traits

A133 Unaccompanied Minors Automatic Forensic Age Estimation (UMAFAE): Applying Artificial Intelligence-Based Methods to Estimate Legal Age in Undocumented Minors

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Learning Overview: After attending this presentation, attendees will understand that Artificial Intelligence (AI) is a very useful complementary tool when estimating the age of minors without family references since it allows obtaining precise and accurate information in a very short time and with less effort on the part of the experts involved in the process of evaluating the subject's identity.

Impact Statement: This presentation will impact the forensic science community by informing attendees that although most age-estimation methods perform reasonably well and are widely used in the scientific community, all of them rely on manual measurements or classifications, which require much time and effort. There is also a high degree of subjectivity (both intra- and inter-observer) in the dental evaluations obtained from some classical methods. Deep learning methods can be used to automatically and accurately predict someone's chronological age, especially in those undocumented minors arriving in Europe without any valid proof of identity, and to efficiently classify between subjects younger and older than 18 years of age.

Legal age estimation is essential for the identification of undocumented minors seeking asylum. In the European Union context, recommendations have been developed by the interdisciplinary German Study Group on Forensic Age Diagnostics (AGFAD) and the European Asylum Support Office (EASO) in order to standardize the assessment procedure and to implement quality assurance. However, due to the absence of good practices, the process is often subjective and inaccurate.

The main aim of the European Union-funded UMAFAE project is to develop and validate methods for estimating legal age by applying AI techniques in order to increase accuracy while reducing estimation times and subjectivity. For this purpose, we are collecting and processing the largest-ever data set of radiographic images (dental and carpal radiographs, computer tomographies of the chest, and cone-beam computed tomographies of the maxillofacial region) to train and validate AI-based methods. The inclusion criteria are: an age range between 14 and 25 years; known biological sex and age; and good quality of the images obtained. In addition, images of subjects with diseases of endocrine or congenital origin, alterations in dental and bone development, subjects with orthodontic history, or with bone trauma in the joints of interest (sternoclavicular, and wrist joint complex) are included to determine the degree of influence of these variables on the accuracy of the algorithms.

In this preliminary work, a sample of 10,848 Orthopantomographies (OPGs) from several countries (Bosnia, Botswana, Chile, Dominican Republic, Kosovo, Lebanon, Malaysia, Italy, Turkey, Uganda, Japan, and Korea) was used to train, validate, and evaluate an AI model for age estimation. As a first step, all images were pre-processed to remove duplicates, inconsistencies, and background noise. We have also segmented the lower left third molar to compare the results using the full OPG (best option) and the third molar only. Several Convolutional Neural Networks (CNN) architectures were trained using those images and metadata (sex and ethnicity) in order to estimate age. Our results in the test set (for the best model in validation) showed a Mean Absolute Error (MAE) of 1.15 years and an accuracy of 88.94% for legal age estimation (an individual age is >18 or not). Our findings are in agreement with previous studies but with a significant difference: we are validating our model on a sample composed of 12 different populations, not only one. Additionally, we are trying to simulate a real scenario by estimating age in samples that have never been seen by the model; that is, the training data does not contain any image from that sample. More specifically, the model showed an MAE of 1.20, 1.49, 1.50, and 1.43 years and an accuracy of 89.77%, 80.32%, 84.81%, and 80.95% for four samples from Russia, Ethiopia, Australia, and Egypt, respectively. We have also developed a model that penalizes age overestimation so that a minor is never classified as a legal adult. This model gets an MAE of 1.77 years and an accuracy of 81.49% in validation, but has less tendency to wrongly classify a minor. Since further experiments are still being performed these results should be considered only preliminary.[1-3]

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Human Identification; Age Estimation; Artificial Intelligence

A134 Macroscopic Differences in Adult Human Femora Are Linked to Body Mass Index

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Learning Overview: This presentation will demonstrate the utility of whole-bone femoral shape in estimating the Body Mass Index (BMI) category for the adult biological profile.

Impact Statement: This presentation will impact the forensic science community by demonstrating a relationship between whole-bone external femur shape and the BMI category and obesity status.

Body mass is generally not included when constructing a biological profile from skeletonized human remains because of the lack of precise and accurate estimation methods.[1,2] This is especially true for obese or emaciated individuals. Bone functional adaptation theory argues that bone tissue responds to mechanical strain in order to maintain skeletal element strain within acceptable limits.[3] Increases in body mass result in relatively higher loading of lower limb skeletal elements, producing strain levels that elicit bone formation and potentially alter the external shape of skeletal elements. This project examines the feasibility of establishing BMI category from external femur shape.

Eighty human femora that are part of the Texas State University Donated Skeletal Collection (TXSTDSC) curated by the Forensic Anthropology Center at Texas State University were used in this study.[4] Forty femora are from female donors and 40 femora are from male donors of known body mass and stature at time of death. For the female sample, we selected 20 individuals with a BMI of 19–27 ("moderate") and 20 individuals with a BMI greater than 29 ("high"). In the male sample, we selected 20 individuals with a BMI of 18-26 ("moderate") and 20 individuals with a BMI greater than 29 ("high"). Femora were scanned on a Canon® Aquilion Precision CT scanner, housed in the Division of Cardiology, Department of Medicine, at the Johns Hopkins Hospital. Triangular mesh surface models were generated from image stacks and all surface models were resampled (~25,000 vertices) to improve triangulation. Variation in femoral shape was quantified using a 3D landmark analysis based on the coherent point drift algorithm.[5] Shape variation was summarized using principal components analysis and BMI groups were compared for component scores.

Statistically significant shape differences ($p < 0.05$) were detected in the male sample between moderate and high BMI categories. In the male sample, the high BMI group femora were associated with greater proximal diaphyseal breadths as well as lower anteversion angles. These shape differences between BMI categories were also detected in the female sample, although they only approached statistical significance ($p = 0.065$). These results demonstrate the potential for incorporating external shape of the femur in estimating BMI for the proposes of improving the biological profile.

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Anthropology; Biological Profile; Body Mass

A135   A Critical Validation of Dental Morphology as a Method of Ancestry Estimation in a Historical Forensic Context

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Learning Overview: After attending this presentation, attendees will have a better understanding of the accuracy of dental morphology methods in forensic casework for ancestry estimation and the factors that influence the success of the methods in forensic application.

Impact Statement: This presentation will impact the forensic science community by validating two major dental morphology methods used to estimate ancestry when applied to a forensic context and will highlight both intrinsic and extrinsic factors that may influence the application of the method.

As the field of forensic anthropology weighs the potential benefits and harms of ancestry estimation on a more theoretical level, the shortcomings of existing methods are being reevaluated to assess their practical value with a more critical eye. The preference for craniometric and morphoscopic methods of ancestry estimation has left dental morphology applications relatively undiscussed. The advanced training necessary to score the traits the dental methods are based on may be contributing to their relative disfavor in case work application. When applied, there are two major methods used with distinct considerations. Edgar only classifies individuals into three groups, severely limiting its application in blind forensic casework.[1] Scott et al. was not developed with a modern sample, so it has unknown classification accuracy in forensic application.[2]

Because casework conducted at the Defense POW/MIA Accounting Agency includes a comparison of antemortem records that report ancestry, the application of dental morphology methods can be tracked and assessed with relative ease compared to casework in other forensic contexts. Although dental morphology is not commonly used in the agency overall, a sample of 78 resolved cases using the Edgar or Scott et al. methods was used to identify trends of method application and performance in a historic forensic context.[1,2] Classifications were compared to the reported ancestry of each identified individual based on antemortem records to determine method accuracy.

Edgar was used in 41 cases (3 Black, 1 Hispanic, 37 White males) with an 80.5% overall correct classification accuracy and 89.2% for White males specifically.[1] Scott et al. was used in 38 cases (1 Asian, 2 Black, 1 Hispanic, 1 Native American, 33 White males) with a 60.5% overall correct classification accuracy and 63.6% for White males.[2] Because so few of the cases involved reported ancestry groups other than White, the correct classification accuracy for other groups was not calculated.

Despite the overall accuracy of the Edgar method exceeding that of the Scott et al. method by 20%, a Fisher's Exact Test indicated that the performance of both methods was not significantly different (p=0.08) in this sample.[1,2] The average number of traits used with both the Edgar and Scott et al. methods was 13 traits, with a range of 2–20 and 1–21 traits, respectively.[1,2] A comparison of the number of traits used and the accuracy of the application resulted in a Point-Biserial Correlation Coefficient of 0.230 (p= 0.16) for Edgar and 0.035 (p= 0.83) for Scott et al., indicating a weak relationship for Edgar and no relationship for Scott et al., but neither were statistically significant.[1,2] The results suggest the performance of the methods is more complicated than simply meeting a threshold value of traits. Analyst confidence in the methods and choices during application are possible factors affecting performance.

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Method Validation; Misclassification; Teeth

A136   . . . and There Were Only Teeth: Postmortem Interval Estimation Based on XRD and FTIR

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Learning Overview: After attending this presentation, attendees will consider the possibility of determining the Postmortem Interval (PMI) on teeth samples based on imaging, non-invasive techniques, X-Ray Diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy.

Impact Statement: This presentation will impact the forensic science community by presenting, for the first time, a new approach to determining the PMI of skeletonized remains on the hardest tissues in the body, teeth, leading to accurately distinguishing among remains in the latest stages of decomposition.

PMI is the time elapsed between the physiological death of an organism and its examination. This constitutes one of the most challenging parameters for estimating in forensic science. Early Postmortem Interval (EPMI) is easier to determine based on physicochemical changes in the body, like rigor mortis. In contrast, Late Postmortem Interval (LPMI) is more difficult to estimate, particularly in skeletonized remains. The study of PMI in the latest stages of decomposition, on skeletonized remains, has been focused on the application of proteomics and other physicochemical techniques, like XRD and FTIR. There are several studies assessing PMI on bones applying these techniques, since they are easy to perform, fast, inexpensive, non-destructive, and show good accuracy in estimating PMI. Despite the potential application of these techniques, there are no studies focused on teeth samples. Teeth are the hardest tissues in the human body and remain after everything else has decomposed. Thus, they could be the samples of election moreover when dealing with fragmentary remains and remains exposed to different environmental insults.

The aim of this work is to assess changes in the mineral composition of human teeth applying XRD and FTIR and correlate these changes with the PMI to improve the accuracy of this estimate in latest stages of decomposition.

Forty healthily erupted human adult teeth (molars and premolars) were obtained from dental clinics and stored at times 0, 10, 25, and 50 years. Teeth were cleaned and pulverized with liquid nitrogen using a 6770 Freezer Mill. Then, 100mg of tooth powder were subjected to XRD analysis to determine crystallographic indices (crystallinity and crystal size) and 100mg of tooth powder were used to perform FTIR Attenuated Total Reflectance (ATR) analysis and determine different indices: Mineral to organic Matrix ratio (M/M); Carbon to Phosphate ratio (C/P); mineral crystallinity; and collagen maturity.

According to the XRD results, there are differences among PMIs: both crystallinity and crystal size increased with the PMI. With respect to the FTIR analyses, M/M ratio increased with the PMI, and in contrast, the C/P ratio decreased with the PMI, not finding differences on the other two indices. Applying Pearson correlations, it was possible to demonstrate a positive correlation among crystallinity, crystal size, and M/M ratio with the PMI, and a negative correlation between C/P ratio with the PMI.

The prediction models developed from these variables indicated a high accuracy for the estimation of PMI, particularly at 10 years and 50 years, with success rates of 87% and 80%, respectively.

Overall, these results demonstrated for the first time the feasibility of using XRD and FTIR to determine the PMI on the hardest tissue in the human body, the teeth, and their accuracy to distinguish between long-term PMIs, allowing the application to severely decomposed bodies. Future research will be able to expand these findings, increasing the number of teeth and assessing the impact of environmental and inherent factors on the determination of PMI.

Teeth; Postmortem Interval; FTIR

A137   Geofor: A Forensic Taphonomy Database for PMI Estimation Using a Machine Learning Model

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Learning Overview: After attending this presentation, attendees will understand the limitations of the current state of Postmortem Interval (PMI) estimation in forensic anthropology and how machine learning models provide a more accurate estimation for time since death. Attendees will also learn how to register and use the geoFOR application—a forensic taphonomic database designed to provide practitioners with a platform to enter cases and receive an automated PMI estimation.

Impact Statement: This presentation will impact the forensic community by demonstrating how the geoFOR application can be used to provide a PMI estimation using observations of human remains and associated weather data through a machine learning model.

Estimating the time since death, or thePMI, poses a significant challenge to forensic scientists when human remains are discovered because of limited reliable methodologies, particularly for cases in advanced decomposition. Current PMI estimation methods lack scientific rigor and are often inconsistent, which can impede the successful identification of unknown human remains and the reconstruction of events around the time of death. Despite decades of research, existing studies regarding PMI estimation are significantly hindered due to reliance on small sample sizes, environmental homogeneity, and inconsistent definitions of the stages of decomposition.

To transcend longstanding methodological issues, we developed an ongoing collaborative forensic taphonomy reference database that utilizes ArcGIS® and machine learning models to deliver improved PMI estimations. This tool offers forensic practitioners a platform to enter case information, including demographic data and uniform descriptions concerning characteristics of decomposition, insect, and scavenger activity. The app automates the collection of weather data from the location of discovery using the Global Historical Climatology Network (GHCN) through the National Oceanic and Atmospheric Administration (NOAA). The database currently contains over 2,600 cases derived from medicolegal death investigations and human decomposition research facilities across the United Stattes. Data collection is ongoing as new and existing collaborators enter case information. The size and comprehensive nature of the geoFOR data allow for the application of machine learning methods for estimating PMI. After users submit the details of their case, the app delivers a PMI prediction using a statistically robust regression model. The advanced cross-validated machine learning PMI predictive model results in an R^2 value of 0.8 and users receive a predicted PMI with an 80% confidence interval. Results will be presented as a decision tree from the machine learning model to illustrate how the characteristics of decomposition are used to predict PMI.

This novel method for PMI estimation can help successfully narrow the search parameters for unknown decedents, which can expedite identification and more accurately inform us about the circumstances surrounding their death. The geoFOR app complies with recommendations from the National Institute of Standards and Technology (NIST) for the Standard for Taphonomic Observations in Support of the Postmortem Interval (2022) through the use of a data-driven model that accounts for a wide variety of environmental factors and decomposition characteristics. GeoFOR also follows an Open Science Framework through data sharing that promotes methodological integrity and reproducibility. These principles encourage fairness, equity, and inclusion within the research community.

Postmortem Interval; Machine Learning; Taphonomy

A138   Skeletal Element Recovery Rates From Outdoor Surface Contexts in Louisiana

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Learning Overview: This presentation provides skeletal element recovery rates for outdoor rural and suburban residential contexts in Louisiana and examines variation in recovery rates according to environment, dispersal, who collected the remains, and estimated Postmortem Interval (PMI). After attending this presentation, attendees will better understand how variables of interest affect skeletal element recovery rates and will have empirical data to help guide future research in methodologies for bioprofile skeletal assessment.

Impact Statement: This presentation will impact the forensic science community by providing skeletal element recovery data for forensic anthropology casework from outdoor contexts in Louisiana and by addressing how environment, dispersal, estimated PMI, and who collects the remains affect recovery rates. Such information will provide empirical data to help guide future research in methodologies for bioprofile skeletal assessment.

Human remains subject to postmortem taphonomic processes are affected with regard to both survivability and recoverability. A recent publication discusses the distinction between the two notions and urges forensic anthropologists to make available skeletal element recovery rates for their casework, with a rationale that such documentation can provide an empirical basis to guide forensic anthropological research.[1]

Heeding this appeal, the current study: (1) provides skeletal element recovery rates for select casework from Louisiana, and (2) examines variation in recovery rates according to environment, dispersal, who collected the remains, and estimated PMI. Data were gathered from case files at the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Laboratory. Cases included in this study were recovered between 1980–2023 from outdoor surface contexts in rural or suburban residential areas. Skeletal element recovery data were collated from bone inventories. Other variables, such as the environment in which the remains were found, the minimum and maximum estimated PMIs (PMI_{e-min}, PMI_{e-max}), whether the remains were dispersed or non-dispersed, and whether the remains were recovered by forensic anthropologists or medicolegal personnel, were gleaned from case reports, bench notes, and scene photographs.

Most skeletal elements were recorded as present or absent, and if paired, separated by side; the cranium was considered a single unit. For ribs, vertebra type, carpals, Metacarpals (MC), manual and pedal Phalanges ($Phal_{man}$, $Phal_{ped}$), tarsals, and Metatarsals (MT), only the total number of bones collected was recorded. Descriptive statistics were computed for the collective sample and for dispersed and non-dispersed subsamples. Inferential statistics were also computed to assess variation in skeletal element recovery rates among the other variables of interest. Significant differences among categories were noted if p values were less than .05.

The research sample included 152 cases. The cranium was most frequently recovered (96%, n=146); the hyoid least recovered (26%, n=39). In general, skeletal elements from the torso, and the larger elements from the limbs, were recovered in higher frequencies (88, 63%); smaller skeletal elements were recovered in lower frequencies (57, 26%). Chi-square tests showed that recovery rates were significantly different between non-dispersed (n=47) and dispersed (n=105) cases for all elements except the cranium; therefore, other inferential results are reported only by subsample.

Unsurprisingly, recovery rates for non-dispersed cases were high (72–100% for 35/36 skeletal elements); only the hyoid had a relatively low recovery rate (43%). Chi-square tests revealed no significant differences in recovery rates based on who collected the remains or environment. Student's t-tests indicated significant relationships between recovery rates and PMI_{e-min} and PMI_{e-max} for only 5/36 and 4/36 skeletal elements, respectively; in these cases, an inverse relationship was noted between recovery rate and estimated PMI.

For the dispersed sample, recovery rates were lower than, but mirrored the pattern for, the complete sample. Chi-square tests showed significant differences in recovery rates for 15/36 skeletal elements based on who collected the remains, and for 6/36 elements based on environment. For the former, forensic anthropologists consistently recovered more elements than other medicolegal personnel; for the latter, wooded areas impeded recovery rates more than other environments. Student's t-tests indicated significant relationships between recovery rates and PMI_{e-min} and PMI_{e-max} in 32/36 and 33/36 skeletal elements, respectively; an inverse relationship also was noted between recovery rates and estimated PMI for these elements.

In conclusion, results from this assessment of skeletal element recovery rates corroborate those found in previous studies and provide empirical information that may help guide research into methodologies for bioprofile skeletal assessment. Moreover, these data suggest that environment, estimated PMI, and who collects the remains primarily affect recovery rates in cases where remains are dispersed in wooded environments.

Reference:
[1.]   Scott S, Jantz RL. Survivability versus rate of recovery for skeletal elements in forensic anthropology. *J Forensic Sci*. 2022;67:1758-1765. https://doi.org/10/1111/1556-4029.15087.

Taphonomy; Remains Recovery; Outdoor Surface Contexts

A139 Small but Mighty: A Controlled Observation of Magpie Scavenging Among a Cohort of Human Remains in the Rocky Mountain Region of Colorado

Christiane I. Baigent*, Sothern Illinois University Carbondale, Fairplay, CO

Learning Overview: After attending this presentation, attendees will understand the longitudinal composite of environmental, skeletal, and soft tissue change catalyzed by scavenging magpies.

Impact Statement: This presentation will impact the forensic science community by describing a longitudinal, controlled observation of magpie scavenging, facilitating distinguishment of magpie behavior from the forensically relevant corvid groups with which their behavior is typically grouped.

Black-billed magpies (*Pica hudsonia*) are recognized for their diverse and voracious feeding habits.[1] True omnivores, the magpie diet ranges from fruit and grain to active hunting of small mammals and invertebrates. Carrion is a preferred food source, demonstrated by the adaptive behavior of tracking human and non-human game hunters and exploiting their discarded carrion.[1,2] Because many corvid species cooperatively scavenge, avian scavenging has typically and necessarily been documented in sum, as cooperation precludes species-specific description of scavenging pattern, and behavior.[3,4] Longitudinal controlled study of environment-specific rate and pattern of human decomposition affords the appended opportunity for controlled study of associated scavenger behavior. Toward that end, magpie scavenging among a group of human donors is reported, with an emphasis placed on intraspecies continuity in behavior, and the temporal, anato-spatial, and archetypical presentation of magpie-mediated osseous and soft tissue change, summarily yielding a higher resolution model of magpie scavenging as distinct from typically associated corvid groups.

The impact of magpie scavenging was investigated at Colorado Mesa University's Forensic Investigation Research Station high-altitude satellite facility (FIRS-TB40). FIRS-TB40 is 9,500 feet (2896m) above mean sea level, in the Rocky Mountain Region of Colorado. The local environment is characterized by a complex composite of environmental and meteorological variables that summarily produce a harsh hypoxic environment distinguished by long winters and persistent snow overburden. The structure of scavenger guilds and the behavior of individual species is largely dictated by local environmental variables, among the most prominent of which is protracted overwintering resulting in limited mobility with concomitant reduction in home range size, scarce and limited resources, and metabolism slowed by physiological stress and plasticity.

Magpie scavenging was observed within a cohort of 12 human donors opportunistically placed between March 2020 and October 2021. Field observation persisted through November 2022. Human donors were placed unclothed, supine, on a 45° slope in 10'x10' steel cages and monitored by game cameras programmed to collect both time lapse and motion-activated photographs. The presence of magpies was established through game camera capture, feather shed, scat, and tracks.

Baigent et al. described the succession and progression of scavenger guilds among a porcine cohort placed at FIRS-TB40 in the summer of 2018.[5] Cooperative and competitive behavior between avian groups was observed. While vultures and ravens scavenged cooperatively, magpies demonstrated inter- and intragroup aggression. Subsequent construction of scavenger cages acted as a deterrent to vultures and ravens, precluding access to human remains and affording magpie radiation into the role of apex scavenger. Temporal specificity was not observed in magpie scavenging, though voracity increased in late fall and persisted throughout overwintering. Both primary pattern and mode of tissue removal were distinct, as were the passive byproducts of their behavior. Primary patterns of magpie scavenging included: (1) boring; (2) muscle mining initiated via transdermal tissue plug removal; (3) longitudinal tissue peeling; (4) ribboning of connective tissue; (5) trabecular mining; and (6) both focal (active trabecular mining) and passive skeletal destruction. Additionally, a propensity for focal soft tissue scavenging was observed before progression to a novel intracorpus source was undertaken. Passive byproducts of magpie scavenging included: (1) peripheral soil excavation; (2) beak raking and superficial talon stamping; (3) pseudoburial; and (4) commingling and displacement of skeletal elements.

Due to their propensity for cooperative scavenging, the environmental, soft tissue, and osseous change catalyzed by "avian" scavenging has necessarily been understood in sum. Site infrastructure facilitated the establishment of magpies as the apex scavenger at FIRS-TB40, affording the opportunity for controlled observation of soft tissue and osseous scavenging, yielding a distinct pattern of categorical change.

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3. Sincerbox SN, DiGangi EA. *Taphonomic Signatures of North American Scavengers: Family Corvidae—Corvids—Magpies, Crows, and Ravens, Forensic Taphonomy and Ecology of North American Scavengers, MA*: Elsevier, 2018; 89-94.
4. Komar, D., Beattie, O., 1998. Identifying bird scavenging in fleshed and dry remains. *J. Can. Soc. Forensic Sci*. 31(3), 177-188.
5. Baigent C., Connor MA., Dabbs GR. Introducing Forensic Investigation Research Station (FIRS-TB40): Scavenger Succession and Progression at a High-Altitude Site in Colorado. *Proceedings of the American Academy of Forensic Sciences*, 71st Annual Scientific Meeting, Baltimore, MD. 2019. 25:127.

Scavenging; Taphonomy; Human Remains

A140   The Scavenging and Scattering of Remains: An Experiment to Inform Forensic Practice

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WITHDRAWN

A141   The Fluvial Transport of Human Remains: Forensic Application of an HEC-RAS Model for Predicting Search Parameters for Human Remains Recovered From the Sacramento River, California

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Learning Overview: After attending this presentation, attendees will understand how the relationship between river flow rates and the fluvial transport of human remains can be used to model search parameters for river victims.

Impact Statement: This presentation will impact the forensic community by discussing the use of actualistic research and a hydraulic engineering model to predict fluvial transport rates and search parameters for human bodies deposited in riverine systems. This research provides a tool for forensic practitioners and law enforcement to locate human remains more rapidly in the Sacramento River.

When a deceased person is introduced into a dynamic fluvial system, their remains can be transported a substantial distance from their original point of entry. Search efforts to locate bodies in fluvial systems often involve searches by divers near the point of entry, the use of side scan sonar and underwater cameras, and boats. For search teams, identifying search parameters for deceased persons in bodies of water is especially challenging and involves significant expenditures of time, money, equipment, and personnel.

To date, few studies have been conducted to model the movement of human bodies in riverine systems, such as the Sacramento River. The Sacramento River is California's largest fluvial system, with the highest flow rates in winter and the lowest in late fall. Every year, the river claims several victims due to drownings and boating accidents, with a small number being suicides and homicide body dumps. Our historical study of cases (1972–2012) found a moderate relationship between postmortem interval and transport distance among cases with known points of entry and exit (Pearson's $r=.583$, $p< .001$; $r^2=.34$, $n=66$).

In addition to the case data, this study used two types of rescue mannequins to simulate "sinker" and "floater" body positions (between River Miles 199–80). For the sinker trials, data were collected manually on transport distance for submerged mannequins, and flow velocities and depth were recorded using an acoustic doppler velocimeter. The substrate was also photo-documented and described in field notes. The data indicated minimal transport distances for sinker mannequins, especially under low flow conditions and in shallower waters. The river substrate was also an important factor. For sand/gravel substrates, velocities over 2.0ft/sec were sufficient to move the mannequin whereas cobble substrates required velocities over 2.7ft/sec. For the floater trials, mannequins were released under low (< 6,000cfs), medium (6,001–9,000cfs), and high (>9,000cfs) flow rates. To maximize coverage, the study area was divided into 20 sub-reaches, with three independent trial runs conducted per location for each flow rate condition. For many of the trials, mannequin Global Positioning System (GPS) tracking devices provided real-time data.

The hydraulic simulations used the Army Corps of Engineers Hydraulic Engineering Center River Analysis System (HEC-RAS), which was originally designed for flood control management. The model was used to predict distance from the source given several parameters, such as flow rate conditions and river channel properties, and was calibrated using the mannequin transport data. The model was used to develop SacRiverSearch, a web-based application that can predict search areas for cases where entry location and approximate time of death are known parameters. The search box includes a 95% prediction interval beginning with the float stage of a deceased person. Although developed specifically for the study area, this model can be adapted to other rivers.

Search and Recovery; Taphonomy; Spatial Modeling

A142   Standards for Analyzing Skeletal Trauma in Forensic Anthropology

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Learning Overview: After attending this presentation, attendees will understand the importance of scientifically valid methods for skeletal trauma analysis, why many do not meet *Daubert* guidelines, and the importance of conducting skeletal trauma analyses that meet current published standards.

Impact Statement: This presentation will impact the forensic science community by reviewing the scientific validity of skeletal trauma research. It will discuss recommendations from the national Standard for Analyzing Skeletal Trauma in Forensic Anthropology, recently published by the American Academy of Forensic Sciences' Academy Standards Board (ASB).[1] Additionally, future research and initiatives in skeletal trauma analysis needed to meet the needs of the medicolegal community will be identified.

Skeletal trauma analysis is an integral part of a forensic anthropologist's requested examination of human remains in a medicolegal setting. However, the scientific foundation for trauma analysis, including its underlying theoretical and methodological approaches and the empirical data which support them, has undergone limited critical review. This has resulted in an absence of clarity and uniformity in method application, including an unknown degree of error associated with skeletal trauma analysis methodology. It has also led to subjective and over-interpretation of trauma evidence. For these reasons, skeletal trauma analyses often do not meet the *Daubert* guidelines for legal admissibility of scientific evidence, which can lead to uncertainties about the probative value of skeletal trauma evidence.

This study summarizes an evaluation of over 250 anthropological publications focused on skeletal trauma for their scientific validity and merit. This includes a systematic review of antemortem and acute/perimortem-focused skeletal trauma publications from the *Journal of Forensic Sciences* (1987 to 2023), *FSI International (*2010 to 2023), *FSI Synergy* (2019 to 2023), and *Forensic Anthropology* (2018 to 2023). Data collected from these publications focused on the use of a scientific approach, including defined terminology, clear and repeatable methodology, known error of methods used, and consideration of confounding variables affecting skeletal trauma presentation.

Results of this study indicate that, of the total (n=251) publications examined, the majority (70.5%) were classified as research articles, followed by technical notes (14.7%), case reports (7.6%) and literature reviews or retrospectives (7.2%). Research articles and technical reviews were scrutinized for their use of a scientific methodology; the following key deficiencies were identified: (1) misunderstanding, misuse, or absence of standardized skeletal trauma terminology or definitions (24.7%); (2) unclear, subjective, or inadequate methodology for trauma morphology observations and recording (32.3%); (3) unclear, subjective, or inadequate assessments of trauma mechanism (27.9%); (4) over-interpretation of skeletal trauma data beyond the supporting empirical evidence (58.4%); and (5) non-existent or inadequate inclusion of the limitations of the skeletal trauma study, including assessments of method error and validity as well as a consideration of the numerous intrinsic and extrinsic variables affecting skeletal injury and subsequent trauma interpretation (69.5%).

Standard 147 provides recommendations for skeletal trauma methodology, based on objective (empirically based) measures of fracture data observations, analyses, and interpretations. The use of macroscopic, microscopic (e.g., histology, light microscopy), imaging (e.g., CT, radiography) and other modalities for accurate trauma identification and interpretation are also recommended. The importance of meticulous distinctions between trauma description and interpretation is emphasized. Standardized trauma definitions are also provided.

In addition to these standard requirements, the authors recommend recognition and evaluation of error, bias, and limitations of methodological approaches in future skeletal trauma analyses, based on empirical data (such as that derived from controlled experimental design) in order to mitigate *Daubert* admissibility challenges. They also encourage the development of more formal training opportunities for forensic anthropology practitioners in skeletal trauma methodology, analysis, and interpretation, including an understanding of the legal restrictions and constraints associated with trauma interpretations. Finally, the importance of an interdisciplinary theoretical approach to understanding skeletal trauma, founded upon anthropological, anatomical, and biomechanical principles and functional perspectives, is also discussed.

Reference:
1.   Standard 147. *Standard for Analyzing Skeletal Trauma in Forensic Anthropology*. American Academy of Forensic Anthropology. ANSI Academy Standards Board (2023).

Anthropology; Trauma; Standards

A143   The Use of Outline Analyses to Assess Kerf Floor Shape Patterns in Saw Marks

Alexis L. VanBaarle*, Michigan State University, East Lansing, MI

Learning Overview: After attending this presentation, attendees will have a better understanding of the relationship between kerf floor shape and saw class characteristics.

Impact Statement: This presentation will impact the forensic science community by providing an objective, quantitative analysis of kerf shape that can be referenced in dismemberment casework and future research.

In cases of dismemberment, forensic anthropologists assess the bony cut surfaces (i.e., kerfs) to estimate information (i.e., saw class characteristics) about the potential tool used, which can then aid in investigative and legal proceedings. One such variable, known as kerf floor shape, is defined as the incomplete kerf wall and floor profile. Previous research indicates that saw class characteristics such as tooth shape, saw set, and saw power can be deduced from the kerf floor shape. However, these studies are based on visual categorizations of kerf shapes, introducing subjectivity, and limiting statistical assessments. This study aimed to use Elliptical Fourier Analysis (EFA) to assess the relationship between kerf shape and saw class characteristics quantitatively and objectively.

The study sample consists of 133 incomplete kerf profiles made in 19 anatomically gifted, macerated human limbs using a diverse set of 19 saws. Entrance and exit profiles of the incomplete cuts were captured with a stereomicroscope and cropped at a depth of 3mm to produce a closed outline. The outline images were then imported into SHAPE ver.1.3 to perform an elliptical Fourier analysis to quantitatively represent the kerf shape, followed by Principal Component Analysis (PCA). Eighteen PCs (99% of variation) were exported and analyzed using IBM® SPSS. Multivariate Analyses of Variance (MANOVAs) were performed on all PCs to assess whether there was a significant difference between entrance and exit kerfs, and then to assess the effects of saw set, power, and tooth shape on overall kerf shape. When significant, Analyses of Variance (ANOVAs) were used to investigate which PCs were contributing to the differences and cross-validated stepwise Discriminant Function Analyses (DFA) were performed to evaluate classification accuracy.

There was no significant difference between entrance and exit defects (p=0.31). Significant results (p<0.001) were obtained for all saw class characteristics, indicating tooth shape, saw set, and power each affect kerf shape. PC 2, capturing "W-shaped," flat, and rounded kerf floors, was significant for all three characteristics. PC1, capturing relative kerf width, contributed to power and set, but not tooth shape differences. DFA produced a model 88.0% accurate in assigning tooth shape. Rip saws tended to display relatively flat or stepped kerfs compared to crosscut saws that displayed W-shaped kerfs. DFA classified saw power with 89.5% accuracy. Mechanical saws displayed on average relatively wider kerfs that lacked W-shaped floors, while hand saws displayed narrower kerfs, with and without W-shaped floors. Classification of saw set, however, was relatively low, with only 59.4% correct overall.

The EFA results generally support anecdotal relationships presented in the literature and support the utility of kerf shape in forensic analyses. Results indicate that kerf floor shape (i.e., squared vs. W-shaped) can reliably estimate saw tooth shape and power with at least 88% accuracy. Estimations of saw set, however, are lower and this variable should not be estimated from kerf shape without further investigation. Kerf shape does not vary significantly between entrance and exit profiles. Note that kerf width is indirectly included in analyses given the standardized kerf depth, and PC1 (representing relative kerf width) did contribute to differences in power, but not tooth shape predictions. Overall, these results provide statistical support to kerf shape assessments and provide a foundation for future methodological approaches.

Anthropology; Sharp Force Trauma; Dismemberment

A144   Deep Learning Models for Fracture Detection and Segmentation in Bone Histology

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Learning Overview: This presentation will describe how deep neural networks can be used to automatically detect and segment fracture margins in whole slide images of decalcified cranial bone samples. After attending this presentation, attendees will understand how Artificial Intelligence (AI) can be applied to casework to enhance a practitioner's workflow.

Impact Statement: This presentation will impact the forensic science community as it is the first application of AI based on deep neural networks to histological fracture detection and segmentation, an integral first step in utilizing AI-enabled approaches for estimates of Post-Traumatic Survival Time (PTST).

As AI becomes more established and prominent in diverse fields, researchers should look toward utilizing this technology to benefit their respective fields. For example, digital pathology has implemented the AI subfield of deep learning to enhance diagnostic accuracy and efficiency, most notably in the oncologic application of identifying and quantifying cancerous cells and lesions. As researchers strive to establish methods to reliably estimate the PTST from fracture histomorphology, the capability of deep learning to detect patterns from a training dataset to interpret new data should be explored. This project is the first step in employing such models for histological fracture aging by developing deep learning approaches for the detection and segmentation of fracture margins in histological photomicrographs.

Several image analysis tasks are required in the analysis of a photomicrograph, including detection of the Region Of Interest (ROI), ROI segmentation, and ROI classification. In the context of analyzing a fracture photomicrograph for PTST, detection entails identifying the area of fracture, segmentation is demarcating that area, and classification is estimating the PTST. The study sample consisted of 120 photomicrographs of fractured cranial bone each annotated with a bounding box to demarcate the general fractured area and a precise contour outlining the fracture margins. The photomicrographs were divided into training (N=94) and testing (N=26) samples. Two approaches to automatic fracture detection and segmentation were developed and tested: a Faster R-CNN model and a Segment Anything Model (SAM). The Faster R-CNN uses two submodules: Region Proposal Network (RPN) and shared convolutional features. The RPN proposes the possible locations of the ROI with different aspect ratios to improve object localization, and the shared convolutional features allow reuse of the same set of features for different bounding boxes to enhance and streamline object detection. SAM is a versatile segmentation model pretrained on an extensive image dataset. Its architecture consists of three core components: the feature extractor, responsible for capturing essential features from the input; the prompt encoder, which converts the input bounding box into a meaningful embedding representation; and the mask decoder, which combines the extracted features of the object within the given bounding box to generate an output mask.

After training, the Faster R-CNN model was able to correctly detect the fracture bounding boxes with mean Average Precision (mAP) of 84.61% at Intersection over Union (IoU) threshold of 40%. To generate the precise contour of the fracture, the predicted bounding boxes from the Faster R-CNN model were used as input prompts in the generic SAM model to produce precise contours of the fracture defect. Initial qualitative observations of the SAM predictions show promise, with precise localization of the fracture in 7 of the 26 samples. As SAM has not been fine-tuned specifically for fracture photomicrographs, there is potential for substantial improvement with domain-specific training.

These results highlight the challenges of histological fracture PTST research broadly. The limited sample size makes it difficult to divide the sample for training and testing while maintaining an adequate training sample that represents the variety of fracture morphologies. Moreover, variations in bone samples due to the size, shape, composition, age of the individual, type of injury, and histological stain further make the task challenging.

As fracture detection and segmentation are the foundation of PTST estimates, future work will focus on refining deep learning models to improve accuracy, fine-tuning SAM for fracture photomicrographs, and data augmentation to provide a more robust training sample. This will allow for the second phase of study to commence: the development of the PTST estimation algorithm.

Post-Traumatic Survival Time (PTST); Fracture Histomorphology; Machine Learning

A145   Unexpected Antemortem and Perimortem Rib Fractures in Infants Without Other Significant Injuries

Erin Chapman*, Erie County Medical Examiner's Office, Buffalo, NY; Katherine Maloney, Erie County Medical Examiner's Office, Buffalo, NY; Tara Mahar, Erie County Medical Examiner's Office, Buffalo, NY

Learning Overview: The goal of this presentation is to describe five cases of infant death in unsafe sleep environments in which unexpected rib cage trauma was identified during postmortem examination.

Impact Statement: This presentation will impact the forensic science community by highlighting cases of unexpected trauma in infants and the need for gross and microscopic examination of osseous tissues, especially in the rib cage.

Studies have shown that the gross examination of osseous structures provides a better understanding of the timing and mechanism of injury, especially in juveniles.[1] Five cases of infant death were examined in which an anthropological analysis of the rib cage revealed numerous antemortem and perimortem rib fractures. Cases were selected based on the presence of healing and acute fractures in the absence of evidence of abuse or other significant traumatic injury to the deceased. In all five cases, unsafe sleep environments were confirmed. In all cases, there was no documented history of abuse or Child Protective Services reports. The infants ranged in age from 1 to 4 months old.

A total of 46 antemortem and 93 perimortem fractures were noted in the five infants. Antemortem fractures were predominately concentrated in the posterior rib cage. In all five cases, untrained and/or trained individuals administered manual Cardiopulmonary Resuscitation (CPR).

Although the current study includes antemortem and perimortem fractures, the authors were most interested in the potential cause(s) of the healing injuries. Studies have shown that anterior-posterior compression of the rib cage is necessary to create fractures in the posterior neck of the infant rib.[2] The mechanism of these injuries are consistent with the posterior migration of the rib shaft, which creates a Type II lever between the transverse process of the vertebra and the articulation with the vertebral body and rib head. The posterior migration of the rib shaft creates tensile force that acts upon the rib neck, causing initial failure on the visceral surface of the neck.[2] In other words, normal or appropriate manual CPR performed on an infant should not create posterior rib fractures. However, Love et al. have shown that unintentional anterior-posterior compression of the rib cage occurs in some cases during manual CPR administration in infants.[3]

In all cases, the administration of CPR does not account for antemortem healing fractures in the infant rib cage. The antemortem posterior fractures appeared to be consistent with anterior-posterior compression of the rib cage with initial failure in tension on the ventral neck or head of the rib at the terminus.[2,4] Posterior rib fractures of the neck have a high specificity for abuse; however, similar patterns may occur in accidental situations if the essential components exist.[2]

The cause of these antemortem injuries remains unknown. However, the cause was likely accidental in nature, given the information gathered during the investigation and postmortem examinations. The authors suspect that bed sharing and prior non-fatal rollovers on a soft surface may account for at least some of these healing injuries. The question remains whether or not the necessary posterior migration of the rib shaft happens when anterior compression of the rib cage occurs when the child is supine (in a rollover scenario) due to the soft surface beneath them.

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Trauma; Infant Death; Antemortem

A146 Whole Body Fracture Patterns From Blunt Force Trauma Vary Within Similar Experimental Loading Events

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Learning Overview: After attending this presentation, attendees will have an appreciation for interdisciplinary research in skeletal trauma analysis by learning how experimentally driven biomechanical validation of injury mechanisms can strengthen interpretations of observed trauma in forensic casework. Additionally, the importance of utilizing experimental data to explain differences in injury patterns will be emphasized.

Impact Statement: This presentation will impact the forensic community by demonstrating the need for caution in over-interpreting injury patterns. Further interdisciplinary analyses of skeletal trauma in experimental human specimens are needed to better understand fracture mechanisms and specific causes for differential injuries.

A 31% increase in deaths between 2010 and 2019 was identified for older occupants in car crashes in the United States.[1] In near-side impacts, specifically, small, elderly female occupants were identified as the most at-risk population for fatality from thoracic injury.[2]

Ten Postmortem Human Subjects (PMHS) were included in this study. All PMHS were elderly females over 60 years old (61–94 years), approximately 5th percentile in height (147–167cm) and weight (40–56kg), with osteopenic lumbar spine areal bone mineral Density (aBMD) in order to target a potentially frail demographic. Near-side impact sled tests were conducted that mimicked real-world motor vehicle crash boundary conditions. Boundary conditions for the series included an intruding driver's side door that was controlled using the Advanced Side Impact System (ASIS) on a HYGE™ sled. The sled acceleration matched the acceleration profile of the impacted vehicle with a delta-V of 50kph, while four pneumatic cylinders of the ASIS, covered by a door liner, produced realistic door intrusion. Each subject was seated on a mass-production driver seat, equipped with a side airbag and standard three-point restraint with a pretensioner. Subject seating and positioning were tightly controlled in a realistic driving posture to ensure highly repeatable tests.

Nine PMHS experienced rib fractures, particularly on the impact side, but the locations varied with most fractures occurring anteriorly, and some posteriorly. The upper ribs were most affected, and six PMHS had rib fractures on the non-impacted side. Injury severities ranged from *none* to *severe* according to the Abbreviated Injury Scale (AIS), and number of rib fractures per PMHS ranged from 0–20 rib fractures. Three PMHS had sternum fractures, five had upper extremity fractures, and seven had lower extremity fractures. One PMHS experienced no injuries at all.

The same loading conditions experienced by all ten PMHS resulted in extreme variability in fracture location and severity. This demonstrates that a great number of fractures throughout the body is not necessarily associated with a different or more severe traumatic event when interpreting fracture mechanisms. Common assumptions regarding loading direction, speed, or severity based on overall fracture patterns may not be reliable in trauma interpretations of blunt force trauma.

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Fracture; Blunt Force; Trauma

A147   Structural Violence in Orleans Parish, Louisiana: Implications for Forensic Anthropological Casework

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Learning Overview: After attending this presentation, attendees will have a better understanding of assessing forensic anthropological casework through the lens of structural violence in Orleans Parish, LA.

Impact Statement: This presentation will impact the forensic community by providing an examination of contemporary forensic anthropological case work for evidence of structural violence and how that may assist with the identification of unidentified remains.

In biological anthropology, structural violence refers to how the body, often the skeleton, is affected by systemic imbalances in access to health care, socioeconomic resources, and death care among different populations.[1,2] Osteological evidence often consists of skeletal pathology, antemortem injuries, and perimortem trauma. Since systemically engendered suffering in everyday life (i.e., structural violence) has accumulative effects on the body, anthropologists also consider injury recidivism, or the aggregate of traumatic lesions accumulated by an individual, in research that addresses structural violence.[3,4]

The current study explores the concept of structural violence in contemporary populations living in Orleans Parish (OP) using data culled from the forensic anthropology case files of the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Laboratory. Information on skeletal injury recidivism (considering both antemortem injuries and perimortem trauma) and recovery location were used to assess OP cases to identify patterns of structural violence that potentially could assist with generating leads for cases of unidentified human remains.

Case files from OP in which the decedent was identified, spanning 1991 to 2021, were selected for assessment (n=41). The sample consisted of 32 full anthropological analyses and nine trauma-only analyses. The sample included 31 males and 10 females; ages ranged from 18 to 89 years, with one individual of unknown age. Additionally, 26 individuals identified as Black, 10 as White, 2 as multiracial, 2 as Asian, and 1 as Hispanic. Assessment of case files involved examining reports for: (1) the number of antemortem skeletal injuries, (2) the presence of perimortem trauma, and (3) the type of perimortem trauma. Additionally, data from the United States Census Bureau (demographics and income) were collected for comparison with case file data to identify patterns regarding demographic and socioeconomic variables.

Skeletal evidence of one or more Antemortem Injuries (AMI) was observed in 66% (n=27) of the cases. Only three individuals had a single element with a healed injury. The remaining 24 individuals had two or more AMI involving multiple elements and various stages of healing. Additionally, 46% (n=19) exhibited Perimortem Trauma (PMT). High-velocity trauma and blunt force trauma had the highest rate of incidence at 31.6% each, versus sharp force and thermal trauma at 5.3% each. Of the 19 with PMT, 63% (n=12) also experienced AMI. The assessment of OP cases revealed a high percentage of individuals who experienced AMI and PMT that are consistent with injury recidivism.

Presence of AMI and/or PMT was also compared to sex (M:F), age, social race, Per Capita Income (PCI), and location of recovery. Of the 27 with AMI, 63% identified as Black (16M:1F), 26% White (5M:2F), the remaining 11% included Asian (1M), Hispanic (1M), and multiracial (1M) social races. When examining PMT (n=19), 68% identified as Black (10M:3F), 26% as White (2M:3F), and 5% as Hispanic (1M). From the 12 with AMI and PMT, 7 identified as Black males (68%), 2 each as White males and females (34%), and 1 as Hispanic male (8%). Regarding recovery location, two cases were found in areas with PCI > $40,000, and 35 cases from neighborhoods with PCI < $40,000; the recovery location for four cases was unknown.

Young to middle-aged males found in lower-income neighborhoods represented the largest group experiencing injury recidivism in this study. The assessment identified structural violence involving persons from historically marginalized communities, specifically Black males. The expansion of anthropological assessments to include data that can be socially and culturally contextualized as structural violence enables forensic anthropologists to provide information that will generate leads for unidentified remains and, thus, better serve medicolegal agencies, policymakers, and communities.

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Structural Violence; Identification; Forensic Anthropology

A148   Skeletal Trauma in Suicide Cases (2017–2018) in Puerto Rico After Hurricane Maria

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Learning Overview: After attending this presentation, attendees will understand the importance of analyzing skeletal trauma-related deaths in suicide cases after a severe hurricane.

Impact Statement: The presentation will impact the forensic community by presenting skeletal trauma assessment in suicides after natural disasters.

Natural disasters (e.g., hurricanes) may increase suicide deaths.[1] In 2017, the category 5 Hurricane Maria caused massive destruction in Puerto Rico. The loss of basic living conditions (electricity, water, among others) led to an elevated suicidal ideation risk.[2,3] Within five months, the suicide rate increased 29% compared to previous years.[3] In forensic investigations, suicidal cases present unique challenges for skeletal trauma examination, depending on the mechanism of death. The three most prevalent methods of suicide after Hurricane Maria were hanging, gunshot wounds, and falls from heights.

In this study, the autopsy records from the medical examiner's office at the Institute of Forensic Sciences of Puerto Rico, namely 100 suicide cases that occurred between the years of 2017 and 2018, were analyzed. For that purpose, the cross-platform relational database FileMakerPro® version 14 was used. The inclusion criteria were individuals over 18 years of age, victims of suicide through hanging, gunshot wounds, and falls from heights. Skeletal trauma was recorded according to location in the skeleton.

The analyzed sample was composed mostly of males (89%). The average age of the analyzed individuals was 56 years (+/-16). Regarding the method of suicide, 44 were hangings (males: 41; females: 3), 34 were gunshot wounds (males: 33; females: 1), and 22 were falls from heights (males: 15; females: 7). All individuals who died from falls and from gunshot wounds presented skeletal trauma. In opposition, only 20.5% of the individuals whose mechanism of death was hanging presented skeletal trauma.

Falls from heights affected only the axial skeleton in 14% of the individuals and only the cranial skeleton in 4.5% of the individuals. The appendicular skeleton was never affected alone in this mechanism of death. Much more common was the combination of trauma in different parts of the skeleton (cranial and axial and appendicular: 27%; cranial and appendicular: 4.5%; axial and appendicular: 50%). Interestingly, the combination of trauma lesions in both cranial and axial skeleton was never observed in falls from heights. In deaths by gunshot wounds, 31 individuals (91.2%) presented cranial fractures, and 3 (8.8%) had fractures in the axial skeleton. Finally, in hangings, 77.8% of the individuals presenting skeletal trauma showed fractures in the thyroid cartilage, 33.3% in the hyoid, and 11.1% in the cervical vertebrae.

The results presented here, although preliminary, show the patterns of skeletal trauma more commonly observed in different methods of suicide and are in accordance with the described in forensic literature.[4] In a near future, the aim is to provide a baseline for the forensic sciences community to better understand the interrelationship between trauma-related deaths and their circumstances.

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Suicide; Trauma; Firearms

A149 An Analysis and Reconstruction of Remains from a World War II Mass Grave in the Szpęgawski Forest, Poland

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Learning Overview: This presentation examines human remains from a World War II mass grave located in Szpęgawski Forest in southeast Poland. After attending this presentation, attendees will understand the importance of conducting research on war crimes by highlighting the social impact on the public and the relatives of the war victims. Furthermore, the methodology used when working with cremains and case studies will be presented.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of humanitarian efforts in forensic archaeology, specifically in contexts surrounding war crimes.

The well-planned mass murder of Polish citizens by Nazi Germans was happening all around Poland during World War II in the autumn of 1939. Pomeranian voivodeship, where the Szpęgawski Forest is located, is one of the most affected areas. Educated, disabled, and mentally ill individuals were targeted and exterminated under the pretext of achieving the Nazis' vision of an ideal population, as prescribed by the Führer. No man, woman, or child was treated with condescension. Targeted educated people (e.g., teachers, politicians, policemen, and priests) were killed by gunshot to the head in the forest just prior to being buried. Additionally, disabled and mentally ill individuals who were perceived as "life not-worth-living" were often killed in hospitals by lethal injection, and their bodies were carried out to be buried with other victims. In 1944, when the war seemed to be lost for Nazi Germany, the exhumation and burning of those bodies were ordered to cover up the evidence of the war crimes.[1]

In the Szpęgawski Forest, there are approximately 5,000 people buried in multiple mass graves. They were marked as a single grave until after the war ended. In the summer of 2023, a team of forensic archaeologists and anthropologists excavated suspected regions in the forest to confirm the presence of the remains. Mass grave numbers 19 and 31 were excavated. Mass grave 31 was fully excavated and 19 was divided into four parts, of which only two were excavated.

The analysis of the human remains included assessment of fragmentation, taphonomic changes, degree of burning, sex, age at death, and pathological changes. One aim of the analysis was to estimate the Minimum Number of Individuals (MNI) buried.

Mass grave 31 was found empty and classified as a symbolic grave. Mass grave 19 had multiple layers of cremains and unburned human remains on the bottom of the grave pit. The postmortem changes of these remains (including bone warping, thumbnail fracture, and "sandwich effect") suggest that the bodies were partially covered with soft tissue during the exhumation in 1944, and then burned and reburied. The color of the remains indicated a low temperature of burning (around 200–550°C). The MNI was estimated to be 87 individuals, including six non-adults. Male features were observed more frequently, suggesting a predominance of men among the victims. Two individuals whose remains were not precisely exhumed and were not burned had evidence of gunshot wounds to the cranium, one reflected by a keyhole fracture. Reconstruction of one cranium was possible, and the trajectory of the projectile bullet was estimated.

The ongoing archaeological investigations and analyses conducted at the Szpęgawski Forest serve as a crucial endeavor to honor the memory of the victims and shed light on the atrocities perpetrated during this chapter of history, thereby facilitating the acknowledgment and understanding of the tragic events that transpired during the Second World War in Poland.

Reference:
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Second World War; Mass Graves; Cremains

A150   The Latin American Association of Forensic Anthropology (ALAF): 20 Years of Development

Luis Fondebrider*, Madrid, Spain

Learning Overview: After attending this presentation, attendees will be aware of the developments that forensic anthropology has developed in various Latin American countries, its applications, and its challenges.

Impact Statement: The impact of this presentation will be reflected in the different processes that forensic anthropology has followed in the United States and in the development of the three branches of anthropology in Latin America, with a broader and more comprehensive perspective.

In 2003, the ALAF was created with a meeting of the main forensic anthropologists in Latin America and three independent teams (Argentine Forensic Anthropology Team, Forensic Anthropology Foundation of Guatemala, and Peruvian Team of Forensic Anthropology).

The ALAF brings together professionals from Latin America who work in the field of forensic anthropology and other forensic disciplines. ALAF currently brings together professionals from more than 20 countries and is the largest group of forensic anthropologists in Latin America.

The objectives of the ALAF are to establish professional and ethical criteria for the exercise and promotion of the use of Forensic Anthropology and Archeology in judicial investigations in the different countries of Latin America, as well as the creation of an independent directory that guarantees technical autonomy of forensic anthropological investigations and the development of studies and the quality of practice.

The initial objective in 2003 was to develop a model for the application of forensic anthropology following the specific needs of the region (violence of all kinds), with a perspective that contemplated the needs of victims of human rights violations. In 2012, a Forensic Anthropologist Certification process was created that has been on-going for ten years and has become a successful process in the world. In 2023, a similar certification process will be carried out for forensic archaeologists. To this day, ALAF holds a congress each year in a different Latin American country for five days and has about 120 members.

During this presentation, the different contexts of violence (political conflict, migrants, femicide, organized crime) to which forensic anthropology is applied today in Latin America and its growing role in the medicolegal systems of the continent will be discussed. Also, the achievements and challenges of these 20 years of work will be discussed, as well as the relationship with colleagues in the United States.[1,2]

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Latin America; Families; Certification

A151 Missing Persons in Argentina: The Age Progression Technique to Support Non-Profit Organizations in the Search for the Lost

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Learning Overview: This presentation will show how the age progression technique can be applied to the search for missing persons. After attending this presentation, attendees will understand the importance of involving age progression in the search for the long-lost in order to be able to disseminate updated mugshots according to the current age of the missing.

Impact Statement: This presentation will impact the scientific community by showing how age progression has been applied to the search for missing persons in Argentina. The example case examined will show how, thanks to the international collaboration of non-profit organizations, the process of age progression for the search for the missing is activated and implemented.

In every country in the world, the phenomenon of missing persons is present and focuses the attention of the authorities who have actions to monitor and contain the problem.

Argentina is one of the countries which, despite the difficulties associated with the political turmoil, has undertaken initiatives aimed at countering the increase in the number of missing persons. Among these, the Person Search Coordinated Action against Trafficking in Persons (ACCT) in 2013, the Nacional de Información de Información de Personas Menores Extraviadas, and Alerta Sofía monitor the number of missing persons in Argentina.

Some organizations have taken action to help spread the photos of the missing person in the area. Among these, the Missing Children Argentina (MCA) is a non-profit organization that supports the families of the disappeared. To increase the chances of finding those missing for many years, MCA makes use of the support of age progression, provided by the non-profit organization Italian Missing Children (IMC). MCA and IMC have activated their collaboration since 2020, processing eight cases of digital aging.

The case of a missing girl and her age progression is reported to document the collaborative work between these two organizations. Age progression technique is based on scientific standards of facial aging, developed for medical reasons, especially in cosmetic surgery and orthodontics. To obtain as accurate a result as possible, the IMC made use of photographic material of the relatives of the lost. Photos of parents and siblings were used to identify similar somatic traits and to study their evolution during growth. By using Adobe® Photoshop® graphic software, specific facial morphological features were searched for in the high-resolution reference photos and assembled until a new mugshot of the missing person was obtained as a result.

The photo of the missing individual's face, processed through digital aging, is inserted into a poster with information on the case and diffused by television and social media. The poster indicates the time and place of the disappearance, personal data of the missing person, contacts in case of sighting, photos closest to the moment of disappearance, and those obtained with age progression techniques.

It is important to also provide information to the population on the possibility of requesting the age progression of a person who has been missing for a long time, thus supporting research activities.

Missing Persons; Forensic Anthropology; Age Progression

A152   The Role of Forensic Anthropology in Long-Term Missing Persons Cases in Scotland

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Learning Overview: After attending this presentation, attendees will have insight into the application and role of forensic anthropology in Long-Term Missing Persons (LTMP), "cold cases," voluntary disappeared, and no body homicide cases as they relate to Scotland. Through examination of past cases, this presentation will discuss the benefits and limitations of anthropological involvement in such contexts and what lessons may be learned that can be applied to other body recovery and identification challenges, particularly in relation to vulnerable persons.

Impact Statement: LTMP cases, often termed "cold cases" by the general public, the media, and society at large, impact not only the immediate family and friends of the missing individual, but have the potential to affect whole communities for decades. In Scotland, forensic anthropologists are routinely involved in such cases from an early stage, either through planning, assisting in a ground search where bone fragments have been identified, or in the recovery and examination of human remains. This presentation will impact the forensic science community by sharing the experience of the forensic anthropology practitioners in Scotland in relation to these cases, enhancing the awareness of the benefits of the early involvement of anthropologists in such investigations.

Vulnerable persons, including the elderly, those with medical conditions such as dementia, poor mental health, or those who live at the fringe of society are more likely to be the subject of LTMP cases than other groups, and are also more likely to go missing without being reported to the police.[1] The expansive and rural nature of the majority of Scotland makes the task of investigating, and searching forLTMPs and those who have voluntarily disappeared particularly challenging, with discoveries of human remains often being the result of rural land-management activities or chance discoveries by outdoor enthusiasts. This presents challenges for the recovery of human remains, including effects of taphonomy, scavenging, and the lack of safe and easy access to the remains.

In recent years, forensic anthropologists based at the University of Dundee, Scotland, have been involved in various stages of investigations relating to individuals who have voluntarily disappeared and LTMPs, including those believed to be the victims of homicide. The early involvement of forensic anthropologists in such investigations has provided the opportunity for relevant information and advice to be provided during the planning of search activities and for appropriate attendance in the search, recovery, and postmortem examination of any recovered human remains. This presentation will, through the presentation of past cases, provide insight into the benefits of early involvement of forensic anthropologists in the search planning for LTMPs. Further case studies will illustrate the positive impact that the attendance of a forensic anthropologist during the subsequent search and recovery activities, including where fragmented and non-human remains are located; and finally, the benefit that the involvement of the forensic anthropologist in the postmortem examination of the remains can have on the success of the investigation. The particular context of voluntary disappearance and vulnerable persons will be considered in light of the specific challenges presented by these cases.

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Missing Persons; Unidentified Persons; Human Remains

A153   FORDISC® and AncesTrees: Limitations and Considerations for the Identification of Individuals From Central and South America

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Learning Overview: The goal of this presentation is to present new data on population structure and population history from individuals from Central and South America through the interpretation of population-affinity estimations obtained by two online applications (FORDISC® and AncesTrees).

Impact Statement: This presentation will impact the forensic science community by providing forensic practitioners with a theoretical and practical background for the application of two popular online softwares, FORDISC® and AncesTrees, targeting issues for population-affinity estimations on human remains from Central and South American.

Extreme conditions leading to rapid skeletonization in some areas of the United States-Mexico border can make identification difficult.[1] From the demographics gathered for biological profile estimation, biogeographical origin can impact the choice of subsequent methods for sex or age-at-death assessment.[2] AncesTrees and FORDISC® are two computerized statistically based methods for estimating population affinity.[3,4] To determine the reliability of group allocation using these applications, different target samples should be tested. The aim of this study is to explore the reliability of these applications in estimating the origin of individuals from Central and South America, comprising population groups that likely form the migratory flow prevalent across the United States-Mexico border.

A total of 50 adult individuals (25 males and 25 females) identified as Mexican, Mexican American, Hispanic, New Mexican, or Latino were selected from the New Mexico Decedent Image Database.[5] Seventeen cranial measurements were collected from Computed Tomography (CT) scans. Compiled measurements followed protocols outlined by AncesTrees and FORDISC®. Parameter values were inserted into the online platforms, with the settings for the application determined as non-prior background information. For FORDISC®, both the Howells' and the Forensic Anthropology Data Bank (FDB) datasets were tested to explore any potential differences related to the chronology of the reference samples, as well as allowing for a comparison between AncesTrees and FORDISC® historical samples. All allocations are reported in the results, with Posterior Probability (PP) and Group Membership (GM) with values ≥0.80 being considered for further interpretation of the group allocation.

Results show that AncesTrees allocated individuals as European (48%) and East Asian (32%), with the remaining biogeographical population sample allocations representing less than 10%. For the FORDISC® FDB, 26% of the sample was estimated as White and 24% of the sample was allocated as Hispanic, with the third-highest number of individuals being associated to Japanese (16%), Guatemalan (12%) and Chinese (12%) samples. Regarding the FORDISC® historical dataset, 24% of the individuals were classified as European with the next highest allocation being for East Asian samples (40%). Very few individuals obtained a PP higher than the threshold for FORDISC® estimations while GM for AncesTrees was over 0.80 for around 62% of the sample

An accurate reconstruction of the biological profile is crucial for positive identification. Most of the population allocations obtained here using two commonly used online platforms present trends that suggest individuals from Central and South American origin would fall within Hispanic and European populations as well as East Asian samples. This research provides insights on population structure and population histories, as the patterns observed may be linked to social and political history.[6,7] Future research, including larger and more diverse samples, will confirm our results and provide further information on human variation and inter-population differences. A consensus on the terminology as well as a revision of the information used for grouping the samples is recommended to ensure consistency between forensic practitioners and researchers. Moreover, larger and more representative datasets are needed to increase the accuracy and reliability of identification of unknown individuals in a forensic context, especially for areas with high migration flows such as the United States-Mexico border.

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Population Affinity; Craniometrics; FORDISC®/AncesTrees

A154 The Forensic Border Coalition South Texas Cemetery Survey: Insights From a Forensic Science and Humanitarian Effort Addressing Unidentified Burials and Unidentified Remains

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Learning Overview: This presentation reviews the Forensic Border Coalition's (FBC) South Texas Cemetery Survey, 2015–2017. After attending this presentation, attendees will understand the challenges to finding, accounting, and conducting forensic and humanitarian interventions in Texas and the implications to missing and deceased migrant accounting in other fatal international border zones.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the FBC conducted this South Texas Cemetery Survey in the Texas Rio Grande Valley region of the Central America-Mexico-United States migration corridor, assessing available death documentation of unidentified remains likely corresponding to migrants. The FBC consulted local cemetery and funeral home information and conducted physical surveys of multiple burial sites.

Through a review of the present research, its purpose, and its conclusions, the FBC shares: (1) a brief examination of the increase in deaths of persons in migration at the United States southwestern border, focusing on increased deaths in Texas; (2) background on the formation of the FBC in response to addressing missing migrants and migrant deaths; (3) the FBC's Texas Cemetery Survey Project development, methods, and outcomes; and (4) recommendations for actionable changes based on the experiences of this project.

Field Work Summary: Between July 22, 2015, through April 1, 2017, the FBC conducted site visits, cemetery surveys, and gathered public records (i.e., financial, death certificates, records of death, indigent burial records, NamUs Unidentified Person records, field surveys of cemeteries with unidentified burials likely to correspond to migrants, etc.) to examine challenges related to unidentified remains in Texas. Then, the FBC mapped 133 unidentified remains of probable migrants from seven South Texas counties (64 Hidalgo, 43 Cameron, 12 Starr, 14 Kenedy, 0 Willacy, 0 Jim Hogg; due to an existing collaborative relationship with the Brooks County Sheriff's Office and Operation Identification, the FBC did not survey cemeteries in Brooks County). This number is a conservative estimate because many migrants are never recovered. This is also a conservative estimate because of the limited and inconsistent documentation of where and how many unidentified burials exist in those counties.

The FBC's key findings point to severely lacking practices. There is a lack of data on migrant deaths, a lack of recordkeeping, and a lack of coordination of records among participating agencies. In some counties, the FBC found that if DNA had been collected on unidentified remains, it had not been entered into national databases, making it impossible to identify remains, even if relevant family reference samples exist and have been entered into these databases. In other cases, DNA samples were not collected before the remains were buried. The degree of fragmentation and failure to effectively manage the forensic cases of unidentified human remains in Texas need to be the core targets efforts for change.

The FBC initially planned that the seven counties would comprise only Phase I of several surveys. The findings, however, caused the FBC to pivot its efforts in Texas to address immediate needs indicated by the Survey (e.g., exhumations) and to adapt other southwestern border-wide activities to more effectively address the challenges related to migrant death. In the concluding discussion, the FBC shares five critical recommendations for supporting the processes around finding and investigating unidentified human remains of probable migrants that directly affect Texas counties and are relevant to the whole southwestern border and are informative to other fatal migration corridors such as the Mediterranean.

Anthropology; Unidentified Persons; Human Rights

A155   Shifting Routes: Expanding Migrant Decedent Identification Efforts in Texas

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Learning Overview: After attending this presentation, attendees will better understand major shifts in migrant death investigation practices in Texas over the past ten years.

Impact Statement: This presentation impacts the forensic science community by exposing practitioners to the challenges, advancements, and changes in migrant decedent identification in Texas, which has implications for all jurisdictions handling transnational unidentified persons cases.

Migrant death along the United States-Mexico border persists at disastrous rates while many Texas border jurisdictions remain unable to access medicolegal services or feasibly manage decedent identification. In 2012, in response to an influx of migrant death in South Texas, Operation Identification (OpID) was founded as a humanitarian initiative of the Forensic Anthropology Center at Texas State (FACTS) to assist border counties with recovering, identifying, and repatriating migrant decedents. Specifically, OpID began as a project to assist with exhumations, forensic anthropology analysis, and DNA submission for the long-term unidentified buried in cemeteries throughout the Rio Grande Valley. As a member of the Forensic Border Coalition (FBC), OpID participated in the three-year South Texas Cemetery Survey to document the burial locations of migrant decedents and generate counts of unidentified human remains in the region. As a result of this survey, OpID and partners exhumed 230 long-term unidentified decedents from four counties between the years of 2013–2021, of which 31 have been identified.

However, shifting migration routes and increased death in under-resourced West Texas regions have created a new state of emergency necessitating additional responses. Throughout the past two years, OpID has adapted to include case management of more recently recovered individuals from counties that have little or no previous experience overseeing cases of unidentified migrants. Of the 225 cases OpID has received from direct agency transfers, 40 have come from 11 border counties not originally included in the South Texas Cemetery Survey. Additionally, at the request of county officials, throughout the fall and spring of 2023, OpID has focused efforts on exhuming and identifying 26 individuals who perished in Eagle Pass, Maverick County, in the summer of 2022. As a new epicenter of migrant death, Maverick County experienced an average of one death per day this past summer and began burying decedents without investigative efforts due to lacking storage—a situation mirroring Brooks County in 2012 that requires urgent intervention.

While increased identification hypotheses for these and other recently recovered individuals partially contribute to the sharp increase in identifications facilitated by OpID—from an average of five per year through 2021, to 26 in 2022, and 23 to-date in 2023—this also reflects the need for continued capacity building in border jurisdictions. Although extensive community outreach, training efforts, and stakeholder collaboration have improved case management in the Rio Grande Valley, particularly in Brooks County, the situation in West Texas and ongoing barriers to decedent identification call for the development of sustainable disaster management infrastructure.

The objective of this presentation is to discuss the challenges, advancements, and changes to migrant death investigation in Texas through the experiences of OpID. After recounting the trajectory of the OpID project over the past ten years, this presentation highlights trends in migrant death management along the Texas southern border through recent case examples. It is the goal of this discussion to promote the development of centralized agencies for migrant death investigation in Texas and to advance the integration of disaster victim identification approaches in jurisdictions managing high numbers of transnational unidentified deaths.

Identification; Migrants; Medicolegal Death Investigation

A156   Necrosilence: GIS Visualizations of Migrant Mortality in South Texas, 2009–2020

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Learning Overview: After attending this presentation, attendees will better understand geographic and temporal trends of reported migrant deaths in South Texas, as well as the implications of "empty map spaces" or necrosilences.

Impact Statement: This presentation will impact the forensic science community by addressing two persistent issues specific to the Texas-Mexico border migration context. First, it establishes a 12-year mortality profile. Second, it introduces the concept of necrosilences and their implications to both forensic and humanitarian work and usage of geospatial tools.

The study area, South Texas, is comprised of ten counties with some of the highest reported migrant mortality incidences, 2009–2020. A total of 1,412 records of migrant decedents were obtained from local sheriffs, justices of the peace, and Border Patrol. We applied Analyses Of Variance (ANOVA), spatial statistics, and cluster analysis to test the relationships of mortality point locations throughout South Texas. We used RStudio 3.5.3 ANOVA and independent t-tests to determine whether there were any differences in migrant mortality between counties and, in a separate set of tests, whether migrant mortality varied significantly by season. ANOVA is an aspatial type of analysis. To look at spatio-temporal patterns, we applied a suite of spatial analytical methods, starting with the Mean Center (MC), Standard Distance (SD), and Directional Distribution (DD) of mortality locations, which were calculated in ArcGIS® Pro 2.9.3. We used Getis-Ord Gi* in ArcGIS® Pro to test for mortality clustering of migrant mortality locations in relation to neighboring features in the study area, specifically private land ownership, as well as by year the remains were found and in relation to distance to United States Border Patrol (USBP) structures (ports of entry, stations, checkpoints) and to roads. Finally, to examine patterns of spatial and temporal distribution together, we used SaTScan™ v10.0.

Two important aspects of the patterns of migrant mortality in South Texas emerge from spatial analysis and Geographic Information Systems (GIS) mapping. To start, the 12-year South Texas migrant mortality spatiotemporal profile illustrates a deathscape that approximates the "second border" of Border Patrol checkpoints.[1,2] Visually, this is illustrated by the majority of DD ellipses having a NW-to-SE orientation, as well as by county-level MCs falling near at least one USBP checkpoint (within 0.22–17.00km). Further, Getis-Ord Gi* results showed that migrant decedents recovered farther away from or in between Border Patrol structures tended to be significantly more than expected, and decedents relatively closer to Border Patrol structures were fewer than expected.

The second important takeaway was that there were large swaths of South Texas that were devoid of migrant death. These "empty" areas are emblematic of necrosilences. That is, instances where there is a lack of access or accounting rather than absence of death. Necrosilences are the result of harmful inactivity, obfuscation of death, or failing to intervene in "letting die" situations. As pertains to the visualization and generation of spatiotemporal profiles of migrant mortality, necrosilences are informative. Smuggling of migrants is a highly coordinated process, and it is probable that once migrants are past the border itself, the process of being moved from stash house to stash house via vehicles creates the illusion of blank spaces, especially between the Mexican border and Border Patrol checkpoints.[3] Rather than being free of deaths, these areas likely are not searched regularly or are otherwise inaccessible due to lack of permission, personnel, or other resources. Key "empty" areas include all of Starr, Zapata, Willacy, and Cameron Counties, the northern portion of Hidalgo County, and the southern portions of Brooks and Kenedy Counties.

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GIS; Migrants; Human Rights

B1   What's In It For Me (WIIFM)?: Streamlining Sexual Assault Evidence From Collection to Analysis to Achieve the Best Outcome for the Survivor

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Learning Overview: This presentation will use result outcomes to demonstrate how the Saint Lucia Forensic Science Laboratory was able to streamline its sexual assault evidence collection kit to focus on DNA-based evidence collection in combination with a DNA-first approach to analysis. After attending this presentation, attendees will better understand the value of a shorter and more focused collection system for sexual assault evidence that allows for a faster turnaround time for analysis, while producing probative DNA results to assist the trier of fact in sexual assault cases.

Impact Statement: This presentation will impact the forensic science community by showing that a ten-step sexual assault evidence collection process is effective in collecting enough probative biological evidence for the effective investigation of sexual assault matters through a DNA-first approach. Forensic DNA analysts will also learn how to use the quantitation step and the Capillary Electrophoretic (CE) instrument to make decisions about stopping at quant or triaging cases for autosomal or Y-chromosomal Short Tandem Repeat (Y-STR) profiling.

Evidence collection after a report of a sexual assault is pivotal in proving or disproving the alleged events. In Saint Lucia, evidence collection is usually conducted in the Emergency Room (ER) of our two main hospitals on the island by on-duty medical doctors. It means that all "emergencies" are attended to ahead of a sexual assault survivor. This results in inordinate waiting times for the examination and re-traumatization of the survivor by the whole ER experience. Couple that with an almost 20-step evidence collection process as obtained previously, involving pubic/head hair combing, plucking or fingernail scraping, and drawing of blood.

The Saint Lucia Forensic Science Laboratory decided in 2011 to streamline the Sexual Assault Evidence Collection Kit (SAECK) to ten steps in keeping with the recommendations for standardized evidence collection by the National Protocol for Sexual Assault Medical Forensic Examinations by the United States Department of Justice.[1,2] The kit would be focused on biological evidence collection for subsequent DNA analysis, with considerable time savings for both the sexual assault survivor and the attending ER physician. Then in 2022, the DNA Unit transitioned to a new CE platform along with using new, more sensitive chemistries for extraction of sexual assault evidence such as the Casework Direct System by Promega® Corporation and the Erase Sperm Isolation Kit by PTC Laboratories.[3-6] We used the CE instrument to establish the DNA mass equivalent of our stochastic threshold Relative Fluorescence Units (RFU).[7-9] We are therefore able to identify the most probative samples for DNA profiling at the quantitation step. This approach not only improves the turnaround time for analysis in the DNA Unit and helps to reduce the backlog of cases, but also gives the laboratory the opportunity to provide feedback to the evidence collectors to improve on the quality of evidence collection at different steps in the process.

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Forensic DNA; Evidence Evaluation; Forensic Nursing

B2   The Remains of the Crime: An Assessment of Body Fluid Identification Over Time

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Learning Overview: After attending this presentation, attendees will consider the possibility of recovering body fluids, particularly blood and semen, up to three months old as rapid immunochromatographic tests were able to successfully identify them in different types of fabrics.

Impact Statement: This presentation will impact the forensic science community by presenting a validation study of blood and semen stability on different types of fabric over time and the sensitivity of rapid immunochromatographic tests and by assessing the possibility of recovering total DNA from the samples, thus leading to both the identification of body fluids and DNA isolation.

Among different methodologies to quickly identify body fluids at crime scenes, rapid immunochromatographic tests represent the simplest and quickest. They are based on antigen-antibody reaction against a specific human protein. Particularly, blood rapid immunochromatographic tests are based on the detection of hemoglobin, while semen tests are based on the detection of Prostate Specific Antigen (PSA). These are the most common body fluids encountered at the crime scene. One of the factors that could hamper the correct identification of these fluids is the substrate where they were deposited. Another factor could be the time. It must be considered that crime scene investigation could occur days or even months after the crime was committed. Currently, there are few studies analyzing the possibility of detecting body fluids over time and comparing among different substrates.

Based on these premises, the main aim of this work was to validate the detection of blood and semen samples from denim, cotton, and leather aged up to 3 months and analysis of nuclear DNA recovered from these samples.

Twenty microliters of blood and semen (three replicates per timepoint) were deposited in denim (black and white), cotton (black and white), and leather. The samples were recovered after 1 day, 2 days, 1 month, and 3 months. The fabrics were kept at room temperature under natural light conditions. Blood and semen were identified by SERATEC® HemDirect Hemoglobin Test and SERATEC® PSA Semiquant, respectively. The sample was swabbed for 30 seconds and the swab was diluted in 400ul of extraction buffer. This mixture was shaken for 10 minutes at room temperature. After this period, three drops of buffer were added in each cassette. DNA was extracted from the buffer applying silica-based columns, and later quantified by human-specific quantitation based on quantitative Polymerase Chain Reaction (qPCR). PowerPlex® Fusion 6C System was applied to generate the Short Tandem Repeat (STR) profiles.

The results showed that it was possible to detect blood and semen up to three months after deposition, although with different band intensities. Black denim and cotton showed stronger bands than white denim, cotton, and leather. Total DNA from all sample tubes was successfully recovered, obtaining full DNA profiles with different qualities. Leather, black denim, and cotton samples showed the highest qualities in DNA profiles up to three months. These findings indicate that it is possible to recover and identify blood and semen samples up to three months after deposition and also obtain DNA profiles.

Future research will be able to expand the results, assessing the detection of body fluids in other fabrics and different environmental conditions.

Bodily Fluid; Fabric; LFI

B3   Dry Vacuum Recovery of DNA From Fabric

Emily Sam-Zee-Moi*, John Jay College of Criminal Justice, New York, NY; Mechthild Prinz, John Jay College of Criminal Justice, New York, NY

Learning Overview: After attending this presentation, attendees will be informed of an alternative non-destructive method of collecting touch DNA from fabric by using a dry vacuum and how it can be used for testing fabric evidence.

Impact Statement: This presentation will impact the forensic science community by presenting an optimized method that may be more efficient for DNA collection and may affect how different types of evidence are processed for DNA recovery. The success of the non-destructive dry vacuum method makes this process a valuable addition to the forensic science toolbox.

Touch DNA has proven to be a reliable forensic tool when investigating sexual assaults and other crimes. Current methods of DNA recovery from fabric include cutting, swabbing, scraping, and tape lifting. A Swedish tape lifting method was shown to be effective for DNA collection for plain woven fabric.[1] A new method using a dry vacuum technique has been successful in recovering touch DNA from written documents. This method uses suction to gather DNA onto a dry or moistened filter, which can then be processed to release the DNA and create a DNA profile.[2] The dry vacuum collection is an alternative non-destructive option for DNA collection from fabric.[3] Different filters and moistening agents may increase DNA yields for the dry vacuuming method. This experiment was designed to test the success of the dry vacuum technique on pieces of cotton fabric that had been briefly touched as well as the effectiveness of different moistening agents and filters.

Samples were gathered by having both female and male volunteers rub their faces, followed by rubbing their hands together and gripping DNA-free 15cmx15cm cotton fabric squares. A total of four fabric samples and one buccal swab were collected per donor. This method of collection was designed to simulate an assault with the suspect grabbing the clothing of their victim. The fabric was then vacuumed using either an irradiated glass pipette fit with a Puritan® cotton swab moistened with water, 10% Tween® 20, or an irradiated plastic pipette tip with the filter moistened with water or 10% Tween® 20. A vacuum hose was attached to the narrow end of the pipette or tip, allowing for the wide end to suction the fabric. DNA was then extracted using a QIAamp® DNA Investigator Kit on the QIAGEN® QIACube® extraction robot. All samples were quantified in duplicate using Quantifiler® Trio.

Quantities from each donor varied; however, results show that the dry vacuum technique can collect a sufficient amount of touch DNA from fabric (>7pg/uL) to be used for DNA analysis from all donors. Water as a moistening agent allowed for a higher amount of DNA to be collected. There was no distinguishable difference when using a glass pipette versus a plastic pipette tip. The dry vacuum method is an efficient non-destructive method that can be implemented in any DNA laboratory that has a vacuum pump or a bench vacuum outlet.

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DNA Recovery; Fabric; Dry Vacuum

B4   **An Initial Investigation of the Applied Biosystems® SeqStudio™ Genetic Analyzer for Human Identification Purposes**

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Learning Overview: This presentation will focus on educating attendees about the use of the innovative SeqStudio™ Genetic Analyzer with the GlobalFiler® Polymerase Chain Reaction (PCR) amplification kit for human identification purposes.

Impact Statement: This presentation will impact the forensic science community by sharing the determined limits and best practices for the fluorescence-based SeqStudio™ Genetic Analyzer system on mock casework samples.

Capillary Electrophoresis (CE) is the conventional method used to separate and detect DNA fragments for Human Identification (HID) purposes.[1] Over the years, multiple genetic analyzers have been developed; however, the platform of interest for this research is the latest Applied Biosystems SeqStudio™ Genetic Analyzer. The instrument includes an all-in-one reagent cartridge that contains four capillaries and can detect six different dyes.[2] When performing CE, many factors, including injection time, sensitivity and reproducibility, stutter, inhibition and degradation, and mixture performance must be considered.[3] Previous research has not yet fully established all capabilities and thresholds involved with the use of the SeqStudio™ coupled with the GlobalFiler® PCR amplification kit for HID purposes. This project aims at investigating these limitations and determining the best practices for this instrument.

This research requires performing different evaluation studies using a standard quantitative Polymerase Chain Reaction (qPCR)-to-CE workflow. DNA samples are quantified using Quantifiler® Trio DNA Quantification kit on the QuantStudio™ system, amplified with the 24-loci GlobalFiler® PCR kit on the ProFlex system, genotyped on the SeqStudio™, and profiles analyzed using the GeneMapper® ID-X v1.6. The first study to perform is the injection study that requires injecting samples at different injection times to determine the optimal injection time in order to generate reliable data. A reproducibility study is also conducted to determine the ability of the SeqStudio™ to reproduce genotypic results across multiple runs alongside a sensitivity study to determine the optimal range of target DNA (from 1ng/ul to 7pg/ul) to amplify when using the GlobalFiler® PCR kit. Furthermore, stutter and intra-locus and intra-/inter-color balance studies are performed to establish the amount of stutter produced at each Short Tandem Repeat (STR) locus and the overall balance among loci and dyes, respectively. A mixture study is conducted to define the detection of DNA donors in two-person (e.g., 1:1, 1:3, 1:5, 1:9, 1:19) and three-person (e.g., 1:1:1, 1:1:3, 1:1:5, 1:1:9) mixtures. Inhibition and degradation studies are also performed to assess the typing performance on inhibited and degraded mock samples stored at room temperature.

For the injection time study, one male and one female DNA sample were quantified and diluted to 0.5ng/ul and 1ng/ul and run on the SeqStudio™ at 1, 3, 5, 7, and 10s injection times in triplicate. Overall, a general decline in the max, med, and min Relative Fluorescence Units (RFU) values for alleles at all loci within each dye channel was observed as the injection time decreased. The injection times ranging from 1s to 5s provided optimal results at both concentrations, demonstrating the lowest values of standard deviation observed across all loci. The assessment of min and max peak height reproducibility was also completed for each injection time as well as heterozygous allele balance with no significant variation being reported. Moreover, full genotype reproducibility at all loci was observed across multiple runs at the concentrations tested. For the sensitivity study, no locus or allelic dropouts were observed from 1ng/ul down to 125pg/ul; further analysis is in progress to determine the overall locus/allelic dropout rates at lower concentration inputs.

In conclusion, our preliminary findings support the potential of the SeqStudio™ Genetic Analyzer in forensics, and the ongoing comprehensive evaluation will help elucidate further the best practices for this low-throughput platform.

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Forensic Science; DNA; STR

B5   A Morphological and Genetic Characterization of the Psychoactive Species *Psilocybe Cubensis* From Forensic Samples

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Learning Overview: After attending this presentation, attendees will have insight on the applicability and usefulness of a different type of analyses regarding forensic fungal samples through results obtained from morphological and genetic research of casework samples conducted at the Croatian Forensic Science Centre, "Ivan Vučetić."

Impact Statement: This presentation will impact the forensic science community by emphasizing the ongoing necessity of extending routine analyses of forensic fungal samples. Upgraded data deriving from complementary morphological and genetic analyses subsequently could lead to improvements of legal acts considering psychoactive fungi.

Consummation of psychoactive fungi containing Psilocybin (PY) and Psilocin (PI) causes less intensive LSD-like effects.[1] Although physically non-addictive, long-term use can lead to tolerance and psychical addiction, the reason why PY and PI are illegal in a majority of European Union countries, with fungi subsequently seized as forensic samples. Since PY/PI are not yet synthesized in spores, mycelia and sclerotia, routine toxicology methods on these kinds of samples are useless.[2] On the other hand, morphological (macroscopic and microscopic) analyses inevitable in research, taxonomic, and phylogenic fungal studies are somewhat difficult or even impossible to proceed on forensic samples with too dry, too young, or scarce-fruiting bodies, ground to powder, or lacking some of the macroscopic features. Applying molecular-based techniques can overcome some of the abovementioned limitations in identification process.

Hence, the main goal of this study was to present the usefulness of genetic analysis on fungal forensic samples to confirm their accurate species identification.

Within more than 200 described psychoactive species, *Psilocybe cubensis* (Earle) Singer and its varieties are most popular among users worldwide due to their proven potency, wide distribution, and ease of cultivation.[3-5] *P. cubensis* is classified in section *Cubensae* of the genus *Psilocybe* and distinguished from *Psilocybe subcubensis* only by one distinctive feature: the size of basidiospores.[6,7]

A total number of 63 forensic fungal samples seized in Croatia from 2010 to 2014 were morphologically analyzed and genotyped with genetic markers, including complete Internal Transcribed Spacer (ITS) of nuclear DNA and a large portion of 28S gene from 5' end (nuclear-encoded large subunit ribosomal RNA genes, nLSU-rDNA).[8,9] Macroscopic analysis using several taxonomic keys (6, 7, 10-13) resulted in five successfully identified species with *P. cubensis* as the most common (56/63), confirming its popularity. Microscopic analysis of a total 1,219 spores from *P. cubensis* samples, positioned 934 spores (76.62%) within range of basidiospores size for *P. cubensis* and 1,127 spores (92.45%) within *P. subcubensis* established range.[6] Obtained values well encompass both species without discrete grouping, rather showing overlapping and continuous transition from minor size values corresponding with *P. subcubensis* up to major values corresponding with *P. cubensis*.

These findings indicate that *P. cubensis* and *P. subcubensis* cannot be differentiated based only on size of basidiospores and are conspecific, (i.e., both belonging to the same species).

ITS and LSU sequences were successfully determined from 47/56 (83.92%) samples, each represented by only one haplotype. In addition, established haplotypes matched completely (100%) with *P. cubensis* haplotypes from the GenBank database. These results strongly confirm previously established results from morphological analyses. Eligible subsequent study should refer to the genetic analyses of *P. subcubensis* reference samples that served for initial species identification back in 1978.

Overall findings pointed out that molecular data can revise established fungal taxonomy classification based on genetic variations within species, inter- and intra-species phylogenetic relationships as well as the origin of shared morphological or phenotypic features among taxonomic groups.[14-18]

In summary, results imply that limited data obtained from toxicological analyses of forensic fungal samples are not adequate for species identification while morphological and genetic analyses represent actual necessity.

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Psychoactive Fungi; Morphological Analysis; Genetic Analysis

B6   The Persistence of Wearer DNA on Clothing After Laundering

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Learning Overview: This presentation will provide attendees with a better understanding of the evidentiary value of laundered clothing by showing the effects of three different wash conditions on the persistence of wearer DNA.

Impact Statement: DNA analysis is one of the most requested services provided by forensic laboratories. This presentation will impact the forensic science community by informing attendees that in order to make the best use of resources, laboratories need to recognize which evidence is most suitable for DNA testing. Laundered clothing may be considered unlikely to yield interpretable DNA results. Understanding the effect of different wash conditions on the persistence of wearer DNA will assist investigators and forensic examiners in deciding whether laundered clothing should be analyzed for DNA.

Introduction: Clothing is frequently recovered as evidence in criminal investigations. Processing these items for wearer DNA may provide relevant information for investigators to identify the perpetrator of the crime. However, if the clothing is laundered before it is recovered, the quantity of DNA that remains may be insufficient for Short Tandem Repeat (STR) profiling. This study was conducted to evaluate the persistence of wearer DNA after laundering and assess the quality of the resulting STR profiles.

Methodology: DNA was collected from 30 worn hats by swabbing half of the interior sweatband. The hats were divided into treatment groups and were laundered using one of three methods: machine-wash hot, machine-wash cold, or hand-wash lukewarm.

Post-wash samples were collected by swabbing the other half of the sweatband. All samples were extracted using the QIAamp® DNA Investigator Kit. DNA was quantified using a Rotor-Gene Q® real-time Polymerase Chain Reaction (PCR) cycler and the QIAGEN® Investigator Quantiplex™ HYres Kit. If necessary, sample concentration was performed using Microcon® DNA Fast Flow Centrifugal Filter Devices. STR profiles were generated using the GlobalFiler® PCR Amplification Kit, SeqStudio™ Genetic Analyzer, and GeneMapper® *ID-X* Software. Persistence was evaluated by comparing: the proportion of pre- and post-wash samples meeting the validated amplification target, total peak heights across the profiles, locus dropout, and degradation curves.

Results: Statistical significance between pre- and post-wash sample values within each treatment group was measured using either a two-proportion *z*-test or a one-tailed, paired *t*-test. A one-way Analysis of Covariance (ANCOVA) test was used to compare peak height sums, locus dropout, and degradation slopes of the post-wash samples between the three treatment groups. The pre-wash values were used as the covariate in the statistical analysis. For all analyses, a *p*-value < 0.05 was needed to indicate significance.

The proportion of pre- versus post-wash samples to meet the amplification target was not significantly different in any treatment group. Peak heights were found to be significantly lower for post-wash samples than pre-wash samples in the machine-wash cold and hand-wash lukewarm treatment groups; however, the decrease in peak heights observed in the machine-wash hot treatment group was not statistically significant. Post-wash samples from all treatment groups displayed significantly greater locus dropout and steeper degradation slopes than pre-wash samples. Comparing post-wash samples from different treatment groups indicated no significant difference in persistence between wash conditions.

Conclusion: This study shows that wearer DNA does persist after laundering and should be considered a potential source of DNA evidence. Although post-wash samples were found to yield significantly less DNA and lower quality profiles, it was still possible to generate profiles suitable for comparison. Approximately 55% (n=29) of post-wash samples resulted in STR profiles with alleles detected at 6 or more of the 21 autosomal loci tested. Many of these samples were undetected by real-time PCR, indicating that low quantitation values should not preclude further testing if concentrating the samples is an option. This study found that neither water temperature nor laundering method had a significant effect on the persistence of wearer DNA.

Wearer DNA; Persistence Post Laundering; STR Profiles

B7   A Multi-Species Reference Ladder Using Two PCR Multiplexes for the Rapid Identification of Most Domestic Animals for Forensic Purposes

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Learning Overview: Following this presentation, attendees will be informed about a rapid method of identifying an unknown biological sample by comparing it to a reference ladder for ten domestic animals and one human reference as well. The multiplexes simultaneously amplify mitochondrial DNA (mtDNA) markers for a dog, cat, rabbit, chicken, goat, sheep, pig, cow, horse, and donkey and one human mtDNA marker that provides species identification based on length heterogeneity of the markers.

Impact Statement: This presentation will impact the forensic science community by providing a rapid screening method to identify unknown biological samples that can be found at a crime scene involving humans and pets or for cases involving crimes against animals.

Forensic identification techniques used in cases involving animals, animal abuse, or commingled at crime scenes often rely on blood or shed hair analysis to identify the species. While there are quantitative Polymerase Chain Reaction (qPCR) or Short Tandem Repeat (STR) commercial kits available for DNA testing of domestic animals, some only test for one animal species at a time. The qPCR kits are used in food fraud cases where, for example, a 100% beef hot dog, may actually contain dog, and these kits can quickly identify that adulteration.[1,2] However, if the food was adulterated with lamb, for example, those species-specific kits would not be able to confirm that mixture of meat products. In other examples, crime scenes often find hair, tissue, or blood at the scene. Common presumptive tests will test for human evidence. However, the scene can often be mixed with non-human DNA evidence—evidence that could provide additional leads in a case. To date, no presumptive method provides a rapid identification of multiple unknown species as the source of the evidence—critical to know before moving to STR analysis.

This project used mitochondrial DNA cytochrome B gene marker to create a reference ladder and design two multiplex kits that can *simultaneously* detect dog, cat, rabbit, donkey, goat, sheep, chicken, pig, cow, horse, and human DNA using a traditional forensic workflow. Labeled universal forward primers and species-specific reverse primers were used for simultaneous amplification of 11 species in two multiplexes.[3,4] The individual species' PCR products were column-cleaned, then mixed together to form a reference ladder with bin sets for precise identification of a sample. The validation of the ladder and kits was tested against mock crime scene samples, blinded to the analyst, of blood found in soil, on wood, carpeting, and clothing. The source species was able to be identified using the reference ladder and multiplex. In addition, the kits were tested on various dog food samples to verify ingredients on the labels did indeed match the meat products used in the production of the kibble. Ongoing research continues to expand the reference ladder and multiplexes by adding rat, mouse, guinea pig, and ferret species.

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Mitochondrial DNA; Animal Abuse; PCR

B8   Advancing Semen Detection Techniques With Sperm Tracker STK® in Real Case Scenarios and Diverse Environments

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Learning Overview: In this presentation, attendees will gain valuable insights into the cutting-edge field of semen detection at crime scenes. Attendees will learn about the innovative Sperm Tracker STK® and its role in forensic investigations. This presentation will delve into real case scenarios where STK® has been employed, showcasing its effectiveness in identifying semen traces and contributing to solving criminal cases. Attendees will also explore various conditions under which STK® has been tested, providing a comprehensive understanding of its reliability and versatility. By the end of the presentation, attendees will have a deeper appreciation of the importance of semen detection in forensic analysis and the significant impact that STK® can make in advancing investigative practices.

Impact Statement: This presentation on semen detection using Sperm Tracker STK® will have a significant impact on the forensic science community. By introducing this innovative tool, attendees will gain insights into advanced forensic techniques and how it significantly improves the accuracy and reliability of semen detection. Real case scenarios demonstrate the potential to solve cold cases through the analysis of, for instance, degraded samples. Moreover, the implementation of STK® will enhance crime scene investigations, allowing for more effective identification and preservation of semen traces. This presentation is expected to stimulate further research and collaboration within the community, driving continuous improvements in forensic science and its applications. Overall, our findings will empower investigators with a powerful tool in semen detection, leading to more efficient, accurate, and just outcomes in criminal investigations.

Forensic science plays a crucial role in criminal investigations, and the detection of bodily fluids at crime scenes is essential in establishing a link between suspects and victims. Among these fluids, semen is of particular importance due to its potential as a key piece of evidence in sexual assault cases and other crimes.[1] Traditional semen detection methods have limitations in sensitivity and specificity, requiring the exploration of innovative technologies to improve forensic practices. This work presents a comprehensive study on the utilization of the Sperm Tracker STK® for semen detection, with a focus on its effectiveness in real case scenarios and different environmental conditions.[2]

The objective of this study is to evaluate the impact of the Sperm Tracker STK® on forensic semen detection, elucidating its potential benefits for the forensic science community, by offering enhanced sensitivity and enabling the detection of trace amounts of semen that may have been missed using traditional techniques.

Through a series of controlled experiments and field tests, we assessed the performance of the Sperm Tracker STK® in various challenging conditions. These included scenarios with degraded semen samples on hair and skin specimens, varying environmental factors (i.e., humidity and temperature), and different storage conditions over time. The results demonstrate that the Sperm Tracker STK® consistently is a valuable alternative to traditional methods, with a higher rate of semen detection in the tested conditions, leading to more robust evidence collection and helping to ensure the integrity of the investigative process.

Moreover, the application of the Sperm Tracker STK® to real case scenarios has led to breakthroughs in investigations, including its use for casework related to suspected sexual assaults. The analysis of semen samples has proven crucial in establishing the identity of perpetrators and providing closure to victims' families.

In conclusion, Sperm Tracker STK® represents a groundbreaking advancement in forensic semen detection. By empowering investigators with this advanced technology, we aim to strengthen the foundations of forensic science and promote justice in criminal investigations.

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Forensic DNA; Semen; Crime Scene Investigation

B9 Into the "Sexome": Genital Microbiome Transfer and Applications

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Learning Overview: After attending this presentation, attendees will have gained a better understanding of the genital microbiomes and its potential for forensic application.

Impact Statement: This presentation will impact the forensic science community by presenting support for genital microbiomes as potential evidence in sexual assault evidence in the absence of spermatozoa.

The preferential lysis-based differential extraction method is the conventional approach for sexual assault evidence processing. However, this workflow relies on the presence of spermatozoa to identify male perpetrators, making it limited in certain cases where spermatozoa may not be present. To address these shortcomings, there is a growing interest in using microbiome sequencing as a potential alternative, especially with the availability of next-generation sequencing technologies.

The purpose of this research was to investigate the potential post-coital transfer between vaginal and penile microbiomes in genuine samples. To achieve this goal, pre- and post-coital vaginal and penile swabs were collected from two donor couples. Microbiome libraries were prepared by targeting specific regions V3 and V4 of the 16S ribosomal RNA gene, commonly used in microbial community profiling.

The results of the study not only support previous literature findings, such as the primary genus found in vaginal microbiomes being *Lactobacillus*, but they also contribute to the relatively limited knowledge on penile microbiome communities.[1] Notably, post-coital vaginal and penile microbiomes displayed even greater diversity compared to their pre-coital state, suggesting potential changes or interactions resulting from sexual activity.

While further research with a broader range of samples will be conducted in the future, the observed differences in diversity between circumcised and uncircumcised penile microbiomes suggest potential variations in the persistence of coital transfer in different microenvironments. This finding aligns with previous studies that have shown that circumcision status can influence microbiome composition, which could have implications for the analysis of sexual assault evidence.[2]

Overall, this research contributes to the understanding of the dynamics of microbial communities in the context of sexual activity and has implications for forensic investigations. By utilizing microbial next-generation sequencing, this study highlights a promising avenue for gaining probative information, even in cases where conventional preferential lysis-based approaches may not be sufficient.

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Microbiome; Sexual Assault; 16S Sequencing

B10 **The Adaptation, Optimization, and Validation of a Semi-Automated DNase I-Based Differential Extraction Procedure on the Beckman Coulter Biomek NX^P Automation Workstation**

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Learning Overview: The goal of this presentation is to describe the development of an automated separation of sperm from non-sperm cells for sexual assault samples using a DNase I-based method prior to DNA purification.

Impact Statement: This presentation will impact the forensic science community by providing a more automated and less hands-on, faster, more efficient and less labor-intensive method for processing sexual assault cases.

Processing of evidence in sexual assault cases is time consuming, which has led to high backlogs in many localities. A key challenge has been to separate DNA in sperm cells from DNA in epithelial cells prior to purification in order to simplify the mixture prior to DNA profiling. The Differential Extraction (DE) method developed by Gill et al. and still routinely used in forensic laboratories, is based on the higher density of sperm cells and their resistance to cell lysis in the absence of a reducing agent such as Dithiothreitol (DTT) relative to epithelial cells.[1]

Following lysis of epithelial but not sperm cells in buffers containing proteinase K and detergent, samples are centrifuged to pellet sperm cells (Sperm Fraction [SF]), leaving lysed epithelial cells in the supernatant (Non-Sperm Fraction [NSF]). Removal of the NSF and repeated washing of the SF prior to DNA extraction is used to develop separate fractions for DNA profiling.

While effective, the process is challenging to fully automate, and the repeated wash steps are slow, labor intensive, can lead to loss of sperm, and provide more opportunities for cross contamination. Multiple procedures to improve upon the differential lysis/centrifugation method have been developed, including laser-, acoustic-, pH-, pressure-, affinity- and enzyme-based approaches. Critical aspects of new methods are to demonstrate that they work as well or better than methods currently in use in the forensic laboratory and an ability to seamlessly integrate into existing workflows, which reduces the impact on casework output.

The Virginia Department of Forensic Science (VADFS) optimized and fully integrated an automated DNase I-based differential extraction method into the current automated DNA processing pipeline using the Promega® DNA IQ™ DNA isolation system and the Beckman Coulter® Biomek NX^P automation workstation. DNase I is a nuclease that degrades DNA to oligonucleotides and is used in this method to remove residual non-sperm DNA from the sperm cell pellets, thus removing the need for repetitive wash steps.

Starting from reports by Garvin et al. and Wong and Mihalovich and the VADFS's current DE procedure, DNase I digestion of SFs was performed using a new method developed for the Biomek NX^P. [2-4]This was performed following manual epithelial cell lysis and pelleting of the sperm cells to remove the non-sperm cell lysate. We report the results of testing multiple enzyme concentrations, incubation times and temperatures, and semen dilutions using vaginal and buccal mock sexual assault samples from multiple donors. We also report the effects of commonly encountered vaginal contaminants and aging on results and tests to measure cross contamination rates in order to validate the optimized protocol on the Biomek NX^P.

We found that multiple conditions worked comparably to the current VADFS standard DE protocol with respect to Y-DNA yields (measure of male DNA), ratios of autosomal-DNA to Y-DNA yields (A/Y ratios, which measure the relative quantity of female DNA), and the quality of sperm fraction DNA profiles (peak balance, profile completeness, etc.). No evidence for increased rates of cross contamination was observed due to the automated DNase 1 treatment. In less than one-half the time (e.g., ~80 minutes versus ~180 minutes for 13 samples, including epithelial cell lysis) needed by the current VADFS DE method, the automated DNase I DE method transfers DNase I-treated SFs into the deep well sample plate in which, along with other casework samples, the SFs will be purified for DNA using the existing automated extraction procedure.

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DNA; Sexual Crimes; Automation

B11　From Tecan to Hamilton: Material Modification of Applied Biosystems™ PrepFiler™ Automated Extraction

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Learning Overview: After attending this presentation, attendees will better understand the validation process that was undertaken by DNA Labs International to bring the HID NIMBUS® Presto System online.

Impact Statement: The presentation will impact the forensic science community by demonstrating how the HID NIMBUS® Presto System was brought online and could increase the processing capabilities at forensic laboratories around the world.

As advancements are made in the forensic science world, some of the most common questions revolve around how we make the process more efficient and accurate. The use of robotic systems to assist in laboratory processing has been used in the forensic community for decades to ensure reliability of results while also increasing efficiency. This allows forensic scientists to focus their efforts on more complex tasks like screening items of evidence, data interpretation, and mixture deconvolution.

Since 2013, the Tecan Freedom EVO® 150 Robotic Workstation has been dutifully extracting, quantifying, normalizing, and amplifying tens of thousands of samples every year. It has been a valuable tool with the capability of performing both extraction and quantification with minimal user intervention. However, as with all robotic platforms, at some point these years of glory must come to an end. Similar to the vehicles we drive every day, repeated use causes breakdown over time and at some point, it becomes time for a new instrument.

DNA Labs International began exploring alternative options for high throughput extraction for non-differential samples in 2022 and the HID NIMBUS® Presto System was taken into consideration. A large point to consider was that the same extraction chemistry currently in use on the Tecan could also be employed on this robotic platform consisting of the Hamilton™ Microlab™ NIMBUS® HD Liquid Handler in conjunction with the KingFisher™ Presto Purification System. Additionally, the processing time would be a fraction of the current processing time. Having dealt with the headaches of custom scripting in the past, the pre-made scripts offered on this system were also appealing. In 2023, the system was purchased, and training and testing went underway. This presentation will highlight the testing results, including sensitivity and modifications to the previous protocol as well as plans for the implementation of downstream quantification, normalization, and amplification platforms.

Robotic System; Forensic DNA; Validation

B12   An Evaluation of the Effectiveness of Flow Cytometry in Sperm Testing in Mixed Semen Specimen

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Learning Overview: The goal of this presentation is to introduce attendees to a method with highly accurate and effective cell separation to identify sperm cells from samples mixed with sperm cells and female epithelial cells by flow cytometry.

Impact Statement: This presentation will impact the forensic science community by providing forensic personnel with an additional reference method for detecting cases involving mixed sperm cells in sexual assault cases.

The presence or absence of sperm cells is one of the key pieces of evidence in the detection and investigation of sexual assault crimes. Separating male sperm cells from female epithelial cells in mixed samples and obtaining a higher concentration of suspect sperm cells while reducing interference from female epithelial cells is one of the challenges forensic personnel faces. In practical criminal cases involving sexual assault, the conditions of mixed sperm cell samples from the female vagina are often less than ideal. Factors such as postmortem changes, decomposition, or a small number of male sperm cells within a background of a large amount of female epithelial cells further increase the difficulty of analysis. Therefore, developing techniques with highly accurate and effective cell separation is one of the challenges that forensic personnel must overcome to clarify the case. Flow cytometry, which possesses the characteristic of cell separation, can collect cells with high specificity, achieving the desired separation effect. These characteristics can be utilized in the identification of samples containing female cells and sperm cells in sexual assault cases.

In this study, a mixture of female oral epithelial cells and male sperm cells was prepared to simulate samples from sexual assault cases, and flow cytometry analysis was performed on the samples. The Forward Scatter (FSC) and Side Scatter (SSC) parameters of flow cytometry were used for analysis. The results showed that there were two cell populations in the mixed samples (located in regions R1 and R2), demonstrating that flow cytometry can indeed classify mixed samples into distinct groups. Furthermore, to achieve significant cell classification, this study evaluated several antibodies to enhance the cell classification effect. CD52, ACRV1, MOSPD3, JLP, DEFB126, PH20, and ADAM23 antibodies were selected to label male sperm cells, and the results showed that CD52 antibody labeling had the best effect (88.7% positive in region R2). CD324, CD326, and CD227 antibodies were selected to label female oral epithelial cells, and the results showed consistent effects for all three antibodies (positive rates in region R1 were 92.6%, 91.4%, and 92.2%, respectively). Additionally, mixtures containing different amounts of male sperm cells were examined to evaluate the limitations of the flow cytometry technique. It was found that when the sperm cell concentration was below 10^3, the positive signal of sperm cells was too low, which could potentially affect subsequent DNA profiling analysis. Therefore, in addition to utilizing the differences in surface antigens between male and female cells, this study also used parameters such as differences in nucleic acid content and exclusion of the CD45 signal to improve the classification of sperm cells and female cells in mixed samples, providing forensic personnel with an additional reference method for detecting cases involving mixed sperm cells in sexual assault cases.

Examination; Forensic Science; Sexual Crimes

B13   An Examination of Vaginal Swab Cross-Reactivity in Presumptive Saliva Testing

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Learning Overview: After attending this presentation, attendees will be informed about the use of presumptive saliva testing on vaginal swabs when investigating sexual assault kits. This examination involved comparing two presumptive saliva tests commonly used within the forensic science community and determining if menstrual cycle impacted the rates of cross-reactivity.

Impact Statement: This presentation will impact the forensic science community by providing an update to the collection of sexual assault evidence to include the collection of vaginal swabs prior to the collection of urine. Additionally, this research allows practitioners to be aware of the limitations of presumptive saliva testing.

Background: Forensic laboratories use presumptive saliva testing to identify probative staining as well as in Sexual Assault Examination Kits (SAEKs) to corroborate victim statements. Two types of presumptive saliva tests are currently used within forensic testing: one which detects the enzymatic activity of amylase and one which detects the amylase enzyme itself. Amylase is a key component in saliva that aids in the digestion of complex carbohydrates and is either produced by the salivary gland, salivary amylase, or the pancreas, pancreatic amylase. Amylase is present in various bodily fluids, including saliva, urine, breast milk, tears, perspiration, nasal secretions, and feces. An ideal presumptive saliva test would be sensitive enough to detect minimal amounts of amylase and specific enough to only detect amylase produced by the salivary gland.

Aims/Purpose: The purpose of this study was to determine the rates of cross-reactivity when testing vaginal swabs and urine with RSID™-Saliva and Phadebas® Amylase test methodologies as these fluids are known to contain non-salivary amylase. Additionally, this study noted the menstrual cycle phase from each participant to determine if menstrual phase increased the cross-reactivity rate. This research provided foundational knowledge on rates of false positivity when using presumptive saliva testing on samples that may contain vaginal fluid and/or urine.

Methods: Biological females were asked to abstain from any sexual contact for five days prior to collection. All participants collected eight vaginal swabs, followed immediately by collecting one urine sample. Testing was performed in duplicate on all samples using both RSID™-Saliva and Phadebas® . RSID™-Saliva is an immunochromatographic test method which detects the presence of the amylase and is ran on a test cartridge, while Phadebas® detects the enzymatic activity of amylase within a test tube. When performing the RSID™-Saliva testing, samples were extracted in either manufacturer provided Extraction Buffer or ddH$_2$O to determine if the extraction method altered the test's specificity.

Hypothesis: It was hypothesized false positives would be seen on vaginal samples when participants were either in the follicular or luteal phase of their menstrual cycle, as amylase levels are highest during these phases. It was also hypothesized the urine samples would not have any false positive results.

Results: RSID™-Saliva had a false positivity rate of 10% for urine samples and an inconclusive rate of 10% for vaginal swabs, while Phadebas® had a false positive rate of 20% for urine samples and a false positivity rate of 5% for vaginal swabs. An inconclusive RSID™-Saliva result was determined when the test line was present but too faint to be able to testify about its presence in court.

Conclusion: RSID™-Saliva and Phadebas® both had detectable false positives when testing urine and/or vaginal swabs. There are multiple potential causes of this cross-reactivity, including the host's natural presence of pancreatic amylase within the vagina and amylase produced by bacteria present within the vaginal cavity. This research provides the groundwork for additional studies that can aid in determining best practices for detecting salivary amylase on vaginal swabs, such as testing a smaller portion of the collected swab. Additionally, this research allows for a recommendation to be provided to Forensic Nurse Examiners (FNE) to collect vaginal swabs prior to urine to prevent inadvertent urine deposition on the vaginal swabs.

Saliva; Forensic DNA; Sexual Crimes

B14   Mitochondrial DNA Sequencing for Forensic Human Identification in a Saudi Arabian Context

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Learning Overview: After attending this presentation, attendees will be aware that: (1) mitochondrial sequencing of HVI, HVII, and HVIII resolution increases with whole genome sequencing; and (2) validation of Massively Parallel Sequencing (MPS) analysis consistent with Sanger-Type Sequencing (STS) for Mitochondrial DNA (mtDNA) typing of samples that may be encountered in a forensic laboratory.

Impact Statement: This presentation will impact the forensic science community by developing strategies for a complete mtGenome sequencing platform and being the first to investigate mtGenome different haplotypes and observe the hypervariable regions of the Saudi population in order to evaluate their application in forensic sciences.

The mtDNA is inherited maternally; it has developed from one population to another in the presence of polymorphisms, hence it is widely used as a marker in molecular evolution, population genetics, and forensics. mtDNA has some unique features that make it a particularly useful forensic marker such as absence of recombination across many generations, maternal inheritance, accumulation of mutations over time and multiple copies per cell. Therefore, it is particularly characteristic and informative in kinship analyses.

The sequence of a particular mtDNA genome is its haplotype, which is a genetic locus inherited as a unit. mtDNA genomes that are not identical, but closely related, are said to be within the same haplogroup (a collection of related haplotypes with the same ancestral mtDNA molecule). mtDNA can be assigned to a haplogroup based on the characteristic mutations that it contains; some of these are within the hypervariable region, whereas others are dispersed around the genome; the evolutionary relationship of different haplogroups can be shown as a phylogenetic tree. There is much mtDNA sequence data produced from different global and regional populations. Information from the haplogroup for any mtDNA provides us with a rational arrangement of the mtDNA according to recognized phylogenetic principles. However , it demands a reliable platform for representation of data to be able to draw meaningful and simplified information about mtDNA distribution.

Currently, STS is the primary method to develop mtDNA profiles in forensic laboratories. However, this method is laborious, time consuming, and, therefore, subject to human error. The application of MPS to forensic genetics has rapidly expanded in recent years due to its potential to dramatically increase sample amount, detection resolution, workflow, and cost efficiency, especially when compared to Sanger sequencing.

The creation of regional reference databases is recommended for all forensic loci, but even more important for lineage markers. While there is already a large amount of mtDNA sequence data produced from different global and regional populations, limited mtDNA data exists for the Kingdom of Saudi Arabia, with most previous studies focusing on medical genetics aspects of the population, and so do not necessarily reflect forensic genetics population studies.

This study investigates the Saudi population from a mitochondrial perspective and aimed to establish a mtDNA forensic database; a total of 300 samples were analyzed that were collected from Saudi nationals.

Samples were collected, then DNA was extracted and quantified. After primer optimization using the chosen sample and primer concentration, samples generated a high-quality mtDNA control region dataset. The entire Control Region (CR) was amplified, sequenced, and analyzed by STS using six forward and reverse sequencing primers that generated full coverage with overlapping sequences across the entire mtDNA control region, representing 66.2% unique haplotypes and 7.5% shared. Whereas, with MPS of the whole mtDNA, 47% of these mitochondrial types were unique and 19% haplotypes were shared. The polymorphic sites of the whole mtDNA genome MPS were found in the CR with a total of 119 polymorphic sites and 85 in CR Sanger analysis, a large number of unique mitochondrial haplotypes suggesting the high mtDNA, especially in mtDNA control region for forensic identification context.

Haplogroups were assigned and showed that J, R0, and H are the major haplogroup with almost half of the population samples and L2, L3, and M for the rest of the sample haplogroup analyzed between both Sanger CR and MPS mtDNA whole genome. This frequency distribution of mitochondrial haplogroups shows that Saudi Arabia is genetically related to the Middle East and Africa, which was expected due to its geographical location.[1-86]

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Genetics; Mitochondrial DNA; Statistics

B15   An Application of Chemometrics-Assisted ATR/FTIR Spectroscopy for the Identification of Human Biological Stains Exposed to Different Environmental Conditions and Estimation of the Time Since Deposition

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Learning Overview: After attending this presentation, attendees will be informed about new applications of a non-destructive method (infrared spectroscopy) in forensic investigations.

Impact Statement: This presentation will impact the forensic science community by helping investigators at the crime scene, without consuming the sample, to rapidly discriminate the type and the species of origin of unknown biological stains and to establish their Time Since Deposition (TSD) in order to identify and collect only samples related to the criminal event.

Introduction: Different types of biological stains can be found at a crime scene, and sometimes they may be recovered days after exposure to different environmental conditions. The utility of a biological evidence consists in the ability to obtain a DNA profile that can be compared to the ones of a suspect, a victim, or can be inserted in a DNA database.[1] Therefore, it is important to correctly identify biological stains and to discriminate them according to date of deposition.[2,3] Traditional analytical methods are destructive and, in cases of scarce samples, they may compromise further analysis such as DNA typing. Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) has great potential for forensic investigation since it rapidly provides detailed information about the chemical composition of a sample by means of the generated spectrum.[4] Here we evaluate the suitability of ATR/FTIR in combination with chemometrics for the differentiation of biological stains onto porous and non-porous substrates when exposed to different environmental conditions.[5,6] The aim of the study is to develop an innovative rapid, low-cost, and effective method that allows, without consuming the sample, one to rapidly discriminate unknown biological stains and establish their TSD in order to identify and collect only samples related to the criminal event.

Material and Methods: Blood, saliva, and semen will be collected from two adult anonymous donors (one woman and one man) in accordance with the Ethic Committee for Research and Animal Experimentation of the University of Alcalá. First, 100µL of each biological fluid will be deposited onto two substrates (paper and plastic) as representatives of porous and not porous substrates. Each stain will be divided in four parts and kept in different conditions for one week: 18-25° indoors; at 2-8°C in a fridge; in direct sunlight (>25°C); and at high humidity (>50°C). Temperature and humidity conditions will be monitored by a datalogger. Infrared (IR) spectra measurements will be performed at 24h, 48h, 72h, and 7days using an FTIR Spectrometer Nicolet iS10 equipped with the software OMNIC v9.6. Each sample will be measured five times to assure measure reproducibility. Samples will be measured between the range 2,000–800 cm-1.[7] IR spectra will be pre-processed using The Unscrambler X, then data will be used to make Orthogonal Partial Least Square Discriminant Analysis (OPLSDA) models using the SIMCAv17.02software, useful to differentiate samples according to type, donor sex, and TSD.

Results and Discussion: ATR/FTIR relies on vibration of the molecules' bonds after their excitation with electromagnetic radiation.[8] In previous studies, we have demonstrated the suitability of ATR/FTIR spectroscopy for the identification of bloodstains and for the differentiation of human and animal oral fluids stains as well as for the estimation of their TSD.[9-11] Here we expect to demonstrate that the IR spectral signatures of blood, saliva, and semen allow their identification independently from the substrate where fluid is deposited and to observe spectra changing as each sample composition degrades over time when exposed to different environmental conditions. In addition, we expect to be able to construct a chemometric model able to differentiate stains according to type, donor sex, and TSD.

Conclusions: Rapid non-destructive methods (IR Spectroscopy and Raman) appear to have great potential in forensic science for many applications.[12] Challenges in the analysis of forensic samples are due to the wide range of biological samples, variation in the sample amount, high heterogeneity of the substrates, and exposure to factors that favor degradation (i.e., heat, humidity) and limit the samples available for the analysis. The present study is based on a novel approach to face a relevant forensic issue consisting in the rapid non-destructive identification of biological stains and in the TSD estimation and to expand knowledge in the field.

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Crime Scene Investigation; Bodily Fluid; Age Estimation

B16   Cellular Autofluorescence for the Determination of Time Since Deposition of Blood Samples

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Learning Overview: After attending this presentation, attendees will have a better understanding of the relationship between the autofluorescence of cellular components of dried blood and Time Since Deposition (TSD) over a period of six months.

Impact Statement: This work will impact the forensic science community by assisting in developing a screening technique for TSD of all bodily fluids to assess the probative value of biological evidence at a crime scene.

Knowledge of the relative time evidentiary biological material was deposited at a crime scene, referred to as the TSD, would assist investigators by determining which pieces of evidence are most relevant to a case and help with reconstruction of events. One of the most commonly encountered body fluids at a crime scene is blood, which has a complex matrix containing several endogenous fluorophores. Various methods for estimating TSD of bloodstains have been developed, primarily spectroscopic analyses centered around the oxidation of hemoglobin or the fluorescence lifetime of tryptophan residues. However, none of these methods to date have been validated for use in the forensic laboratory for several reasons, including lack of precision and reliability of TSD estimates, instrumentation, time, labor costs, and importantly, the consumption of the evidentiary sample. Flow cytometry measures the optical properties of cells by interrogating them with a laser, exciting either endogenous fluorophores or hybridized fluorescent tags. As a fast and non-destructive method, flow cytometry is a promising technique for use in forensic science. Past applications of flow cytometry in the field have focused on differentiating between contributors or cell types in sexual assault casework and has only recently been explored for age estimation of body fluids. Studies estimating TSD of epithelial "touch" and saliva samples have found a positive correlation between autofluorescent intensity and TSD.[1,2]

Thus, this study expanded on that research by investigating the relationship between autofluorescence and TSD for blood. Blood samples from ten donors were deposited in triplicate onto microscope slides (three separate stains for each replicate), air dried and maintained at ambient conditions, and collected after designated time periods ranging from fresh(t=0) to six months. One stain was designated for the analysis of whole blood and another for analysis of only the White Blood Cell (WBC) component, both using flow cytometry. The third stain was for DNA characterization at the time points. To obtain the WBC component, red blood cells were lysed using Ammonium-Chloride-Potassium (ACK) Lysing Buffer prior to characterization using flow cytometry. Samples were collected after the assigned interval using a sterile cotton swab moistened with deionized water and air dried. Prior to data gathering using a flow cytometer, samples were filtered through a 100μm mesh.

Results showed that over the course of six months, the fluorescent intensity of WBC samples increased with increasing TSD, with fresh samples showing median intensity ~2,250 Relative Fluorescence Units (RFUs) and six-month samples showing median intensity of ~15,000 RFUs. The whole blood data showed comparable but distinct trends showing little to no autofluorescence shifts between 0 and 7 days and then linear increases with time from 7 days until 6 months. This trend, consistent with that previously recognized in saliva and "touch" samples, is promising for the development of a screening technique to determine the TSD of unknowns at a crime scene using a comparison with a fresh sample from a person of interest. To test compatibility with the operational workflow of a DNA case-working unit, a series of blood samples were analyzed for TSD, collected from the flow cytometer, and then subjected to a DNA profiling workflow. Results showed that cellular DNA could be recovered with high efficiency (>95%) and that probative profiles could be generated that were consistent with the original contributor.

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Bloodstains; Flow Cytometry; Time Since Deposition

B17   The Accuracy of Semen Presumptive Testing

Heather Rogers*, University of Central Oklahoma, Oklahoma City, OK; Rhonda Williams, University of Central Oklahoma, Edmond, OK

Learning Overview: This presentation will use data collected from semen presumptive tests to demonstrate the importance of having accurate test kits in forensic science. After attending this presentation, attendees will better understand the importance of considering the possibility of false positive results when conducting semen presumptive testing with actual evidence.

Impact Statement: This presentation will impact the forensic science community by showing that presumptive tests have a chance of producing inaccurate test results. In the past, there has been very little research done on the accuracy of semen testing, and there hasn't been a single study done where researchers compare RSID Semen™, Seratec PSA™, and ABAcard P30™. Investigators should take from this study that they need to confirm their results with secondary testing.

Semen presumptive testing is used to detect the possible presence of semen on evidence or at a crime scene. Semen presumptive tests work by detecting a particular enzyme, such as the Prostate-Specific Antigen, or PSA for short, and semenogelin. However, these tests are prone to false-positive results because PSA and semenogelin can be found in other parts of the body or even outside of the body. Despite this, some agencies still use semen presumptive test results at trial as a confirmation of semen.

In this study, three rapid immunochromatographic test kits were used to test the accuracy and sensitivity of semen presumptive test kits. One semen presumptive test used in this study was the Rapid Stain Identification Series, also known as RSID Semen™. RSID Semen™ works by indicating the possible presence of semen by detecting the semen component, semenogelin.[1] Semenogelin is the main component of the human semen coagulum and is highly concentrated in seminal vesicle fluid.[2] The other two test kits used were Seratec PSA™ and ABAcard P30™. They indicate the possible presence of semen by detecting the PSA, one of the components of seminal fluid.[3] PSA is needed to degrade the other components of semen to keep the semen sample from coagulating.[3]

The study aims to determine the sensitivity and specificity of semen-presumptive tests used in forensic labs. This study aims to determine which of the three test kits had a higher accuracy rate. All three test kits were used to test 26 samples, most of which included no traces of semen. A variety of hygiene products, as well as urine samples, breast milk samples, blood samples, and vaginal swabs, were tested to determine how often false positive test results occur in each of the test kits. Some tests were expected to have a higher rate of false positive results. There is also expected to be a difference in sensitivity between the test kits. This study is important to forensics because these tests are still widely used by forensic labs. Several past studies have produced false positive results with all three presumptive tests.[4-6] This is dangerous because semen presumptive results have been used at trial as evidence. The results of this study will be able to help investigators determine which test kit they should use on evidence. This is vital information because using the wrong test could affect the investigation. The use of the correct kit will lead to a more accurate investigation.

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Semen; Accuracy; Bodily Fluid

B18   The Developmental Validation Testing of the Integrated Quantifiler™ Trio-HRM Assay

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Learning Overview: This presentation will introduce the forensic DNA community to the optimal run conditions and limitations of the integrated Quantifiler™ Trio-HRM assay that allows for mixtures to be discerned earlier in the forensic DNA workflow.

Impact Statement: The completion of the project defined herein will impact the forensic science community by contributing to saving time and resources, which are limiting factors in forensic labs, that are consumed in the lengthy mixture detection, analysis, and reanalysis process. Furthermore, the additional information that will be provided about an evidentiary sample could help shape or redirect the path of an investigation prior to the development of a Short Tandem Repeat (STR) profile.

The forensic DNA analysis process is currently incapable of providing information about the number of contributors in an evidentiary DNA sample at an early stage. Although guidelines exist for resolving mixtures, these measures are often reactive rather than proactive and require substantial time and effort. To meet this need, we integrated a High-Resolution Melt (HRM) mixture screening assay into the quantitative Polymerase Chain Reaction (qPCR) step of the forensic workflow producing the integrated Quantifiler™ Trio-HRM assay. The assay, when coupled with a sequential statistical prediction tool, allows for the identification of the contributor status (single-source vs. mixture) of a sample. Data was previously presented demonstrating that the integrated Quantifiler™ Trio-HRM assay produced hDNA quantitative values, M:F ratios, degradation indices, and DNA profiles consistent to those obtained when the standard Quantifiler™ Trio assay was used. When 56 single-source and 16, 1:1, 2-person mixtures were used to evaluate the integrated Quantifiler™ Trio-HRM mixture prediction assay on the QuantStudio™ 6, an overall accuracy of 79.2% was obtained in which 57 of the 72 samples tested were correctly classified as single-source or a mixture.[1]

Many factors determine the complexity of a mixture, including the number of contributors, quantity of DNA from each contributor, and the quality of the DNA. Further, all new methods used for forensic DNA analysis must undergo developmental validation prior to being implemented in labs.[2,3] Thus, a select group of essential developmental validation studies were conducted to assess a variety of sample conditions and determine the performance limits of the newly developed integrated Quantifiler™ Trio-HRM assay. Initial developmental validation testing for this assay included reproducibility and accuracy testing for single-source samples and mixtures that had varying numbers of contributors or varying ratios. For each study, 10-40 samples were evaluated using the final formal protocol for the integrated Quantifiler™ Trio-HRM assay and the predictions for single-source vs. mixture samples were compared to knowns to determine the overall accuracy.[1]

First assessing for reproducibility, the assay was found to be 59% reproducible, 10/17 samples tested were concordantly predicted as a single-source or mixture across runs. Ten of the previously evaluated two-person mixtures were remade at the following mixture ratios: 1:2, 1:5, 1:10, and vice versa. Additionally, five of the existing two-person mixtures were remade with a third, fourth, and fifth contributor at equal ratios. Overall, 80.0% of the ratio mixtures and 90.3% of the increased contributor mixtures were accurately predicted as mixtures. Further studies will be conducted to evaluate mixtures with ratios from 1:20-1:100 and 100:1-20:1 and to assess the sensitivity of the assay using concentrations that range between 0.01-60 (ng/ul). Once the aforementioned validation studies are completed, mock evidentiary samples will be collected and tested using the final protocol established for the integrated Quantifiler™ Trio-HRM assay.

With these developmental validation studies and a user-friendly web interface (in development), the application will be ready to deploy to accredited practitioner laboratories for external testing. Upon successful validation and implementation, this tool will provide forensic examiners with a powerful way to screen and triage evidence items prior to the end point of analysis.

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Mixture Analysis; DNA; Validation

B19   A Qualitative Analysis of Natural, Synthetic, and Semi-Synthetic Cannabinoids in Vape Products by GC/MS

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Learning Overview: This presentation will provide the forensic science community with a new method to qualitatively analyze vape products that contain cannabinoids using a simple sample preparation and analysis by GC/MS. After attending this presentation, attendees will also have a better understanding of what other cannabinoids may be found in today's vape products.

Impact Statement: This presentation will impact the forensic science community by providing a closer look into what natural, synthetic, and semi-synthetic cannabinoids are found in vapes on the market in the Northeast United Sates that may be leading to adverse effects.

The use of vaping devices to consume cannabis, and more recently synthetic and semi-synthetic cannabinoids, has surged in the past two decades in individuals of all ages. More recently, the recreational market for vaping devices has pivoted, largely to skirt legal regulation, and now includes "alternative" cannabinoids. Vaping devices (vapes) are electronic products that allow users to inhale an aerosol that contains nicotine and/or other substances such as cannabinoids and is seen by many as a healthier alternative to smoking. Vapes that contain natural, semi-synthetic, and synthetic cannabinoids are of concern due to adverse effects and negative user experience. Additionally, while some cannabis vapes are regulated in states where cannabis has been legalized, there remain gray markets for all types of cannabinoid-containing products that may not be regulated. Overall, this subject area contains limited research as rapid evolution continues in cannabis and cannabinoid markets. To assess the cannabinoids found in vape products a qualitative GC/MS method was developed to provide chromatographic resolution and diagnostic mass spectral characterization for 18 target cannabinoids: CBD, CBG, CBN, D^8-THC, D^9-THC, D^{10}-THC, HHC, 9(R)-HHCP, THCV, D^9-THCO, THCM, THCB, THCH, THCA-A, THCP, D^9-THC-C8, ADB-BINACA, and MDMB-4en-PINACA.

The GC/MS method employed an HP-5 column (5% phenyl-methylpolysiloxane) on an Agilent® 5973 N coupled to an Agilent® 6890N MS detector with a scan acquisition mode from 40 to 400amu. The e-liquid from the vapes were diluted in 1mL of methanol and spiked into a 50ug/mL solution of methanol with the two internal standards of amitriptyline and strychnine for analysis. Identification was performed by chromatographic retention time and mass spectral comparison to an in-house library database generated from purchased reference standards. Validation in accordance with the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) guidelines was performed for interferences, sensitivity, and stability. Interference studies were designed to assess interferences from the matrix, internal standard, and analytes of interest. Assay sensitivity was determined using purchased reference standards and Signal to Noise (S:N). Finally, stability was evaluated for analytes in prepared samples.

To date, the method has been applied to over 50 diverse vape products (the sample population includes commercial, seized, and collected vapes) to accurately determine what cannabinoids along with new emerging THC isomers are present in various vape products: analysis continues, and future results will be provided. The study contains a unique sample subpopulation of vaping devices collected from teenagers and provides insight into differences in products among different populations. Our analysis shows that the most common cannabinoids found in vapes used by teenagers (among our limited samples) are D^9-THC, D^8-THC, CBN, and CBG. Analysis of authentic samples has shown major differences between labeled cannabinoids and the cannabinoids that were detected in the vapes.

This is a validated GC/MS method that can qualitatively identify 18 different cannabinoids including natural, alternative, and semi-synthetic cannabinoids in vape products. It has given insight into the unlabeled or mislabeling of cannabinoids within products, along with the most common cannabinoids found in vapes used by teenagers in the Northeast United States, some of which may be leading to adverse effects. Increasing the awareness of unlabeled or mislabeled cannabinoids within vaping products could positively influence a person's decision on whether to use vapes.

Vaping; Mass Spectrometry; Cannabis

B20   Quantifying Cannabinoids in Complex Matrices

Michael W. Gilbert, Pinellas County Forensic Lab, Largo, FL; Reta Newman*, Pinellas County Forensic Laboratory, Largo, FL

Learning Overview: After attending this presentation, attendees will understand how to validate streamlined analytical procedures to quantify cannabinoids in plant material, oil, and waxes.

Impact Statement: This presentation will impact the forensic science community by illustrating how laboratories keep pace with increasing analytical demands associated with recent marijuana regulation.

The marijuana industry is regulated by the 2018 United States Department of Agriculture (USDA) Farm Bill that defines hemp as cannabis containing less than 0.3% delta-9-Tetrahydrocannabinol (Δ9-THC) by dry weight and by multiple state statutes that govern medicinal and recreational use. Without innovative solutions and additional resources to help meet laboratory accreditation requirements, the increased analytical burden of quantifying Δ9-THC in a complex matrix is creating an unstainable environment for publicly funded laboratories.

This study validates a novel analytical test kit for high-throughput testing applications in plant, oil, and wax products using Gas Chromatography/Flame Ionization Detection (GC/FID). A quantitative validation study that assessed accuracy, precision, measurement uncertainty, calibration models, reportable range, sensitivity, specificity, carryover, interference, and analyte stability was completed for each matrix in one week. Results show that validated testing procedures are accurate (% bias <20%) and precise (% CV <20%) and that the validated reportable range meets statutory requirements for hemp products. Similar results were obtained for oil and wax. Suitability was further assessed for drug chemistry cases by evaluating a certified reference hemp standard over multiple non-consecutive days. Δ9-THC and cannabidiol were adequately separated and quantified at the statutory limit for Δ9-THC (< 0.3%).

The pre-manufactured test kit is a custom-product designed to meet individual laboratory Quality Assurance/Quality Control (QA/QC) requirements and comes equipped with matrix-matched, injectable-ready standards and quality controls to speed sample analysis. Laboratories only extract unknown specimens with custom extraction solutions tailored for specific standard operating procedures. This increases laboratory efficiencies by alleviating many analytical burdens associated with the intricate task of preparing standards and controls required for accreditation.

Cannabis; Hemp; Method Validation

B21   Seized Drugs: Carrier Gas Conversion of Helium to Hydrogen for GC/MS and GC/IR Instruments

Cody Woltz*, Orange County Crime Lab, Santa Ana, CA; Sadie Kasten*, Orange County Crime Lab, Santa Ana, CA

Learning Overview: After attending this presentation, attendees will be informed about the Orange County Crime Laboratory Seized Drugs conversion of Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography/Infrared Spectroscopy (GC/IR) instruments from helium as a carrier gas to hydrogen as a carrier gas. The hardware changes required for the different MS instruments will be discussed. The attendees will also learn about how GC/MS methods were optimized to make the best use of hydrogen as well.

Impact Statement: This presentation will impact the broader forensic science community because most Seized Drug labs still use helium as a carrier gas, despite the growing cost and limited supply. This presentation hopes to show that there are alternatives that other labs can consider.

The cost of helium has been increasing over the past five years. With consistent supply potentially becoming an issue in the future, alternative options to helium should be considered for Seized Drug analysis. The most popular alternative is hydrogen as a carrier gas, but there are three major drawbacks to hydrogen. The first issue is that hydrogen can potentially be dangerous as an explosion or fire hazard. The second issue is that hydrogen can react with certain compounds and solvents, causing issues with Electron Ionization (EI) spectral matching with instrument libraries acquired utilizing helium. The third issue is that there is a loss in sensitivity when comparing hydrogen GC instruments to helium GC instruments.[1]

Safety issues can be mitigated by using features available on most GC instruments in combination with using hydrogen generators to prevent the build up of excess hydrogen. Seized Drug sections are capable of mitigating the sensitivity issues as typical drug submissions require only a small amount of material for GC/MS or GC/IR testing. Most drugs that Seized Drug sections typically see are not reactive with hydrogen. The few drugs that are reactive such as PCP or tramadol can be reacquired using the new hydrogen conditions for better EI spectral matching.

The Orange County Crime Laboratory Seized Drug section first experienced issues obtaining helium in 2018 due to price and availability from contracted sources. Since then, the section has converted most of their GC/MS and all of their GC/IR instruments to hydrogen. This presentation will cover the following points: (1) changes made to EI sources on different models of GC/MS instruments to allow for the use of hydrogen; (2) method optimizations made to both GC/MS and GC/IR instruments to mitigate issues with hydrogen; (3) drawbacks that were encountered using hydrogen that had to be mitigated; and (4) the overall impact that these changes have had on the Orange County Crime Laboratory Seized Drug section.

Reference:
1.   Agilent EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion User Guide; Agilent® Technologies: Santa Clara, CA, 2022; 1-36

Seized Drugs; Gas Chromatography; Hydrogen

B22 **The Structural Characterization of Nitazene Analogs Using Electron Ionization/Mass Spectrometry (EI/MS)**

Emma Hardwick*, Huntsville, TX; J. Tyler Davidson, Sam Houston State University, Conroe, TX

Learning Overview: After attending this presentation, attendees will have learned about conserved fragmentation pathways and diagnostic ions observed for 17 representative nitazene analogs using Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS). Attendees will learn about shifts in fragmentation behavior induced by the type and location of substitution to the core nitazene structure and how to apply this information to the identification of novel nitazene analogs.

Impact Statement: This presentation will impact the forensic science community by furthering our understanding about the EI/MS fragmentation of nitazene analogs with varying substitutions to their core structure. The identification of diagnostic ions that enable the differentiation of nitazene analogs based on the type and location of substitution will assist with the development of more accurate and reliable screening and confirmatory GC/EI/MS methods. Finally, the characterization of nitazene analogs with common substitutions across the nitazene core will help to establish a baseline for the identification of novel nitazene analogs.

Recent changes in legislation within the United States and China have led to a shift in the Novel Synthetic Opioid (NSO) drug market away from fentanyl analogs toward a new emerging class of NSOs known as 2-benzylbenzimidazoles or more commonly, nitazenes. The gold standard analytical technique used for the identification of seized drugs is GC/EI/MS based on the ability to gather both retention time and structural information through the combination of Category B and Category A techniques, as defined by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) and the American Society for Testing and Materials (ASTM) E2329-17. However, given the recent emergence of the nitazene analog class of NSOs, little information is known about the EI/MS fragmentation behavior of these compounds. In addition, many of the initial nitazene analogs to emerge have been classified as Schedule I controlled substances, leading to an influx of novel nitazene analogs designed to circumvent legislative restrictions. Therefore, there is a need to understand the impact of substitution to the core nitazene structure on the observed EI/MS fragmentation behavior, including the identification of diagnostic fragment ions, to assist seized drug analysts with the identification of novel nitazene analogs. The central hypothesis of this research is that the type and location of substitution to the core nitazene structure will impact the observed EI/MS fragmentation behavior, enabling the identification of diagnostic ions and characteristic ion ratios that can be used to differentiate even closely related nitazene analogs.

This study examined 17 representative nitazene analogs, including nitazene, metodesnitazene, isotodesnitazene, protodesnitazene, 5-methyl etodesnitazene, 5-aminoisotonitazene, menitazene, 4'-hydroxy nitazene, *N*-pyrrolidino 4'-hydroxy nitazene, *N*-piperidinyl 4'-hydroxy nitazene, iso-butonitazene, *N*-desethyl etonitazene, *N*-desethyl isotonitazene, *N*-pyrrolidino isotonitazene, *N*-pyrrolidino metonitazene, *N*-piperidinyl etonitazene, and *N*-piperidinyl protonitazene. These compounds contain representative substitutions to the benzimidazole ring, benzyl group, and ethylamine functionality of the core nitazene structure, with at least two examples for each type and location of substitution. All standards were prepared at a concentration of 100ppm and analyzed using an Agilent® GC/EI/MS. The standards were analyzed individually to avoid issues with co-elution given the importance of collecting pure EI mass spectra for spectral characterization.

The results indicate that even though many nitazene analogs produce similar EI mass spectra, there are subtle differences in low abundance fragment ions that are due to the location and type of substitution to the core nitazene structure. For example, the base peak for nitazene analogs with two ethyl groups on the amine moiety is observed at *m/z* 86, which is formed through an α-cleavage adjacent to the ethylamine moiety. Any observed shift in the base peak indicates modification to the ethylamine moiety, such as the observation of a base peak at *m/z* 58 for *N*-desethyl etonitazene, which has only a single ethyl substitution to the amine functionality. Likewise, the formation of fragment ions through a benzylic cleavage (e.g., *m/z* 135 or *m/z* 149) provides information about the type of substitution on the benzyl group. Typically, this substitution is an alkoxy substitution, which fragments further to a common fragment ion at *m/z* 107 through the loss of a neutral alkene. Finally, differentiation of compounds with different substitutions to the benzimidazole ring is based on observed shifts to the fragment ion formed through an α-cleavage adjacent to the benzimidazole ring (e.g., *m/z* 324 for *N*-desethyl isotonitazene), as well as additional secondary fragmentation through the loss of the alkoxy chain (e.g., *m/z* 282). Even though several of these diagnostic ions are observed at relatively low abundance, they provide helpful information for the differentiation of nitazene analogs, especially for compounds with similar retention times.

Novel Synthetic Opioids; Analogs; Mass Spectrometry

B23   The Effect of Solid Copper Bullets on Barrel Rifling Characteristics

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Learning Overview: This presentation will utilize 3D bullet scans to demonstrate how solid copper bullets affect barrel rifling characteristics. After attending this presentation, attendees will better understand the degree to which the individual characteristics of barrel rifling persist after the successive firing of solid copper bullets.

Impact Statement: This presentation will impact the forensic science community by showing the effects of solid copper bullets on barrel rifling characteristics. Specifically, it will illustrate these effects on the enhanced polygonal rifling characteristics of the Generation 5 Glock® Marksman Barrels, which are present in some of the most common pistols in the United States.

Research on the persistence of barrel rifling features is typically conducted on one of the most common ammunition types on the market, full-metal jacket ammunition. However, solid copper bullets are becoming more commonplace, especially in the hunting industry. This is due to a push for lead-free ammunition supported by environmental legislation. As solid copper bullets become more widely used, crime laboratories will see an increase in the number of solid copper bullets submitted as evidence. For firearms examiners to better understand the effects of solid copper ammunition on the persistence of barrel characteristics, more research is needed. The purpose of this study is to determine if solid copper bullets can be identified after successive firing.

This study will include the successive firing of 500 solid copper bullets through a new Glock® 19 Gen 5 chambered for 9mm Luger® ammunition. The first ten, last ten, and every tenth bullet will be collected for a total of 68 bullets. These bullets will be scanned using the Cadre Forensics TopMatch-GS 3D System. The three-dimensional scans of the land-engraved areas will be used to compare the individual characteristics left on the bullets by the barrel rifling.

In contrast to previous studies that involved qualitative visual examinations, this study will be based on quantitative data. The TopMatch System's built-in algorithm will be used to generate similarity scores for each comparison. Similarity scores range from zero to one, with higher values indicating greater similarity between the features being compared. The algorithm's results will be used to support conclusions, and the similarity scores will allow for statistical demonstrations on the similarity between the fired bullets.

The results from this study will show if solid copper bullets affect barrel rifling characteristics to the point where identifications are no longer possible after 500 rounds. The three-dimensional scans from this study will be uploaded to the National Institute of Standards and Technology Ballistics Toolmark Research Database to allow other researchers and professionals to understand the effects of solid copper bullets. The results will also benefit firearms analysis by showing how solid copper bullets affect the rifling characteristics of Glock® barrels. Glock® is one of the most popular pistol manufacturers, so Glock® pistols are often involved in crimes. Understanding how solid copper bullets affect Glock® rifling characteristics will help firearms examiners better analyze such evidence.

3D Technology; Ballistics; Bullet

B24   The Loss of Latent Fingerprint Visual Quality: Natural Degradation vs. Depletion Series

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Learning Overview: After attending this presentation, attendees will understand the visual differences existing between "natural aging" vs. "depletion series" of latent fingerprints to estimate "absolute" vs. "relative" time-since-deposition, respectively, as examined by 2D and 3D imaging analyses.

Impact Statement: This presentation will impact the forensic science community by providing novel research methods and techniques to investigate the changing topography of latent fingerprints. In the current project, two different imaging approaches are combined (2D and 3D) to provide a robust interpretation of how fingerprints naturally age or deplete to obliteration using computer software, increasing the objectivity of results. These will undoubtedly contribute to the larger pool of data necessary to explain the mechanisms of visual degradation of fingerprints' topography.

Despite its pervasiveness as crime evidence, fingerprints are considered circumstantial evidence and, thus, must be reinforced with unassailable expert testimonies. A suspect (in this case, the actual perpetrator) of a crime could easily justify that their fingerprints were placed months, days, or hours before the crime was committed. If a reasonable alibi is at hand, these fingerprints can be then invalidated as evidence and the perpetrator may walk free. On the other hand, an innocent person whose fingerprints just happened to be at the crime scene could unnecessarily be implicated. Either situation of a false negative or a false positive is undesirable for the victims of a crime, the innocent, and society based on fair justice for all. In practice, the age of fingerprints is a topic that has been implicitly or explicitly discussed in at least 28 court cases from 1961 to 2011 as reported in the literature, and it is a reoccurring issue in crimes involving fingerprints.[1]

This experiment addresses a question embedded within the general goal of estimating the age of latent fingerprints by utilizing optical means alone: can we objectively determine whether a fingerprint has naturally aged or has been depleted to obliteration? In this project, 3,000+ 2D and 3D fingerprint imaging data were compiled between 2018 and 2022 and were statistically examined and compared based on their "natural aging" vs. "depletion series" patterns. Although these are both temporal mechanisms of the loss of fingerprint ridge integrity, the term "aging" refers to the "absolute age or time-since-deposition" of the impression whereas "depletion" refers to the "relative time of deposition in a series of continuous touches," usually within a few minutes. This distinction is important when attempting to reconstruct the specific order of events at a crime scene. Indeed, depletion series are very relevant in cases of two or more fingerprints falling within the same "absolute age range," mostly because of the inherent limitations of the technologies employed at the time of providing an estimate of age with its standard deviation. Then, a method to distinguish fingerprints deposited in a narrow timeframe is by their "relative position" within a depletion series.

The substrate of choice was glass microscope slides exposed to indoor dark conditions. For the "aging" set, fingerprints were aged up to one year, whereas for the "depletion" specimens, up to 24 fingerprints were sequentially deposited. In all cases, sebaceous-rich impressions were created from 41 males and 34 females. The topographical parameters for discerning "aging" vs. "depleting" modifications have included data from 2D and 3D fingerprint images: Blue-Green metric (BG) from the Universal Latent Workstation (ULW) and Sa metric from an Optical Profilometer (OP), respectively.[2] ULW provides metrics on the levels of visual quality (clarity) of a fingerprint and the area extension (mm^2) of "discernable and identifiable" ridges (BG). The OP provides data on the average height of ridges (Sa), among many others. These data were input into a Microsoft® Excel® spreadsheet and IBM® SPSS for further statistical analysis. Preliminary results revealed that Sa metric was more sensitive at detecting differences over time and across depletion series than BG values. At the same time, 2D and 3D metrics were able to distinguish the temporal processes visually and statistically. The results of this study are key to the goal of quantitatively and automatically defining the aging parameters of fingerprints and fueling research and innovation in fingerprint aging analysis.

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3D Technology; Fingerprint; Aging

B25 The Optimized Separation of Cannabis Terpenes/Terpenoids Via GC/EI/MS

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Learning Overview: After attending this presentation, attendees will learn how to optimize the separation of 23 terpenes in a standard terpene mix by Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS).

Impact Statement: This presentation will impact the forensic science community by informing attendees how this optimized method will improve the identification of terpenes in cannabis samples.

The cannabis plant (*Cannabis sativa* L.) produces a variety of compounds covering numerous chemical classes such as cannabinoids, flavonoids, terpenes, and terpenoids. The terpenes and terpenoids are recognized as important constituents in cannabis products by growers and consumers. They are responsible for the aroma characteristics in addition to a variety of purported medical benefits. Terpenes are oligomers of isoprene units, whereas terpenoids are oxidized forms of terpenes.

Analysis of these compounds is typically performed by GC using liquid or gas injection (headspace). Due to the wide boiling point range of these compounds, operational cannabis laboratories most often perform the analysis using liquid injection. However, separation of the numerous and wide variety of compounds in the relatively short period of time needed for high throughput is challenging. Separation of terpenes and terpenoids is necessary for proper identification and quantitation. The identification and quantification of these compounds has become increasingly important with the state-by-state movement towards cannabis legalization and the purported medical benefits of all compound classes in the plant.

In the first phase of the project, we determined that separation (selectivity) of terpenes and terpenoids commonly found in consumer products was improved by modifying GC column stationary phase chemistry and temperature programming. In this current phase, we focused on modifications to the column dimensions. These variables included internal diameter (0.32, 0.25, and 0.18mm), mobile phase thickness (0.32, 0.25, and 0.18um), and stationary phase composition within the column (100% dimethyl polysiloxane vs. 95% dimethylpolysiloxane/5% diphenyl polysiloxane). These conditions were compared using a combination of two standard terpene mixtures available from commercial sources, for a total of 23 terpenes to be separated. Along with the improved separation in this phase, we also created a ChemStation® library of these compounds using the AutoQuant® feature for rapid identification. This improvement in selectivity ultimately achieved identification of these compounds.

Further work in this project will include quantitation of each compound, further optimizing resolution by testing a 1,4-phenylene stationary phase (which has a different polarity profile than di-phenyl), and implementation of these techniques on commercially available botanical samples. To date, the best separation was with a DB-5ms column (20m x 0.18mm x 0.18um load). Full baseline separation was accomplished for 21 of the 23 compounds in the standard terpene mixture.[1-3]

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Cannabis; Separation; Chromatography

B26   The Quantitation of Furanocoumarins in Grapefruit Supplements Using High-Performance Liquid Chromatography

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Learning Overview: After attending this presentation, attendees will understand the amounts of two furanocoumarins, Bergamottin (BGT) and 6'7'-Dihydroxybergamottin (DHB) in over-the-counter dietary grapefruit supplements and other products reported to contain grapefruit extract measured by High-Performance Liquid Chromatography (HPLC).

Impact Statement: This presentation will impact the forensic science community by informing attendees of the amounts of BGT and DHB in County Life® Chewable Acerola Vitamin C Complex, NutriBiotic® Defense Plus, NutriBiotic® Grapefruit Seed Extract, and Solaray® PhytoEstrogen & Grapefruit One Daily supplements with grapefruit extract. These furanocoumarins are important because they inhibit the CYP34A enzyme. Taking these supplements with drugs that are inhibited by this enzyme leads to increased drug concentrations that may cause potential harmful side effects and/or incorrect cause of death. Current Food and Drug Administration (FDA) guidelines do not require that the furanocoumarins be listed as an ingredient; thus, individuals using these products are often unaware of their presence. Our study will inform the forensic community of the amounts of furanocoumarins in these supplements and compare to the amounts of these furanocoumarins known to be present in grapefruit juice, since consumption of 240mL of grapefruit juice inhibits the enzyme.[1] Extraction efficiency and analytical figures of merit for the quantification of these furanocoumarins using an Agilent® 1200 HPLC will be reported.

In today's society, many people try to improve or maintain a healthy lifestyle by taking Over-The-Counter (OTC) dietary supplements and vitamins to obtain the nutrients that the body needs. While this may seem like the ideal route, many individuals do not realize that OTC dietary supplements are not regulated by the FDA under the normal set of regulations.[1]

Consuming grapefruit is known to result in health benefits, such as anti-inflammatory, anti-cancer, and anti-obesity.[2] As such, grapefruit extract or grapefruit seeds are commonly found in OTC supplements. The labels on these supplements, however, do not list the furanocoumarins present. Since two of these furanocoumarins, BGT and DHB, have been known to cause Drug-Drug Interactions (DDIs), several investigators have measured their concentrations in a variety of matrices such as supplements, grapefruit juice, lime juice, grapefruits, cosmetics, essential oils, and parsnip seeds.[1-12]

This work reports on the concentrations of these analytes (after methanol extraction) in different lot numbers of County Life® Chewable Acerola Vitamin C Complex and NutriBiotic® Defense Plus supplements as well as NutriBiotic® Grapefruit Seed Extract and Solaray® PhytoEstrogen & Grapefruit One Daily supplements. A statistically significant difference was found in the amounts of DHB and BGT among different lot numbers of the Acerola supplements and no significant difference for the same analytes among different lot numbers for the NutriBiotic® supplements. The overall mean concentrations of BGT and DHB in the NutriBiotic® supplements were ~11.7 and 11.4 times greater than those for the same analytes in the Acerola supplements, respectively. The amounts of BGT and DHB measured for one tablet were still well below those reported to cause DDIs in one serving of grapefruit juice. It is expected for the levels measured in the grapefruit seed extract and the One Daily supplement to be significantly lower. In addition to these supplements, there are a large number of natural products (noted above) that contain significant amounts of BGT and DHB. Without knowledge of possible DDIs from taking excessive amounts of supplements and/or combining with natural products, there exists the possibility of accidental overdose. The results of this work can help inform the forensic community about these potential effects and provide them with the extraction efficiency and analytical figures of merit for the quantification of these furanocoumarins using an Agilent® 1200 HPLC.

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Furanocoumarin; Grapefruit; Drug-Dric Interaction

B27　Ag-Ligand Ion Complexation: An Alternative Approach for Hemp and Marijuana Differentiation

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Learning Overview: After attending this presentation, attendees will be informed about the differentiation of hemp and marijuana using Ag-ligand ion complexation and a semi-quantitative decision-point assay. Attendees will learn how the presence of unique product ions of Ag-ligand cannabinoid complexes allows for the differentiation of Δ9-Tetrahydrocannabinol (Δ9-THC), Cannabidiol (CBD), Tetrahydrocannabinolic Acid (THCA), and Cannabidiolic Acid (CBDA), enabling the development of a semi-quantitative method to differentiate hemp and marijuana using a conservative 1% administrative threshold.

Impact Statement: This presentation will impact the forensic science community by providing a method for the differentiation of hemp and marijuana using Ag-ligand ion complexation and a semi-quantitative decision-point assay. Differences in preferential binding affinity of Δ9-THC, CBD, THCA, and CBDA to [Ag(PPh3)(OTf)]2 result in the formation of unique product ions in positive ionization mode, which can be then used to differentiate hemp and marijuana using a semi-quantitative decision-point assay.

The 2018 Farm Bill defined marijuana as *Cannabis sativa* L. or any derivative thereof that contains greater than 0.3% Δ^9-THC, whereas hemp contains no more than 0.3% Δ^9-THC on a dry weight basis. Due to the classification of marijuana as a Schedule I controlled substance, it is imperative that seized drug analysts are able to perform both the qualitative identification and quantitative or semi-quantitative analysis of the total THC content in potential marijuana samples, which includes Δ^9-THC and its acidic precursor THCA. The main cannabinoids present in hemp and marijuana are the structural isomers CBD and Δ^9-THC, which complicates analysis when using soft ionization sources due to their identical chemical formula and only slightly different structural arrangement, resulting in nearly identical product ion spectra. The differentiation is further complicated by the presence of additional cannabinoid interferences. The central hypothesis of this research is that hemp and marijuana can be differentiated using Ag-ligand ion complexation and a semi-quantitative 1% decision-point assay, even in the presence of other cannabinoid interferences.

An Agilent® 6530 quadrupole Time-Of-Flight (qTOF) mass spectrometer with direct injection Electrospray Ionization (ESI) was used for the analysis of the Ag-ligand ion cannabinoid complexes. The following cannabinoids were analyzed with and without the presence of [Ag(PPh3)(OTf)]2: Δ^9-THC, CBD, THCA, CBDA, cannabichromene (CBC), Δ^8-tetrahydrocannabinol (Δ^8-THC), $\Delta^{6a,10a}$-tetrahydrocannabinol ($\Delta^{6a,10a}$-THC), cannabinol (CBN), cannabigerol (CBG), exo-tetrahydrocannabinol (exo-THC), and cannabicitran (CBT). The corresponding product ions were collected for abundant precursor ions present in the full scan spectrum with collision energies of 15-45 eV, using a 4 Da isolation width. Spectral comparisons were used for characterization and to determine unique product ions capable of differentiating the Ag-ligand cannabinoid complexes.

The semi-quantitative decision point assay provides a more conservative approach by setting a 1% administrative threshold to reduce the potential for false positives. The 1% administrative threshold is set by fortifying authentic methanolic extracts of cannabis with a Δ^9-THC-d_9 internal standard at 1% by weight. The resulting total THC abundance is then normalized to the Δ^9-THC-d_9 abundance. If the value is larger than 1, then the sample is considered marijuana, whereas if the value is less than 1, then the sample is not considered marijuana. The total THC abundance is determined by removing the contribution of CBD and CBDA from the analytical signal of Δ^9-THC and THCA using THC:CBD and THCA:CBDA unique product ion calibration curves and the abundance of the respective unique product ions at *m/z* 421 and *m/z* 465. The total THC content is the combined abundance of the precursor ions at *m/z* 683/685 and *m/z* 727/729, which correspond to Δ^9-THC and THCA, respectively.

The results indicate that hemp and marijuana can be differentiated using Ag-ligand ion complexation and a semi-quantitative decision-point assay due to the formation of unique Tandem Mass Spectrometry (MS/MS) product ions. Due to the difference in preferential binding affinity of the cannabinoids to [Ag(PPh3)(OTf)]2, Δ^9-THC and CBD can be differentiated by unique MS/MS product ions for CBD at *m/z* 421, *m/z* 353, and *m/z* 231. In addition, THCA and CBDA can be differentiated by unique product ions for CBDA at *m/z* 465 and *m/z* 379. The unique product ion calibration curves allow for the removal of CBD and CBDA contribution and the semi-quantitative determination of the total THC content. To highlight the method capabilities, 20 blind methanolic extracts of authentic cannabis plant material were analyzed and classified as either marijuana or not marijuana. The results support that the developed approach provides a method to both qualitatively identify and semi-quantitatively assess the total THC content in methanolic extracts of cannabis plant material, enabling the differentiation of hemp and marijuana without the need for lengthy chromatographic separations.

Hemp; Marijuana; Tandem Mass Spectrometry

B28   A Workflow for the Rapid Analysis of Trace Drug Residues Collected From Used Paraphernalia

Meghan G. Appley*, National Institute of Standards and Technology, Gaithersburg, MD; Elizabeth Robinson, National Institute of Standards and Technology, Gaithersburg, MD; Edward Sisco, National Institute of Standards and Technology, Gaithersburg, MD

Learning Overview: This presentation will show results from the development and implementation of the Rapid Drugs Analysis and Research (RaDAR) project being conducted at the National Institute for Standards and Technology (NIST). After attending this presentation, attendees with be aware of the method developed for analyzing trace drug residues collected from used paraphernalia, including both qualitative and quantitative results.

Impact Statement: This presentation will impact the forensic science community by introducing a workflow for rapid qualitative and quantitative analysis of trace drug residues collected from used paraphernalia. Preliminary results will also provide information on the drug landscape for several geographical regions across the United States.

Understanding the illicit drug landscape is a critically important task for law enforcement, forensic science, and public health entities as it can better inform investigations, scheduling decisions, addiction treatment approaches, and overdose prevention efforts. To accomplish this, timely, standardized, and complete—identification of drugs, adulterants, and cutting agents—analyses are required. From a forensic science perspective, this is often difficult because of high backlogs and the need to only report controlled substances.

In this project, a platform that is capable of providing rapid, near-complete, and standardized data was developed to capture the drug landscape in real time. A combination of ambient ionization mass spectrometry and traditional chromatography-based mass spectral techniques have been deployed to enable qualitative and quantitative analysis of trace drug residues from used or suspected paraphernalia. The workflow for this effort involves collection of drug residues from law enforcement, public health, or forensic personnel that is then mailed to the laboratory for analysis. An initial screening of the sample is completed, within 24 hours, using Direct Analysis In Real Time-Mass Spectrometry (DART®-MS) to quickly identify and report back all drugs, adulterants, and cutting agents found in a sample. A subset of samples is also tested using chromatography-based techniques for confirmation of newly identified compounds or to quantitate the relative proportions of compounds within a sample. These results are typically provided back within a matter of days.

The RaDAR project was first piloted in October 2021, through a collaborative effort with public health , forensic science, and law enforcement agencies throughout the state of Maryland. The results from the pilot where able to provide critical information on the influx of xylazine into the state's illicit drug supply, the emergence of nitazenes, and other drugs trends that had not been previously found. This information was important to public health and law enforcement entities but also proved to be critical in acting as an early warning indicator to forensic laboratories so they could prepare for new compounds in their case samples.

Since the initial pilot project, RaDAR has grown to include additional regions throughout the country. In addition to discussing the development of the platform, key findings, regional trends, and information of the latest drug trends will be provided in this presentation.

Rapid Drug Screening; Mass Spectrometry; Direct Analysis in Real Time (DART®)

B29   A Homemade Bomb Mistaken for a Terroristic Attack: A Variety of Forensic Analyses Can Help Substantiate the Investigations

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Learning Overview: After attending this presentation, attendees will understand how useful a complete forensic analysis, including chemical analysis, on the cadaver can be in finding out the composition of an explosive device and the position of the victim in relation to it, thus helping investigations.

Impact Statement: This presentation will impact the forensic science community by providing an emblematic example of an explosive-related event and its management from a medicolegal point of view to obtain useful evidence to help investigators reconstruct the assumptive terrorist attack.

An explosion is an exothermic reaction that occurs when an explosive chemical substance (liquid or gaseous) reacts with oxygen, causing the rapid release of energy in the form of heat, noise, and pressure waves (called blast waves). In these reactions, lots of metallic fragments and gaseous particles also spread at very high speed, being able to reach objects and people, causing different grades of damage, depending on their distance from the epicenter of the explosion. Evaluating these fragments, their composition, and their distribution on the body can provide useful clues for investigations.

We present an emblematic case that occurred in May of 2012 in a city in Southern Italy when a bomb blew up near a high school. In the explosion, nine people were injured and one 15-year-old girl died shortly after she was taken to the hospital for the wounds provoked by the detonation. A complete postmortem examination of her body (including Computed Tomography [CT] scans, autopsy, histopathological and immunohistochemical analyses) was performed; we also performed a complete quantitative and qualitative chemical analysis of the commonest metals using an Inductively Coupled Plasma/Atomic Emission Spectrometry (ICP/AES). The CT scans and the autopsy revealed right otorrhagia, partial amputation of the right forearm, the widespread presence of splinters and other metal particles embedded in subcutaneous tissues, and skin burns on 90% of the body. Moreover, these investigations revealed left costal and scapular fractures, left hemopneumothorax, bilateral lung contusions and left pleural tears, presence of soot inside the trachea and the bronchi. Surprisingly, no lesions were found in the abdomen.

As mentioned above, a standard histopathological study was also carried out using hematoxyline-eosin staining. The main findings were acute pulmonary emphysema, massive intra-alveolar and subpleural hemorrhages, gas embolization, and heat-induced alterations of the tracheal epithelium. Skin samples, which were collected near the burns, were stained with sodium rhodizonate and Perls Prussian blue and examined with a phase-contrast microscope. These stainings, specific for the detection of traces of lead, barium, antimony, and iron, confirmed the presence of these heavy metals in the samples. Heat-induced alterations in cutaneous tissues were also confirmed thanks to immunohistochemistry. These samples showed strong positivity to antibodies anti-Heat Shock Proteins (HSP-27, HSP-70, HSP-90).

Considering all the findings, we could assume that the girl died from a severe primary blast lung injury, caused by the detonation of a bomb, without a blunt or penetrating chest trauma. In addition to this, the information about type, quantity, and distribution of the explosive residues was obtained by using an ICP/AES on lyophilized samples of skin containing heavy metals.

The results of our study and the parallel analyses performed by the police helped identify the chemical substances and the materials used to make the bomb, thus leading to the arrest of the culprit, an Italian citizen who used to deal with agricultural fuel for his job, who admitted having made the bomb detonate with a remote for personal revenge.

In conclusion, a complete postmortem study and the assessment of the composition, quantity, and distribution on the body of fragments spread by an explosion can provide important evidence for the reconstruction of explosion-related events.

Explosion; Tissue Analysis; Fragments

B30 Hidden Traces: An Alternative Analytical Scheme for Seized Drug Analysis

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Learning Overview: After attending this presentation, attendees will have learned about an alternative screening method for seized drug analysis using trace residues generated through sample weighing as part of routine casework processing. The attendees will be informed about the ability to rapidly screen for seized drug residues remaining on different types of weigh paper using Direct Analysis in Real Time-Mass Spectrometry (DART®-MS) and a comparison of the screening results to more classical Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS) analysis.

Impact Statement: This presentation will impact the forensic science community by demonstrating a rapid screening method for the detection of seized drug residues remaining on different types of used weigh paper generated through routine casework processing. Incorporation of this alternative screening method provides a rapid approach to acquire high-quality screening results prior to confirmatory analysis using GC/EI/MS that has the potential to improve laboratory throughput relative to traditional analytical schemes.

The traditional seized drug analysis process involves weighing an evidence submission prior to analysis. Typically, presumptive testing is performed with relatively rapid and inexpensive testing such as color tests or immunoassays, followed by more discriminating techniques such as GC/EI/MS. However, the drug residue transferred during the weighing process creates an opportunity for an even more rapid and reliable screening technique using DART®-MS. Rather than treating used weigh papers as chemical waste, the weigh papers can be used as a sample introduction method containing a representative sample of the weighed seized drug evidence. The central hypothesis of this research is that DART®-MS analysis of different types of used weigh papers will enable a rapid and reliable screening method for seized drug evidence without any additional sample preparation. The ability to rapidly identify controlled substances without additional sample preparation enables more targeted confirmatory analysis and a more efficient seized drug analysis process.

A DART® JumpShot® ionization source was coupled with an Agilent® 6530 quadrupole Time-Of-Flight (qTOF) MS to rapidly analyze the used weigh paper samples. The DART® ionization source, which used helium as the source gas, was operated in positive ionization mode with a 350°C gas temperature and a 350V exit grid potential. The MS was operated in positive ionization mode with a scan range of *m/z* 40-450. Collision-Induced Dissociation (CID) was used to generate additional fragment ions under low (0 eV), medium (30 eV), and high (60 eV) activation conditions using All Ion Fragmentation (AIF). The controlled substances analyzed in this study were PCP, heroin, fentanyl, methylphenidate, phentermine, cocaine, methamphetamine, amphetamine, alprazolam, lorazepam, and diazepam. These compounds were analyzed pure and in simulated 2-, 3-, and 4- component mixtures at two different ratios with a total concentration of 50ppm. 10μL of each sample was spiked onto Whatman® 3 filter paper and glassine weigh paper to assess differences in substrate performance. Method validation was performed for glass capillary, Whatman® 3 filter paper, and glassine weigh paper to assess the selectivity, matrix effects, accuracy, repeatability, reproducibility, limit of detection, and robustness. All compounds were identified using the National Institute of Standards and Technology (NIST) DART®-MS Forensics Database and Data Interpretation Tool (DIT).

The results indicate that Whatman® 3 filter paper and glassine weigh paper can both be used as sample matrices for seized drug residue screening following routine sample weighing. Although there were slight differences in validation performance between the different weighing matrices, reliable detection of all controlled substances was observed. When the validated method was applied to 35 authentic seized drug samples that were acquired from a local forensic laboratory, the results demonstrated that powdered samples were readily retained by the weighing matrices in detectable quantities. However, matrices used to weigh capsules, tablets, and drug paraphernalia did not retain sufficient residue for reliable detection. The authentic seized drug samples were chosen to represent realistic combinations of controlled substances and cutting agents, mixture ratios, and quantities of evidentiary sample remaining on weigh papers to accurately assess the developed screening technique. All screening results were compared against GC/EI/MS confirmatory results and any discrepancies were investigated further. The developed DART®-MS screening method of used weigh papers provides an alternative screening technique that requires no additional sample preparation and provides accurate and reliable results for powdered seized drug samples.

Drug Analysis; Rapid Drug Screening; Direct Analysis in Real Time (DART®

B31   Matricide: When Psycho-Social Fragilities Lead to Murder

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Learning Overview: After attending this presentation, which involves analyzing three cases of matricide that occurred between 2021 and 2023 in the province of Ferrara, Italy, attendees will understand that the psycho-social and family context in which matricides develop is the real starting point for the analysis and prevention of this type of crime.

Impact Statement: This presentation will impact the forensic science community by underlying the reasons, the psycho-social contexts and the pathological mother-child bond that lead a child to murder his/her own mother.

The murder of one's own mother, called matricide, is a rare event that has always attracted media interest because of its inherent nature as a violation of ethical and moral principles on which society is founded. Although each crime stands on its own, common pathological family dynamics can often be observed among these types of crimes, especially concerning the mother-child relationship. It turns out, in fact, frequently marked by an ambivalent relationship, connoted by both a strong emotional and economic dependence on the mother and a constant search of self-affirmation. In addition, the father is often a passive or absent figure. Studies show that the perpetrator generally suffers from a psychiatric illness (sometimes already known, sometimes diagnosed later), related mainly to psychotic or paranoid profiles with difficulties in social integration.

This report aims to illustrate three cases of matricide that occurred between 2021 and 2023 in the province of Ferrara, Italy, with the purpose of understanding and exploring the reasons and socio-familiar contexts that lead the offenders to murder their own mothers.

The retrospective analysis of the three matricides that occurred in the province of Ferrara in the period between 2021 and 2023 was carried out by a compilative-comparative method. It proceeded to examine the crime scene investigation, the autopsy evidence, histological, toxicological and immunohistochemical analyses, as well as the killers' interviews with the judicial authorities. Thus, clinical and psychiatric evaluations were analyzed. Such in-depth research made it possible to highlight as much information as possible regarding the pathological-forensic aspects of the crime, the perpetrators, and the victims as well as the relationship between them.

The data analyzed showed that two out of three murders were committed by the male child. Both sons lived in their mother's home; the daughter, although not living in her mother's apartment, resided in the same building, and her home was owned by her parents. All perpetrators were adults, ranging in age from 38 to 52 years, and were unmarried and unemployed. One of the victims was under the age of 70; the other two were elderly and retired. Two out of the three victims were in good general health; one was bedridden and unable to walk and perform activities of daily living. Autopsy and histo-pathological investigation, as well as the analyses of the historical-circumstantial data, showed that in two cases, the cause of death was by asphyxiation by suffocation with a pillow; one case was attributable to acute nitrite intoxication by poisoning (ingestion). Interviews with the murderers revealed that in all cases there was a conflictual relationship with the mother, who was considered a dominant, intrusive, and judgmental figure. In all the cases analyzed, the father was absent. The offenders' social relationships were characterized by extreme isolation. Psychiatric examinations conducted in the period after the crime revealed that all the murderers suffered from a psychiatric disorder: chronic psychotic disorder, paranoid personality disorder, and anxiety-depressive disorder with multiple personality disorders. Only one perpetrator was determined to be suffering from diminished responsibility because of his acute psychotic crisis at the time of the act and his sentence was reduced.

In conclusion, the findings of this preliminary study confirm the data in the literature highlighting the complex socio-familial context in which matricide cases occur. Psychological and relational fragilities between mother-child and between the perpetrator himself and society are often the real starting point for the analysis and prevention of this type of crime.

Femicide; Violence Against Women; Victim-Offender Relationship

B32   Quantitation of Lysergic Acid Diethylamide (LSD) Using HPTLC

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Learning Overview: After attending this presentation, attendees will gain a better understanding of using High-Performance Thin-Layer Chromatography (HPTLC) with an internal standard to quantify LSD.

Impact Statement: This presentation will impact the forensic community by demonstrating that HPTLC can be a useful and viable alternative tool in the quantitation of LSD.

In recent years, TLC has fallen out of routine practice for the analysis of seized drugs in the forensic science community. This technique does not always provide the resolution or sensitivity necessary to accurately quantify substances of interest. However, with the advancement of HPTLC, quantitation of analytes can be done with a green, fast, and reliable method.

LSD can be a challenging compound to analyze and quantify. Using Gas Chromatography/Mass Spectrometry (GC/MS) to analyze and quantify LSD can cause issues due to it being thermally labile and a polar compound. LC methods can be used to quantify LSD, but these are usually long analyses and require large amounts of solvent to create a calibration curve and analyze samples.

In this study, HPTLC was used to quantify LSD using a mobile phase of 100% acetone. Several other mobile phases were evaluated, but this mobile phase provided the best separation between LSD and N-Methyl-N-Propyl Lysergamide (LAMPA) as well as resolution from four LSD analogs and distinguishing LSD from four other analogs. Thymidine was used as an internal standard at a concentration of 200mg/mL. Six calibrators were used with concentrations ranging from 10–150mg/mL to create a calibration curve. The area ratios between thymidine and LSD showed good precision above the limit of quantitation (CV% <10%). Linear regression of this calibration plot produced a coefficient of determination >0.99, indicating the regression model best explained the data. Residual plots of the data displayed some randomness showing the calibration model was linear. In addition, the Limit Of Detection (LOD) and Limit Of Quantitation (LOQ) were determined to be 27.6ng/band and 83.5ng/band, respectively.

Four blotter paper samples were spiked with known amounts of LSD (90–100mL) to determine recovery of the LSD from the blotter papers. The blotter papers were extracted using 1mL of methanol for 20 minutes in an ultrasonic bath. Thymidine was added as the internal standard, and the area ratio between thymidine and LSD was used to determine the concentration of LSD extracted. Samples were analyzed three times. The average percent recovered was above 55% for all four samples with a CV% <10%.

Results from this study indicate HPTLC is a suitable method to determine the amount of LSD in a sample with good precision, resolution, and sensitivity. In addition, the mobile phase chosen for this study is considered a green mobile phase and had the added benefit of being easy to prepare.

HPTLC; LSD; Hallucinogens

B33   Reviewing the Utility of Fentanyl Test Strips for the Screening of Seized Drugs

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WITHDRAWN

B34   The Odor Behind a Shot: Chemical Characterization of Volatiles From Ammunition

Salem Danielle Smith*, Texas Tech University, Hobbs, NM; Paola A. Prada-Tiedemann, Texas Tech University, Lubbock, TX

Learning Overview: After attending this presentation, attendees will be familiar with the advancing significance of understanding the chemical characterization of odor volatiles emanating from various ammunition along with possible variations due to caliber size and manufacturer, thus initiating proactive developments that directly address firearm threats and enhance sensor and canine-detection applications.

Impact Statement: This presentation will impact the forensic science community by providing a scientific foundation in the understanding of key odor markers emanating from an array of ammunition samples. While various studies have been geared to understanding gunshot residue transfer and composition, no studies to date have focused on understanding the bullet itself as a target odor source. This study presents a foundation to understanding volatile odor signatures from an array of bullets of different brands and calibers to provide forensic personnel as well as law enforcement officials with a basic understanding of odor volatile characterization from unspent cartridges. This effort aids in the development of sensor and canine technology aiming to identify ammunition samples on the scene and in various operational scenarios.

Due to the increase of mass shootings in the past decade, firearm detection is a critical tool in gun violence prevention. Within law enforcement applications, canine firearm detection teams are a valuable tool to combat these threats; however, there is a lack of scientific research and knowledge as to the chemical odor profile of this source of target odor evidence. Although previous studies have been focused on an understanding of gunshot residue components as they relate to firearm discharge events, this project is focused on using ammunition as a direct source for odor profiling for purposes of body worn/concealed weapon detection.

This study provides novel odor profiling instrumental analysis from a range of ammunitions comparing headspace odor profiles of 9mm caliber, 40 and 45 Automatic Colt® Pistol (ACP) live bullets. Utilizing Solid-Phase Microextraction (SPME) headspace sampling and Gas Chromatography/Mass Spectrometry (GC/MS), frequently occurring compounds were present across the sample matrices, including hydrocarbons such as dodecane, tetradecane and hexadecane, and acids such as 1,4-benzenedicarboxylic acid, dimethyl ester. Tetradecane was a common odor marker across all sample matrices. Nonanal, a compound reportedly found in smokeless powders, was emitted sporadically throughout the ammunition samples; however, frequently occurring in one 40 ACP brand. Statistical clustering analysis was implemented to analyze ammunition chemical odor profile clustering to monitor variations of odor signatures across sample groups.

The evolutionary advancements in manufacturing ammunition changes identification and interpretation paradigms over time, while, to date, there is no study emphasizing the evaluation of volatile odor signatures from brand ammunition and contrasting caliber size. By extracting Volatile Organic Compound (VOC) odorants from the various types of existing ammunition, the identification of volatile odor signatures can allow for the future creation of chemical training aids for detection training purposes as well as provide foundational knowledge for continued sensor development in routine firearm-related incident investigations and a step toward preventative detection.

Ammunition; Volatiles; Canines

B35   An Analysis of Improved Methods for the Genetic Identification of Cremated Skeletal Remains

Kadir Dastan*, Kadir Dastan, Istanbul Health and Technology University, Istanbul, Turkey

Learning Overview: After attending this presentation, attendees will be aware that the results of this study indicate that low levels of nuclear DNA can be recovered from the cremated bone with partial Short Tandem Repeat (STR) profiles obtained from combined and concentrated extracts; however, exaggerated stochastic effects such as increased stuttering, allele fall, and peak height imbalance were observed in some profiles due to the low amount of initial template and the use of an increased cycle Polymerase Chain Reaction (PCR), thus complicating the interpretation of the profile.

Impact Statement: This presentation will impact the forensic science community by sharing the results of this study.

Introduction: The cremation process consists of burning the corpse at temperatures of up to 1,000ºC, reducing it to bone remains or bone fragments, which are often ground to a consistency similar to that of sand. In the identification of forensic cases, it is critical to obtain information on the victim's identity from burnt bone and teeth remains.[1] When bone and teeth are burned, both their physical and chemical properties change significantly, which prevents anthropological evaluations and successful DNA profiling.

Background: Heat causes difficulties in the identification techniques in which bones and teeth are used, depending on the degree of temperature exposure. Therefore, it is necessary to evaluate the results of observation and analysis, and the information obtained in a certain order, with the changes occurring in bone and teeth during the burning period. The identification of burnt or buried bones and teeth has always been a difficult process due to the diminished quality and quantity of DNA, and it is still a challenge.[2] Due to heterogeneity in DNA due to the heterogeneity within the bone, unfortunately, the only reliable method for obtaining DNA from cremated samples such as burnt bones and teeth in the literature is still not available.[3]

Results: Previous research on DNA analysis of cremated remains has shown limited success.[4] This study examines adjustments or improvements throughout the DNA analysis workflow to improve methods of identifying human cremated remains. DNA was extracted from bone fragments using a commercial silica-based method or an enhanced extraction method that modifies the commercial kit to determine which process resulted in maximum DNA recovery. Several extracts from a single bone fragment were combined and then concentrated with an Eppendorf® Vacufuge. All extracts were quantified using the real-time Polymerase Chain Reaction (PCR) to evaluate the recovery of nuclear DNA. Concentrated extracts were amplified using the GlobalFiler® PCR amplification kit with an increased PCR cycle protocol and analyzed using capillary electrophoresis. Multiple samples from individual sets of remnants were examined to assess the consistency of the results.

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Genetic Identification; Forensic DNA Analysis; Cremated Skeletal Remains

B36   **An Examination of the EZ2 Connect Fx for Extra-Large Volume DNA Extraction and Purification From Skeletal Samples**

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Learning Overview: This presentation will provide an overview of a DNA extraction and purification method from skeletal samples using the extra-large volume DNA extraction protocol of the EZ1&2™ DNA Investigator® Kit on the EZ2® Connect Fx system.

Impact Statement: Efforts to enhance DNA extraction methods for human identification have resulted in an abundance of methodologies, each with specific recommendations and requirements. In this study, we aim to demonstrate the applicability of an alternate DNA extraction and purification method utilizing the EZ1&2™ DNA Investigator® Kit and the EZ2® Connect Fx system. This presentation will impact the forensic science community by showcasing a method that offers easy adaptability, allowing laboratories to meet their individual needs by adopting a single, flexible protocol.

DNA extraction from skeletal samples plays a crucial role in identifying unidentified human remains, as DNA remains well-preserved within the tough hydroxyapatite matrix of bone. However, processing skeletal remains for DNA extraction presents challenges due to the low-template, degraded nature, and inclusion of Polymerase Chain Reaction (PCR) inhibitors in these samples.[1] Consequently, considerable research has been focused on the refinement of DNA extraction and purification methods from these complex skeletal samples, leading to the development of custom methods with varying parameters for sample input and incubation duration. The implementation of a chosen method is often dependent on the quality or quantity of the sample or the needs of the lab, requiring validation of multiple methods. This study explores the effectiveness of the extra-large volume protocol offered by the EZ1&2™ DNA Investigator® Kit for DNA extraction from challenging skeletal samples. The protocol allows flexibility in both sample input (100–800mg) and incubation length (2-24hrs) within a single method.[2] To evaluate its success, three variations of the protocol were tested and compared against a similar method using ten challenging skeletal samples, including surface exposed, burned, and buried remains.

The first variation involved lysing 50mg of bone powder for 2 hours, followed by automatic purification on the EZ2® Connect Fx. Despite being below the recommended input for the extra-large volume protocol, this input was chosen because this method was compared to the PrepFiler® BTA method, which requires 50mg for automated purification on the AutoMate Express™.[3] Secondly, 250mg of bone powder was lysed for 24 hours before automatic purification on the EZ2® Connect Fx. For comparison, DNA was extracted from 250mg of bone powder using an adaptation of the manual Loreille total demineralization method with MinElute® PCR purification.[1] Lastly, the third variation involved lysing 800mg of bone powder from a single skeletal sample for 24 hours before purification on the EZ2® Connect Fx.

Overall, no significant differences in DNA yield were observed between the extra-large volume protocol and its comparison method. As expected, using 250mg of bone powder resulted in a statistically higher DNA yield than using 50mg of bone powder with the extra-large volume protocol. Additionally, the application of 800mg of bone powder improved both DNA yield and allele recovery. Following traditional Short Tandem Repeat (STR) typing using the Investigator® 24plex QS kit, no significant differences in allele recovery were observed across all methods. Based on these findings, the extra-large volume protocol offers a versatile method to extract DNA from various challenging skeletal samples, catering to the laboratory needs. This single kit can efficiently process samples that require priority processing, have limited starting material, or are extremely challenging (e.g., buried or badly burned remains). The protocol's adaptability, combined with the convenience of automatic purification on the EZ2® Connect Fx, makes it a suitable addition to crime laboratories, with the ability to customize parameters based on their unique requirements.

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Skeletal Remains; Human Identification; DNA Extraction

B37 The Development of a Custom qPCR Multiplex to Quantify Human and Microbial DNA

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Learning Overview: After attending this presentation, attendees will be informed about the development of a novel quantitative Polymerase Chain Reaction (qPCR) multiplex for both human and microbial DNA and how this tool can be used to shed light on the analysis of microbial DNA in forensic investigations.

Impact Statement: With this tool and the information garnered by using it, the forensic science community will gain insight into when microbial DNA analysis would benefit an investigation, including when there is little to no human DNA available. Therefore, the results of this project will aid examiners in deciding which path of DNA profiling to pursue.

The analysis of microbial DNA in forensic science is not new. In recent years, there has been greater interest in determining how and when microbial DNA may be included in an investigation. For samples that are challenged with damaged or degraded human DNA, there is an interest in evaluating if detectable microbial DNA is present. Specifically, touch DNA samples, which tend to contain very small amounts of human DNA, may benefit from the option of microbial DNA analysis.[1] There is much to learn about how the behavior of microbial DNA differs compared to human DNA within the same sample. With the development of a tool that will enable the quantification of both bacterial and human DNA in one assay, the forensic science community will have greater knowledge of what can be expected from a touch DNA sample.

The overall goal of this project is to develop a procedure that will aid forensic investigators in deciding whether they should examine the human DNA or bacterial DNA in a touch DNA sample. Through this project, a tool has been created to conduct a multiplex qPCR analysis on the human and bacterial DNA present in a sample. This multiplex assay provides an easy method for comparing the two types of DNA. With the information of how much of each type of DNA is present, the transfer and persistence of human and bacterial DNA can be examined and compared.

To create this tool, the master mix components of the Quantifiler® HP DNA Quantification Kit were combined with primers and probes designed to target universal regions in the bacterial genome.[2] The standards included in the Quantifiler® HP Kit were used for the human standards. Standards for the bacterial part of the assay were created using synthetic double-stranded DNA fragments, gBlocks, with the sequence of the amplicon for the chosen bacterial primers.

Once created, this assay was tested for repeatability, as were the Quantifiler® HP and custom bacterial assays separately, to determine if the same results could be achieved consistently. Additionally, this set of experiments allowed for the comparison of the behavior of the separate assays to their behavior when multiplexed. The bacterial aspects have been consistently the same between the singular and multiplex assays. However, there were some differences between the human singular and multiplexed assays that appear to be predictable.

The information gained from the repeatability studies have been utilized to evaluate surface samples from objects such as computer mice, keyboards, and mobile cell phones; other researchers have previously examined cell phones as a sample source for bacteria alone.[3] Depending on how much of each type of DNA is associated with each surface, the merit of using microbial DNA when there is less human DNA can be assessed.

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Bacteria; Forensic DNA; Touch Sample

B38   The Reassociation of Highly Degraded Human Remains Found in the East Marshall Street Well Using Insertion/Null Genotyping

Sierra Laveroni*, Virginia Commonwealth University, Henrico, VA; Andrea Malchow*, Virginia Commonwealth University, Richmond, VA; Daniela Frausto, Houston Forensic Science Center, Houston, TX; Michelle M. Woo, Virginia Commonwealth University, Richmond, VA; Baneshwar Singh, Virginia Commonwealth University, Richmond, VA; Filipa Simao, Virginia Commonwealth University, Richmond, VA; Tal Simmons, Virginia Commonwealth University, Richmond, VA

Learning Overview: After attending this presentation, attendees will be informed about the ethical treatment of human remains in addition to an optimized workflow for processing severely degraded human skeletal elements.

Impact Statement: This presentation will impact the forensic science community by providing insight into the most efficient and effective way to obtain genetic information from severely degraded human skeletal remains. Additionally, the benefits of Insertion/Null (INNUL) genotyping in use with highly degraded samples will also be displayed.

The East Marshall Street Well (EMSW) was uncovered during the construction of the Hermes A. Kontos Building on the Medical College of Virginia campus in Richmond, VA. Human remains, artifacts, and other historical items dating to the mid-19th century were discovered inside the well.[1] These items were hastily removed from the EMSW with a backhoe and deposited outside a barricade for archaeologists to sort. This removal process resulted in a severe commingling of the human remains. To re-associate the remains, they were sent to the Smithsonian in Washington, DC, for anthropologists to assess and sort them osteomentrically.[1] This assessment led to "paired" remains, which consist of arm element or leg element matches independently, and "unpaired" remains, which were not able to be matched with other elements. From cranial measurements, it was determined that most of the remains represented individuals of African descent.[2]

Historical records from the university's archives indicate that they were likely victims of grave robbing, anatomical dissection by medical students, and amputation practice.[1,2] Although a significant amount was learned from the anthropological assessment, it was not possible to anthropologically re-associate bones of the upper limbs to those of the lower limbs or to associate any postcranial elements with skulls due to limitations on the methodology as well as the condition of the remains. The study aims to re-associate highly degraded human skeletal groups through INNUL genotyping.

This methodology uses ALU repeats throughout the genome to isolate and detect unique patterns. This method creates smaller amplicons than traditional Short Tandem Repeat (STR) loci.[3,4] The utilization of this chemistry will facilitate the linkage of disassociated and commingled human skeletal groups, such as the arms and legs, ensuring accurate and respectful reburial of individuals.

In this study, 221 "paired" human skeletal elements were sampled using a Dremel® tool, and 40mg of endosteal bonepowder was collected from muscle attachment sites on each element. All bone powder samples were extracted using the InnoXtract™ Bone Extraction Kit following the manufacturer's protocol. The human DNA extracts were quantified using the InnoQuant™ HY kit following the manufacturer's protocol on the QuantStudio™ 6 Real-Time PCR instrument. Any samples that quantified over 25pg were amplified using the InnoTyper™ 21 Human DNA Analysis Kit following the manufacturer's protocol. Amplified fragments were separated on the Applied Biosystem® 3500xL Genetic Analyzer. All data was analyzed using GeneMapper® ID-X software. Likelihood ratios for kinship determination and direct matching of unknown samples were calculated using Familias, a statistical software.

One hundred profiles from the 221 highly degraded bone elements were obtained. Of those 100 profiles, 52 were suitable for analysis. Agreement and disagreement between the osteometric analysis and DNA analysis were examined and a majority of the data showed concordance (87%). However, 13% of the data showed discrepancies between the DNA results and what was previously decided by the anthropologist. Additionally, matches have been made between the upper and lower limb groups. At this time, a total of eight individuals containing both arm and leg elements from the EMSW have been identified. Connecting the upper and lower body was not previously possible when only utilizing osteometric pairing from an anthropologist. Additional testing for all "unpaired" human bone elements is ongoing in furtherance of reassociation efforts aimed at understanding the life histories of these individuals.

In conclusion, integration of the DNA workflow and INNUL analysis along with anthropological assessment have generated more confidence and accuracy in matches during the process of re-associating highly degraded and commingled human skeletal assemblages.

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Degraded Bone; INNUL; Osteometric Analysis

B39   An Overview of Cannabis Seizures and Methods for Distinguishing Between Cannabis and Hemp in Croatia

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Learning Overview: After attending this presentation, attendees will be familiarized with the structure of cannabis seizures in Croatia, along with methodology applied in cannabis casework and the results of cannabis genetic research conducted at Croatian Forensic Science Centre "Ivan Vučetić" (FSCIV).

Impact Statement: This presentation will impact the forensic science community by addressing the challenges in distinguishing between cannabis and certified hemp varieties by routine analysis as well as by applying additional genetic methods.

Cannabis is the most frequently seized and produced illicit drug in Croatia, as it is worldwide. The main psychotropic properties of cannabis are derived from Δ^9-Tetrahydrocannabinol (THC). In accordance with Croatian legal acts, any cannabis plants except certified hemp varieties listed in the European Union Plant Variety Catalogue with a THC content not exceeding 0.2% in the dry plant material are prohibited, along with cannabis resin (hashish), extracts, and tinctures.

The aim of this presentation is to provide an insight into the structure of cannabis seizures confiscated in Croatia from 2018 to 2022. Furthermore, presumptive tests used at the crime scene, routine methods in cannabis casework conducted at the FSCIV are presented in addition to the results of genetic research carried out in order to differentiate cannabis and hemp.

Most commonly seized cannabis material analyzed at FSCIV includes herbal cannabis, cannabis plants, cannabis extracts, and hashish. In the abovementioned period, herbal cannabis was predominant with the average amount of 74.2% of total seizures, followed by cannabis plant seizures (18.7%). Less often-seized material was cannabis extract (4%) and, last, hashish with the average amount of 3% of total seizures.

Upon finding the material suspected to be cannabis, first preliminary tests are applied at the crime scene. The most common presumptive color tests used in Croatia are the M.M.C. International B.V. Cannabis test for THC detection and HEMPTEST®-C for the detection of Cannabidiol (CBD), THC, or Cannabinol (CBN) predominance in tested samples.[1-3]

After crime scene processing, the first step of cannabis casework analysis in FSCIV includes morphology examination. Subsequently, Thin Layer Chromatography (TLC) is used for semiquantitative analysis, Gas Chromatography with Flame Ionization Detector (GC/FID) and High-Performance Liquid Chromatography (HPLC) for cannabinoids quantification, as well as Gas Chromatography with Mass Spectrometry (GC/MS) for qualitative analysis of cannabinoids. However, these methods are not always sufficient for differentiation purposes (i.e., when seeds and immature plants are being analyzed). Furthermore, non-certified potent CBD and Cannabigerol (CBG) varieties present new challenges in cannabis forensic examination.

Nevertheless, the application of genetic methods could provide additional information if routine methods were insufficient for differentiation. Therefore, the selected genetic markers were studied for their potential application at FSCIV. First, nine Short Tandem Repeats (STRs)—ANUCS302, ANUCS303, ANUCS305, ANUCS501, B05-CANN1, C11-CANN1, D02-CANN1, E07-CANN1, and H06-CANN2—were chosen. The results of selected STRs were not sufficient for differentiation between cannabis and hemp. Furthermore, since 98% of the analyzed samples confiscated in Croatia were propagated by seeds, STRs were not useful tool for tracking clonally propagated cannabis and linking the seizures. [4,5] Onwards, genetic research was continued with the THCAS marker developed by Kojoma et al., D589 gene marker and sequencing of THCAS gene.[6,7] The concordance of obtained results for marker reported by Kojoma and D589 markers was observed but these markers also showed lack of specificity for differentiation purpose. However, THCAS gene sequencing demonstrated effectiveness for distinguishing cannabis and certified hemp varieties.

Hence, future study will be dedicated to sequencing the THCAS gene in certified hemp and casework samples, along with establishing the database of THCAS gene variants.

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Cannabis Seizures; Hemp; Genetic Markers

B40   An Efficient Alternative to the Conventional Bone Pulverization Step for Personal Identification in 11 Real Forensic Caseworks

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Learning Overview: After attending this presentation, attendees will be informed regarding the application to real forensic caseworks of the DNA extraction assay, called the TBone EX Kit (DNA Chip Research Inc.), developed to digest bone chips without powdering. We empirically demonstrated the effectiveness of the kit in processing multiple bone samples simultaneously, recovered from different scenarios and exposed to extreme environmental factors, largely simplifying the DNA extraction procedure and the good yield of recovered DNA.

Impact Statement: This presentation will impact the forensic science community by providing additional support for the use of the TBone EX Kit for digesting bone fragments/whole teeth as an effective alternative to pulverization protocols. In addition, the present work turns out to be extremely useful for police force laboratories, to which the various actors of the criminal justice system—such as potential jury members, judges, defense attorneys and prosecutors—require immediate feedback.

DNA identification of human skeletal remains plays a valuable role in forensic field, especially in missing persons and mass disaster investigations. Hard tissues, such as bones and teeth, represent a very common kind of sample analyzed in forensic laboratories because they are often the only biological materials remaining. However, the major limitation in using these compact samples relies on the extremely time-consuming and labor-intensive treatment of grinding them into powder before proceeding with the conventional DNA purification and extraction step. In this context, a novel DNA extraction assay, called the TBone EX Kit, was developed to digest bone chips without powdering.[1] We simultaneously analyzed a total of 18 bone and tooth samples that arrived at our police laboratory and belonged to 11 different forensic caseworks occurring in Sardinia, Italy. The samples were recovered from different scenarios and were exposed to extreme environmental factors, including sunlight, seawater, soil, fauna, vegetation, and high temperatures and humidity. The TBone EX Kit was used prior to the EZ2® DNA extraction kit on the EZ2® Connect Fx instrument, and high-quality autosomal and Y-chromosome Short Tandem Repeat (STR) profiles were obtained for 73% of the caseworks in an extremely short time frame.

This study provides additional support for the use of the TBone EX Kit for digesting bone fragments/whole teeth as an effective alternative to pulverization protocols. We empirically demonstrated the effectiveness of the kit in processing multiple bone samples simultaneously, largely simplifying the DNA extraction procedure, and the good yield of recovered DNA for downstream genetic typing in highly compromised forensic real specimens.

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DNA; Skeletal Remains; Bone

B41   **Investigative Single Cell Genetics: Determining the Probability of a Genotype After Observing Single-Cell Data Enables Robust Database Searches Regardless of Mixture Complexity**

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Learning Overview: After attending this presentation, attendees will better understand the way in which single-cell analysis can be leveraged to improve investigative processes. By constructing a probability distribution across all possible genotypes, the most probable genotypes can be reported and, subsequently, compared to a database.

Impact Statement: This presentation will impact the forensic science community by demonstrating that, regardless of the complexity of the original mixture, single-cell analysis can be leveraged to construct probable genotypes explaining the single-cell data in cases where there is no suspect. The single-cell revolution, therefore, has the potential to positively affect non-suspect cases for which the likelihoods were too diffuse and did not meet the laboratory's search criteria.

In scientific deduction, one of the primary goals of the analyst is to collect data that will discriminate candidate hypotheses. The traditional method of procuring the data is by way of bulk treatments, where the DNA of all contributors is extracted in a single vessel, resulting in identically and independently distributed DNA fragments. If the concentration of any one contributor is low, then allele drop-out can result. With all contributors' DNA being sampled together, the evidential strength (i.e., the likelihood ratio) approaches one as the number of contributors to a mixture increases since these data are comprised of many peaks that are a superposition of peaks associated with distinct individuals. These issues mean that there is scope to improve the robustness of mixture interpretation.

Profiles derived from traditional bulk pipelines are often encumbered by numerous peaks from many, possibly partial, contributors. As the peak heights decrease and the number of peaks increases, interpretation becomes arduous, sometimes requiring numerous propositions across multiple numbers of contributors and taking hours to complete.

One path forward is by way of single-cell analysis, which is defined as a system of laboratory procedures that: (1) isolate each cell, and then (2) extracts and directly amplifies the DNA within the same vessel. By applying single-cell treatments, alleles of each contributor remain coupled and variability due to extract fractionation is circumvented, leading to n single-cell, single-source contributor Electropherograms (scEPGs). The interpretation of these highly resolved signals, therefore, changes from one of determining the weights of multifarious genotype combinations to one that relies on dependable clustering by similarity. With this modification, the number of donors to the admixture no longer affects the ability to discriminate candidate hypotheses. For non-suspect casework, a multi-hypothesis evaluation that estimates the probability of genotype g given the cluster can potentially provide significant gains in investigative genetics. It is for these reasons we expound the legitimacy of single-cell data by evaluating the robustness of the system for investigative purposes. Specifically, we take 630 admixtures containing anywhere from 17 to 75 scEPGs from any of two to five contributors. We cluster the scEPGs into groups based on similarity using a package named mclust in R. This results in k clusters with m scEPGs from a single-source contributor. We then use EESCIt to estimate the probability of these m scEPGs that are in a cluster, C, given g, where G is the contributor's genotype and g is a given genotype within the set of all possible genotypes according to background frequencies. EESCIt then applies the traditional form of Bayes' rule to estimate the P(G=g|C) for each locus allowing a decision threshold, such as 0.998, to define the credible set of genotypes explaining the cluster of cells.

To test the performance method, we report the proportion of loci for which only one genotype is in the credible set and that genotype is the true genotype. This occurred 84% of the time. Further explorations demonstrate when more than one genotype belongs to the credible set, it is affiliated with low peak heights. Specifically, the median total intensity was 11,432 Relative Fluorescence Units (RFU) for clusters with one credible genotype, while the median was 1,825 RFU for clusters where there were two credible genotypes. Mosaic plots demonstrate that this is affiliated with those clusters with two or fewer cells. With highly concentrated probability masses on only one or two genotypes, we show the potential of single-cell analysis to the forensic domain.

Single-Cell Genetics; Database Searching; Investigative Genetics

B42   Locus Abundance Variation by ForenSeq™ DNA Signature Prep Kit Lot

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Learning Overview: This presentation will demonstrate the variability in marker abundance of ForenSeq™ DNA Signature Prep Kit lots. After attending this presentation, attendees will see the need to include lot variation testing in validation studies and perform detailed lot test examinations when conducting Quality Control (QC) testing of newly received kits.

Impact Statement: This presentation will impact the forensic science community by detailing the variability observed across different lots of the ForenSeq™ DNA Signature Prep Kit. The information will benefit laboratories seeking to validate the kit and will provide strategies that may be used for QC testing of Next Generation Sequencing (NGS) typing kits.

The ForenSeq™ DNA Signature Prep Kit is a National DNA Index System (NDIS) -approved, NGS-based library preparation and sequencing kit. The full kit (Primer Mix B), used in this study, includes 27 autosomal Short Tandem Repeats (aSTRs), 24 Y-chromosomal Short Tandem Repeats (Y-STRs), 7 X-chromosomal Short Tandem Repeat (X-STRs), 94 identity **Single Nucleotide Polymorphism**s (iSNPs), 22 phenotypic SNPs, and 56 biogeographical ancestry SNPs (apSNPs).[1] The goal of this study was to assess variation across different lots of the Signature Prep kit. To this end, we evaluated locus abundance among positive control samples, typed in eight separate sequencing runs using five different ForenSeq™ DNA Signature Prep Kit lots. Library preparation for samples followed the manufacturer's protocol with a few modifications. Following the bead cleanup after PCR2, samples were normalized via a quant-based method instead of using the normalization beads. Samples were then pooled and purified using a 0.9X bead purification. Finally, the pool was quantified prior to denaturation to determine the final concentration for sequencing. Sequencing data was processed using ForenSeq™ UAS v1.3. Sample Detail and Phenotype Reports were exported and compiled using custom Python® scripts for evaluation of the sequencing data.

Overall, STRs resulted in the highest percentage of reads across kit lots, accounting for approximately 1% of reads per marker per sample. However, a single STR marker could range from 0.056% to 7.069% of sample reads across the five different lots. Read percentage across lots was most consistent among aSTRs as compared to Y- and X-STRs. When multiple sequencing runs were performed using the same kit lot, the percentage of reads was consistent for each marker type. However, there was high variability in the overall read percentage for each STR marker type between different lots.

In general, SNPs accounted for approximately just 0.3–0.5% of sample reads per marker, which was about two-fold less than STRs. On average, apSNPs resulted in a higher read percentage than iSNPs. Because each SNP accounts for far fewer of the sample reads than most STR markers, differences in read abundance by lot may impact SNPs to a greater degree than the kit STRs. For example, iSNP rs4530059 displayed a 5.3% difference in abundance for the positive control sample between two lots, which translates to a five-fold decrease, for a sample with an ideal DNA input (1ng), from one kit lot to another. Similarly, apSNP rs1805009 displayed a 2.5-fold difference between two kit lots. This issue is particularly important because low quality or challenging samples are more likely to result in allelic dropout. As SNPs make up the smallest percentage of sample reads, the chances of dropout at any given SNP will change with multi-fold differences in locus abundance by kit lot, even if all expected SNP alleles are present in the positive control.

The variability observed between ForenSeq™ DNA Signature Prep Kit lots indicates the importance of performing lot variation experiments during validation. Additionally, the variation highlights the need to conduct QC testing of newly received lots, with detailed data analyses, before using them for forensic casework.

Reference:

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Locus Abundance; Lot Variation; SNPs

B43 Genotyping Strategies for Tissues Fixed With Various Embalming Fluids for Human Identification, Databasing, and Traceability

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Learning Overview: After attending this presentation, attendees will have a clear understanding of the possibility of collecting DNA from various formalin-fixed or embalmed remains for human identification purposes or for providing an additional layer of quality assurance and a last line of defense traceability for specimens in anatomical/pathological collections.

Impact Statement: This presentation will impact the forensic science community by providing informative data when determining which genotyping strategy may be best suited for the identification, re-association, and establishment of a database for the provenance of formalin-fixed human remains either in a forensic or anatomical/medicolegal setting.

Within forensic or anatomical willed-body programs and skeletal collections, whole bodies and their disassociated limbs, bones, and organs are currently identified and tracked using a combination of Radio Frequency Identification (RFID) tags, individual barcodes or labels, written records, and photography. However, if these tracking mechanisms fail, DNA recovered from the formalin-fixed tissues/organs could provide an additional layer of quality assurance and a last line of defense traceability for anatomical specimens. Embalming fluids such as formaldehyde preserve biological tissues; however, they also damage, fragment, and cross-link DNA and protein molecules. As genotyping success is highly dependent on the quantity and quality of the DNA amplified, often DNA profiles from embalmed tissues are only partially complete, are inconclusive, or fail entirely.

This project investigated the success of conventional Short Tandem Repeat (STR) -typing (the QIAGEN® Investigator 24plex and 26plex QS kits) from various soft tissue and bone samples that were fixed with commercial and in-house embalming solutions with a range of formaldehyde concentrations commonly used within anatomical facilities globally (1%–9.5%). Consecutive slices from both legs of five cadavers (to provide skin, muscle, and bone tissues), and the whole heart and one kidney of each cadaver were sectioned and immersed in preservatives for up to three months. In addition, 50 tissue samples from a wider range of embalmed organs and body parts stored for up to ten years and preserved using unknown fixative solutions were also examined to assess a more representative range of samples housed within anatomical and medicolegal collections. Based on our preliminary data, human tissues embalmed with a ~2.5–5% formaldehyde solution would be most amenable to DNA typing for Human Identification (HID) purposes. The tissues most likely to be successfully genotyped were skin and muscle (gastrocnemius) (also bone for Low Template DNA [LT-DNA] samples), and, therefore, these tissues may be prioritized for sampling when establishing the provenance of unknown embalmed remains.

Furthermore, we also investigated whether alternate DNA markers such as Insertion/Deletions (INDELs) using the QIAGEN® Investigator DIPplex kit would provide more probative identifications than traditional STR analysis from these challenging samples. In general, INDELs produced lower/more discriminatory Random Match Probabilities (RMPs) than STRs in the majority of samples when <27 STR alleles were reported.

The results of this project can provide informative data when determining which genotyping strategy may be best suited for the identification, re-association, and establishment of a database for the provenance of embalmed human remains. In addition, this DNA-based approach could provide a higher level of quality assurance within willed-body programs worldwide.

Formalin-Fixed; STR-Typing; Human Cadaver

B44   **The Development of a Facial Phenotype Prediction Method Using Korean 3D Craniofacial Images and SNPs**

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Learning Overview: After attending this presentation, attendees will understand facial prediction methods using facial phenotype-genotypes and 3D facial images. In addition, attendees will be able to explore the potential applications of the methods in the identification of criminal suspects, unidentified bodies, and missing persons.

Impact Statement: This presentation will impact the forensic science community by providing the results of DNA-based facial prediction methods studied within a Korean population group. Additionally, the results from the current study can be compared with previous studies to evaluate the future potential of DNA-based facial prediction methods in the investigation of human identification.

Numerous studies exploring the correlation between facial phenotype and genotype have indicated the possibility of predicting the face of a suspect or un unidentified body using a series of **Single Nucleotide Polymorphisms (**SNPs) extracted from samples obtained at crime scenes. Currently, DNA-based facial prediction technology has been commercialized to provide predicted facial images to law enforcement agencies and individuals seeking suspects or missing persons. However, the reliability of predicted faces for Asian populations has not extensively investigated, and few studies have focused on Asian ancestry, including Koreans. Along with this, it is necessary to identify facial morphological predictors and develop a method for predicting facial phenotypes based on genotypic factors. Therefore, the purpose of this study is to develop a technique for estimating the facial phenotype using high-density SNPs of Koreans to help predict facial phenotypes of criminal suspects or unidentified bodies using genotypes obtained at crime scenes.

Multiple Regression (MR) images of 4,388 subjects (2,959 of males and 1,429 of females) from the GENIE cohort at Seoul National University Hospital Gangnam Center were employed to collect anthropometrical dataset from 3D facial images of the subjects whose SNPs analysis using the Korea Biobank Array (~830K of SNPs) was completed previously. A total of 32 facial landmarks were selected, and 62 measurement values were measured between the landmarks to be analyzed with SNPs.

The association between genotype and facial morphological phenotype was investigated statistically using PLINK version 1.07. The significant differences among the genotypic frequencies of the phenotype were verified using parametric (student's *t*-test and Analysis of Variance [ANOVA]) and non-parametric (Mann-Whitney and Kruskal-Wallis test) methods. The Mann-Whitney and Student's *t*-tests were used for the dominant and recessive models and the ANOVA and Kruskal-Wallis tests were used for the codominant model.

In conclusion, the correlation analysis between the facial landmark-based parameters and DNA-based facial phenotype-genotypes yielded valuable information for effectively predicting the faces of criminal suspects and unidentified individuals.

Forensic DNA; MRI Scan; Facial Reconstruction

B45   **The Development of Reagents, Software, and a Database for Kinship Determination in Missing Persons, Disaster Victims, and Human Rights Violation Cases**

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Learning Overview: After attending this presentation, attendees will understand the usability of a Next Generation Sequencing (NGS) -based assay, software, and database solution for missing persons/disaster victim identification.

Impact Statement: This presentation will impact the forensic science community by demonstrating the ability to utilize a dense Single Nucleotide Polymorphism (SNP) Polymerase Chain Reaction (PCR) multiplex for high throughput kinship determination for missing persons identification utilizing software that includes a database, the ability to build pedigrees, and a highly efficient kinship algorithm with likelihood ratios for relationship determination.

The International Criminal Police Organization (INTERPOL) has developed specific guidelines and protocols for Disaster Victim Identification (DVI) that refers to identification of victims after a mass casualty disaster, armed conflict, or human rights violation. Typically, dental records, fingerprints, or DNA-based methods such as mitochondrial DNA analysis or Short Tandem Repeat (STR) profiling are used for comparison of Antemortem (AM) and Postmortem (PM) samples for conclusive identification. While these DNA-based methods have been successful in DVI, they present several limitations. STRs cannot identify relatives further out than second degree. In addition, these PM samples are likely to be degraded and subjected to environmental insults and do not always provide a full STR profile. Mitochondrial DNA requires a matrilineal relative. To address these limitations, QIAGEN® developed ForenSeq® Kintelligence HT Library Prep Kit and Universal Analysis Software (UAS), including a local database containing AM and PM samples for the MiSeq FGx™ sequencing system.

The ForenSeq® Kintelligence HT system together with NGS (also known as MPS), is based on the ForenSeq® Kintelligence kit. We present the ability to sequence libraries generated with this kit with either 12 PM samples or 36 AM samples per sequencing run to determine relationships out to the 3rd order. The expected relationships were confirmed for samples with known pedigrees. DNA extracted from bones subjected to different insults and dental remains, as well as artificially degraded and low input DNA were utilized to simulate PM samples. DNA from diverse populations were typed to simulate AM samples. To address the forensic community's concerns on privacy, the server supporting the Universal Analysis Software also hosted a local database of these samples. Database management and pedigree tools were developed and integrated into UAS to assist with management of sample data and to calculate likelihood ratios. Overall, we demonstrate that by utilizing a high-throughput NGS library preparation kit, combined with a local database and kinship analysis with likelihood ratios in the UAS, will facilitate DNA analysis of missing persons cases.

Next Generation Sequencing; Disaster Victim Identification; SNP Multiplex

B46 A 353-Plex Panel for Forensic Identification Based on Massively Parallel Sequencing Technology

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Learning Overview: After attending this presentation, attendees will be aware that a Massively Parallel Sequencing (MPS) panel is a powerful tool for individual identification and paternity testing.

Impact Statement: The presentation will impact the forensic science community by sharing our achievement with the attendees, which would promote the development of forensic genetics.

Fluorescence-labeled Capillary Electrophoresis (CE) that detects the length polymorphisms of Short Tandem Repeat (STR) has been widely used for forensic identification over the past 20 years. Conversely, MPS technology is more emphasized nowadays for its significant advantages in forensic DNA analysis with higher loci and sample throughputs, shorter Polymerase Chain Reaction (PCR) amplicons for STR genotyping, and more types of genetic markers detection. There is a wide range of forensic applications involving Single Nucleotide Polymorphisms (SNPs) in genetic analysis, such as kinship inference and phenotype characterization and the sequence of mitochondria DNA (mtDNA) can be used for maternal inferences.

Here, we present a 353-plex panel, which detects more than 350 forensic genetic markers in one amplification. It integrates 205 STRs, 142 SNPs, and 3 hypervariable regions of mtDNA (mtDNA-HVs). In these STRs, 69, 32, and 104 are located in 22 autosomes, X chromosome, and Y chromosome, respectively. With so many alleles in this panel, it is easy to detect more STRs to meet the statistical power for common forensic inferences. The STRs can be detected by sequencing DNA amplicons with less than 400bp in length. Each allele of STRs contains a repeating region and two flanking segments with less than 50bp in length, providing higher genetic polymorphisms of length and sequence. More STRs with shorter amplicons in the panel than that in CE panels result in more detectable STRs in degraded samples. For highly degraded samples, we provide 142 SNPs with shorter amplicons (<150bp), which can reach a great level of forensic statistical power for individual identification (TDP=$1-4.03*10^{-58}$) and paternity testing (CPE $>1-1*10^{-7}$). Evaluation of panel performance showed that only 60 SNPs and 128 SNPs are enough to meet the basic requirement of statistical power of individual identification and paternity testing, respectively. In addition, we also provide 3 mtDNA-HVs for maternal inheritance analyses in specific forensic scenarios.

All markers of this panel can be amplified successfully from as little as 100pg of gnomic DNA. The sequencing libraries are suitable for DNBSEQ™ sequencing platforms with single-end 400bp mode. With only one million reads, all sites can be covered by at least 200 high-quality reads. For the mixed DNA samples, all Y-STRs can be detected when the DNA amount of minor contributor is as low as 20% (female:male = 4:1), which is as well as that in CE panels.

It is easy to obtain high-quality and high-resolution genotype dataset by using this powerful multiplex panel. This high-performance tool could provide far more genetic information for global forensic DNA analyses and inferences.

Forensic DNA Analysis; Multiplex Amplification; Massively Parallel Sequencing

B47   Differentiating Marijuana From Hemp Using an NGS Panel and Rapid Genotyping Assays Targeting Cannabinoid Synthase Genes

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Learning Overview: The goal of this presentation is to delve into the advantages of employing previously discovered potential markers in cannabinoid synthase genes identified by Next Generation Sequencing (NGS). These markers hold promise in facilitating the effective differentiation of various crop types of *Cannabis sativa* through the development of rapid genotyping methods.

Impact Statement: *Cannabis sativa*, known for its cultivation as hemp or marijuana, remains a topic of controversy in the United States. The 2018 Agriculture Improvement Act, legalizing hemp, presents a challenge for law enforcement to distinguish between the two crop types. This presentation demonstrates the use of novel polymorphisms discovered via NGS to develop two rapid genotyping assays, which could help law enforcement differentiate hemp, hemp seed, and illicit marijuana samples.

Genetic markers play a crucial role in identifying crop types, especially in sample types unsuitable for chemical analysis (e.g., trace residues, small leaf fragments, immature crops). Cannabinoid synthase genes are key enzymes in determining a crop's chemical composition or chemotype. However, existing markers often overlook the analysis of pseudogenes in the cannabis genome. Cannabinoid synthase genes are found to be highly repetitive in the cannabis genome and contain many incomplete coding sequences (pseudogenes) that can have 91–95% nucleotide identity to functional synthase genes. The risk of amplification of pseudogenes needs to be carefully evaluated in all synthase-gene targeting methods for genetic testing of cannabis. To address this, we previously developed a custom NGS panel targeting all synthase genes (THCAS, CBDAS, and CBCAS) as well as their pseudogenes. To ensure a comprehensive analysis, a diverse array of sample types was incorporated. These samples included reference *C. sativa* samples sourced from the National Institute on Drug Abuse (NIDA) and the National Institute of Standards and Technology (NIST), along with CBD, CBG, delta-8-THC, delta-10-THC, THCO, and CBDV hemp samples containing. Additionally, the study included hemp seed samples and extracts from seized marijuana provided by the Drug Enforcement Agency (DEA). The identified novel polymorphisms were then incorporated into two fast-genotyping assays.

The study developed a SNaPshot™ assay and a TaqMan™ quantitative Polymerase Chain Reaction (qPCR) **Single Nucleotide Polymorphism (**SNP) genotyping assay for hemp-marijuana differentiation. SNaPshot™, with four markers on the THCAS gene, THCAS pseudogene, and CBDAS gene successfully differentiated five hemp types and hemp seeds from seized marijuana across various locations in the United States, Chile, and the Mexico-United States border. Furthermore, this assay yielded valuable CBD information in the marijuana samples. On the other hand, the TaqMan™ qPCR SNP genotyping assays, using two markers on the THCAS gene and THCAS pseudogene, showed efficient differentiation of the majority of hemp samples from marijuana, offering the benefits of reduced analysis time and lower costs. However, these assays were unable to distinguish hemp seeds from marijuana or provide CBD information of the sample.

In summary, this presentation showcases the extensive findings from the custom-target NGS panel, leading to the development of two robust, rapid genotyping assays. The comprehensive evaluation of synthase genes enhances researchers' understanding of the diversity within and between *Cannabis sativa* crop types. This research offers valuable insights for forensic science and law enforcement in their efforts to accurately differentiate hemp and marijuana varieties.

Cannabis; Cannabinoid Synthase Gene; Pseudogene

B48 Challenges of DNA Profiling After Firefighting Plane Crash: The Influence of Kerosene Biodegradation on Human Remains

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Learning Overview: After attending this presentation, forensic practitioners may comprehend the importance of management of a DNA extraction protocol in order to improve the quantity and quality of DNA, particularly when human remains are negatively influenced by external factors such as kerosene biodegradation.

Impact Statement: This presentation will impact the forensic community by highlighting the role of DNA analysis as the gold standard method in cases when the condition of the remains does not allow identification using classical recognition methods.

The extraction of DNA serves as a fundamental process in biological academia and research. In a forensic DNA context, this procedure takes on even greater significance, acting as the initial pivotal step in generating DNA profiles that can either support convictions or aid in exonerations within the criminal justice system. Nevertheless, this process often encounters hurdles due to the challenging task of obtaining sufficient quantities of uncontaminated DNA, free from environmental inhibitors. These obstacles are further complicated by the wide range of sample types, some of which may contain limited amounts of biological material. Despite advances in methods and technology, certain issues still require attention, such as improving disaster victim identification processes.

This presentation aims to describe the challenges of DNA profiling after a firefighting plane crash occurred in October 2022. Specifically, this presentation focused on the influence of kerosene biodegradation on human remains, describing the complex procedures to identify each human remains recovered in the geographical area of the accident.

At the end of October 2022, during a forest fire on Sicily Island, it was necessary for two men to board their Canadair airplane. While making a drop over a forest fire, their aircraft struck a rock with its left wing, causing it to lose altitude and fall into the forest. The Canadair crashed and, within seconds, was engulfed in flames. Several remains of the two fliers that died were recovered in the next days during complex recovery operations, which were due to the geographical conformation of the area.

Subsequently, the genetic investigations began to identify which biological samples belonged to subject A or to subject B. Specifically, DNA profiling was very challenging for all samples, considering the high contamination by kerosene and the exposure to high temperatures after the explosion. Indeed, several human samples were completely burned. In this scenario, several modifications to the standard DNA extraction protocol were applied to obtain the DNA profiles. Identification was possible thanks to comparison with the reference profiles obtained from personal items and through the comparison with the profiles obtained from the parents. Moreover, blood samples recovered on the glass of the aircraft allowed us to ascertain the presence onboard of subject A.

In conclusion, this presentation highlighted the role of DNA analysis as the gold standard method in cases when the condition of the remains does not allow identification using classical recognition methods (i.e., dental or fingerprint data). At the same time, it is important to apply several modifications to the extraction protocol to improve the quantity and quality of DNA.

DNA; Low DNA; Human Remains

B49　　The Development of a Multiplex SNP Genotyping RT-PCR Kit for Screening of Non-Koreans

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Learning Overview: This presentation proposes that the use of Single Nucleotide Polymorphism (SNP) markers to predict the personal characteristics to narrow the suspect pool and help solve criminal cases, especially those involving foreigners in South Korea. This presentation will demonstrate that real-time multiplex Polymerase Chain Reaction (PCR) methods can be used to rapidly predict accurate characteristics from a small number of SNPs and, furthermore, the stability of SNP markers and the sensitivity and rapidity of real-time PCR can be reconsidered.

Impact Statement: This presentation will impact the forensic science community by showing that analysis of the four selected SNP markers using real-time PCR methods can quickly narrow the pool of primary suspect from evidence. Forensic scientists will learn about the importance of proper selection of SNP markers and their application to forensics through robust validation.

Direct-to-customer (DTC) genetic testing refers to tests that consumers can take directly to prevent diseases based on nutrition, lifestyle, and physical characteristics without visiting a medical institution.[1] As the global DTC genetic testing market is expected to grow at an average annual rate of 10.6% to reach $117.9 billion by 2024, the scale and scope of DTC genetic testing market in South Korea are also increasing.[1,2] In this study, to investigate the forensic applicability of SNP markers used in DTC genetic testing, a sample collected from a single donor was tested by four of the DTC companies in South Korea. As a result, only 6 out of 54 common traits (iron storage and concentration, omega-3 concentration, triglyceride concentration, insomnia, sprinting ability, and handgrip strength) among the four companies were matched, resulting in a matching rate of only 11.11%. These results showed that the test SNPs for DTC genetic test traits were highly unreliable when applied to forensics, and further validation of SNP markers for potential forensic applications was needed.

Therefore, after selecting a few traits that can predict the characteristics of suspects, we decided to create a new multiplex real-time PCR kit using reliable SNP markers for screening of non-Koreans. The selected SNPs are rs17822931, rs671, rs12913832 and rs3827760, which can be used to estimate earwax type, alcohol flush, eye color, and hair, incisor, earlobe, chin shape, respectively.[3-6] Samples were collected from ten Koreans and ten foreigners from different continents using buccal swabs after completing a questionnaire about their ancestry, earwax type, presence of alcohol flushing, eye color, and hair curliness. DNA was extracted using the QIAamp® DNA Mini Kit, and all samples were sequenced after quantification to verify the accuracy of the test SNPs and for comparison with multiplex real-time PCR results. From the result of the experiment, it was confirmed that race could be distinguished at the continental level using phenotype information and allele frequencies for four SNPs, and, in detail, it was possible to distinguish between East and non-East Asians. In addition, the results were consistent with the sequencing results using Capillary Electrophoresis (CE), which verified the accuracy of the multiplex real-time PCR method and has the advantage of obtaining results in a relatively short time. In future studies, we plan to create an NGS SNP panel that can predict phenotype and ancestry by adding a large number of validated SNPs to improve accuracy.

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Forensic SNP Genotyping; Personal Characteristics; Multiplex RT-PCR

B50 **The Utilization of a Single Nucleotide Variant (SNV) Panel for the Confirmation of Sample Identity in Two Clinical Genomics Applications**

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Learning Overview: The goal of this presentation is to address the effectiveness of a panel of SNVs in the context of clinical germline testing results from Chromosomal Microarray (CMA) and Whole Exome Sequences (WES), as a direct method of verifying sample identity. This method would eliminate the need for additional Short Tandem Repeat (STR) analysis, which would add cost and turnaround time.

Impact Statement: This presentation will impact the forensic science community by showing a small pilot initiative that utilized 40 overlapping positions in two genomic assays to verify sample identity. The findings showcase the potential of implementing genomic assays in human identification procedures comparable to STR analysis and the Combined DNA Index System (CODIS).

In the workup of suspected genetic illnesses, patients may undergo both CMA and WES. These tests can be performed on different DNA aliquots from the original specimen or from DNA isolated from different specimens. Currently, sample identity can be confirmed by running additional STR testing on the CMA and WES samples.[1] To eliminate the need for additional testing, a panel of 40 SNVs were selected from the 750,000 SNP markers included in the THERMO FISHER™ Affymetrix® Cytoscan HD.

Variants were chosen for inclusion in the study based on several specific criteria. First, they had to be located within an exon of a protein-coding gene. Second, they were required to be either benign or not known to be associated with genetic conditions. Additionally, the selected variants exhibited a relatively high minor allele frequency in the gnomAD database. Consistent genotype calls were made by Cytoscan HD for these variants. The regions in which these variants were located demonstrated consistently adequate depth in the Agilent® V8 exome. SNVs in regions of recurrent copy number variants were excluded from consideration. Lastly, there was a prioritization to include at least one SNV on each autosome (1-22) and the X chromosome.

A custom Python® script was written to compare the 40 selected positions from the CMA SNP and WES VCF files. A 14 CMA to WES trial was performed and consisted of seven known sample pairs, five unrelated patient comparisons, and two child-to-parent comparisons.

All paired samples had perfect concordance between CMA and WES SNV calls when no-call positions were ignored. Unrelated samples shared, on average, 37% of the genotypes. The comparison between child and parental samples had a 49% similarity.

The adoption of SNV data on a broader scale, along with the utilization of WES, holds considerable promise to exert significant effects on forensic science including increased ability to identify contributors to mixed DNA samples.[2] These advancements possess the capability to improve and refine identification and verification procedures by creating large scale whole exome sequencing databases similar to CODIS, hence playing pivotal role in diverse applications currently reliant on STR analysis both in forensic and clinical settings.

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Identification; Forensic Science; Genotypes

B51   Examining the Risk Associated With Qualitative Exclusions in Forensic DNA Casework

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Learning Overview: The goal of this presentation is to utilize validation data to examine the risks associated with qualitative exclusions of known individuals to forensic DNA mixture samples.

Impact Statement: This presentation will impact the forensic science community through the evaluation of the efficacy of qualitative exclusions in a range of samples, the types of profiles in which false exclusions may occur in forensic DNA casework, and the impact it can have on interpretation.

Based on practical experience, assessing the number of contributors in a forensic DNA sample can be confounded by multiple factors, including allele sharing, the presence of peaks below the laboratory's stochastic threshold, stochastic effects that can result in peak height imbalance, elevated stutter, and the possibility of allelic drop-in. As a result, a single locus that appears to have full representation of all contributors under certain assumptions, and therefore suitable for exclusionary assessments, may be affected by one or more of these factors. This can result in a false exclusion of an individual of interest. Many of the concerns that would make profiles uninterpretable for inclusionary purposes are likewise applicable and can be equally concerning when utilizing exclusionary criteria during manual interpretation efforts (i.e., interpretation without the use of a forensic expert software). Profiles, or portions of profiles, that are determined to be uninterpretable often present with incomplete or limited data. Additionally, profiles in which an accurate determination of number of contributors cannot be made or when the number of apparent contributors exceeds the laboratory's validation parameters may lead to an inconclusive result for comparison to known reference samples.

With the advent and implementation of probabilistic genotyping, available software can more conclusively determine an inclusion or exclusion for complex DNA profiles using statistics and considering phenomenon (e.g., drop-in, drop-out, stutter by allele including longest uninterrupted stretch) that analysts are aware of but do not have the ability to incorporate during qualitative exclusions. In addition, when the software is applied correctly by a trained analyst, assessing performance diagnostics can give additional insight into the number of contributors present in the profile.

Validation data, including known single source and two-person mixtures of varying templates, were used to assess drop-in, stutter, and allelic modeling with the addition of simulated drop-in alleles above and below the laboratory's drop-in cap. Limitations were further explored by varying peak heights of the alleles for the known donors (i.e., modifying major and minor peak heights to evaluate stutter where the peak in stutter position was consistent with the minor donor). In some cases, for the mixture samples, a false manual exclusion could be made based off the apparent number of contributors without accounting for stutter and/or drop-in, whereas STRmix™ was able to account for these phenomena and/or had diagnostic flags (e.g., LR=0 at one locus) that would warrant additional scrutiny by the analyst. Overall, the results of the study demonstrate how readily a false manual exclusion can occur when an analyst does not consider all variables (e.g., elevated stutter, drop-in).

Forensic DNA; Exclusion; Probabilistic Genotyping

B52   STRBase Moving Forward

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Learning Overview: This presentation will inform the forensic community of the new features implemented in the Short Tandem Repeat database (STRBase) website in the past year.[1] A content and interface-driven update to STRBase has been released, allowing greater engagement with the public it serves. After attending this presentation, attendees will be aware of the data being maintained on STRBase and future planned expansions to the site.

Impact Statement: This presentation will impact the forensic community by updating attendees on the priorities and content of STRBase. STRBase is an ever-developing website with the goal of serving the forensic community in both scientific research and practitioner communities. User feedback is valuable and always welcome to help guide how the site can better serve the human identity testing community.

The STRBase website was launched in 1997.[2] At the time, Short Tandem Repeat (STR) markers were new in the forensic community. STRBase was developed to gather information on the new forensic markers for the forensic community. This included information on the markers as well as research and other forensically relevant projects. Over the next 25 years, the site grew with information provided by the forensic community in the form of variant and tri-alleles observed. The National Institute of Standards and Technology (NIST) Applied Genetics Group also contributes research and educational resources.

The STRBase website, up until 2023, was built with HTML pages. The site was several hundred HTML pages and increased until the end when this site was archived.[3] This made maintaining the site complicated. There were a number of issues, including broken links and missing files, that could not be easily fixed in the old format. The archived site is still currently available, but no changes have been made to the site since March 2023. It was decided to move to the .NET platform with a backend database, which would allow for easier maintenance and expansion in the future.

The new website went live in April of 2023 and continues to expand with new content for the forensic community. The site is now more user-friendly, with a simplified layout and search capabilities. It allows users to submit data directly to STRBase using a login. This streamlines the data collection process for variant and tri-alleles, as well as other data types associated with active projects. All information contained in the old site was evaluated, and if it was within the scope of the new site, the content was moved. Not all topics on the previous site are still relevant to human identity testing. Making STRBase beneficial to the forensic DNA community is accomplished by working with the people.

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Short Tandem Repeats; Forensic Markers; STRbase

B53   Ten Years of Developing and Improving CSI Camps, Forensic Science Educational Conferences, and Science Museum Programs Toward Enhancing Interest, Attitudes, and Motivation in Forensic Science

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Learning Overview: After attending this presentation, attendees will learn best practices evolved from ten years of research on developing and improving three informal, inquiry-based, out-of-school engagement programs: (1) Forensic Science (FS) and Crime Scene Investigation (CSI) Camps and Mystery dinners (*n*=10); (2) Teacher Forensic Science Education Conferences (AAFS FSECs) (*n*=5); and (3) Science Museum programs (*n*=2). Attendees will gain knowledge from research results using pre- and post-engagement surveys, modifications to experimental planning and design strategies, methods for transitioning to on-line delivery, and lessons learned for developing public science programs in their universities and other settings, based on forensic science, to introduce Science, Technology, Engineering, and Math (STEM) careers to middle and high school students.

Impact Statement: This presentation will impact the forensic science community by presenting for the first time a ten-year-old study of the effectiveness of public programs in STEM education, including teaching resources and methods empirically tested, that may assist other forensic science educators, program leaders, CSI camp and FSEC directors, and other stakeholders to employ the modules, strategies, and lessons learned toward advancing our students' forensic science and crime scene investigation knowledge, skills, abilities, and confidence.

Tomorrow's science leaders are in our classrooms today. Educating and galvanizing the next generation of scientists is of paramount importance to our future as the impact of science and technology on society continues to expand.[1] A growing body of research indicates informal, inquiry-based, and science-rich resources have value in sparking, sustaining, and extending interest in and understanding of STEM disciplines, underscoring the importance of activities outside of the classroom.[2-9] Studies indicate the public learns more science over their lifetime in informal settings, such as programs in museums, science centers, zoos, parks, or science camps, than in formal settings, like classrooms and seminars.[2]

FS and CSI are by their very nature inquiry-based, inter-disciplinary, and collaborative. The hands-on, engaging, rigorous science and math applications and analyses have great potential to excite students about science, inspire them into STEM careers, and become lifelong science learners.[10-14] Connections of science to the real world are brought to life through the casework and testimony experiences delivered by FS and CSI experts and through hands-on, inquiry-based investigations of mock crimes. Integration of FS into hands-on programs can foster critical thinking and problem solving and "out of school engagements can help to develop high school students' identities as forensic scientists.[10]

In this presentation, we summarize ten years of lessons learned and research on improving three out-of-school engagement programs: (1) FS and CSI Camps and Mystery dinners (1 week, *n*=10); (2) Teacher FS Education professional development conferences (AAFS FSECs (3 days, *n*=5); and (3) Science Museum programs (1/2 day, *n*=2). Research results using pre- and post-engagement surveys, modifications to experimental planning and design strategies, methods for transitioning to on-line delivery and lessons learned from ten years of developing, delivering, and reimagining these programs will be described.

Participants work in teams and are led by FS graduate student counselors. The counselors lead participants through the activities, providing both teaching experiences and more direct interactions for our participants. During the in-person CSI camp, families come together to help solve a case during a mystery dinner. In our museum Adult Overnight Mystery and Girl Scout FS Badge events, participants rotate through different experimental stations, examining and analyzing evidence to solve a crime. Programs culminate in a moot court with participants as expert witnesses and parents playing the jury.

Program improvements from participant feedback, in format, pedagogy and topical content have been used for redesigning our programs. Evaluations supported increasing inter-student engagement and increased hands-on experimentation. Programs now include multiple hands-on activities for each topic. Unexpected experimental results are included in at least one experiment (e.g., spiking expired reagents into one team's kit), providing critical thinking and problem-solving opportunities for students while emphasizing the scientific method and importance of controls.

Our programs have collectively served 504 student CSI campers between the ages of 12–21 years old, and 97 teachers/educators from 21 states; 71% female and 36% underrepresented campers from 15 states and 6 countries have participated in our programs. Both site cohorts have had a majority of female participants (71%).

Outcomes have demonstrated participant improvements in FS knowledge and an increased interest in pursuing FS studies observed based on pre- and post-assessments and evaluation surveys, post-event follow-up communications, and enrollment of some former participants and counselors into FS university programs.

Results of our redesigned program strategies, including detailed agendas, supplies lists, suppliers, protocols, on-line modifications, and teaching resources, may assist other FS educators, program leaders, CSI camp and FSEC directors, and other stakeholders to employ the modules, strategies and lessons learned toward advancing our students FS and CSI knowledge, skills, abilities, and confidence.

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Forensic Science; Crime Scene Investigation; Education

B54   **A Longitudinal Study on the Odor Stability of Decomposition Simulated Training Aids Under Freezing Conditions**

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Learning Overview: After attending this presentation, attendees will better understand odor behavior as a function of storage period under freezing conditions from animal-analog decomposition samples, thus presenting implications for prolonged shelf life and storage conditions for human remains canine detection team training regimens.

Impact Statement: This presentation will impact the forensic science community by depicting odor fate across a prolonged storage freezing condition of up to 16 months, thereby providing the first of its kind longitudinal study on the odor profile of simulated decomposition samples. By evaluating the headspace odor of simulated decomposition training aid samples across this length of time from a range of putrefactive stages, the research presents practitioners within human search and rescue as well as law enforcement personnel who utilize human remains canine detection teams a scientific understanding of how training aids change over time.

The utilization of canines as a biological detector tool has increased immensely as they assist various law enforcement agencies in locating a wide variety of target substances, such as explosives, narcotics, pests, and human remains. Human Remains Detector (HRD) canines—also commonly referred to as cadaver dogs or victim recovery dogs—are an evolved specialty from the search and recovery discipline. HRD canine teams are deployed in operational scenarios needing the recovery of whole bodies or body parts. Thus, these canines are trained on a wide variety of decomposition odor sources, including whole bodies, body parts, tissue, bone, and decomposition fluids. Additional odor sources utilized for training include, but are not limited to, clothing from a deceased victim, blood, soil, and even synthetic training aid formulations. Given that human decomposition is a highly dynamic and variable process, it is imperative for canine handlers to use a wide assortment of training aids to optimize the canine's ability to locate the target substances. Enhanced training on an array of decomposition training odor sources reinforces the canine's odor memory to respond to target odor compounds in operational contexts and thus maximizes their efficacy.

A canine's ability to accurately locate human remains is highly dependent on the training aid's ability to mimic the odor profile of decomposing human remains. Therefore, the chemical odor characterization of these training aids is critical knowledge to further understand odor stability and persistence. One of the biggest challenges within HRD canine teams is the procurement of representative decomposition odor sources for training purposes. Thus, when a canine team can obtain training aids for routine maintenance training and assessments, a crucial factor to consider is optimal storage and containment for preservation of odor samples. To date, there is limited knowledge on the stability of the chemical odor profile from decomposition training aids after prolonged storage periods. To further complicate the situation, the different decomposition stages depict unique odor profiles across the full putrefactive spectrum, thus raising concerns as to optimal protocols to safeguard the integrity of different types of odor sources.

Three animal models (*Sus scrofa*) were laid out on a field and allowed to reach a targeted stage of decomposition (fresh, advanced decay, dry/skeletal). Once the targeted stage had been reached, tissue samples were collected, stored, and analyzed via Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS). The headspace odor concentration of each simulated-training aid was monitored at time of collection (week 0) and analyzed once more approximately 16 months later. Two different vial types were used as containment vessels (clear, amber) to further monitor if either vial would lead to higher indistinguishability. The results depicted a decrease in overall number of compounds detected when analyzed after two years of undisturbed storage. However, primary compounds that were observed at week 0 were also observed during months 14–16. Further statistical analysis will assist in portraying how distinguishable/indistinguishable the odor profiles of these samples were at time of collection versus 16 months post-collection.

Understanding how these odor profiles behave as a function of time concurrently with storage regimens is of high importance as it can gap the bridge of knowledge as to how the chemical odor characterization of decomposition training aids change over time, and how long they serve their purpose as an effective canine training aid.

Decomposition; Odor; Sample Storage

B55   A Pre-Concentrating Polymer Coating for Improved Detection of Fentanyl and Fentanyl Analogs

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Learning Overview: This presentation provides an overview on determining the most suitable acrylate-based polymer coating to pre-concentrate the target analyte vapor of fentanyl and fentanyl analogs based on the behavior of each coating toward the vapor using acrylate base-coated Quartz Crystal Microbalance (QCM) sensors.

Impact Statement: The technology of this research will be used to address the imperative need of improving sensitivity and selectivity of commercially available handheld Ion Mobility Spectrometer (IMS) to be effectively employed in vapor phase, non-contact detection of fentanyl and fentanyl analogs, thus impacting the forensic science community.

Field detection and identification of drugs of abuse is challenging and associated with many hazards. The Drug Enforcement Agency (DEA) currently has no advanced recommendations other than advising the first responders and law enforcement personnel to avoid direct contact with suspected drugs of abuse. Currently, the handheld IMS is an instrument that can be used for trace level, field detection of illegal drugs that utilizes swipe sampling and thus requires risking human exposure. Although research has been conducted to employ the IMS in vapor phase, its low sensitivity to trace level identification is a drawback. Therefore, this study explored the feasibility of deploying a novel pre-concentrator for target analytes of fentanyl and fentanyl analogs, which can be affixed on to the commercial handheld IMS.

The Naval Research Laboratory (NRL) in their prior efforts have found and tested a library of acrylate-based coatings that have enhanced analyte pre-concentration. The most favorable acrylate-based coatings that facilitate pre-concentration of vapors were investigated using a QCM. A QCM measures the frequency changes of a Quartz Crystal Resonator (QCR) as an analyte is introduced, and the mass deposited on the surface can be determined. By using acrylate-based sensors, or coated QCRs, the extent of vapor adsorption and desorption is observable, and therefore the ability of a coating to pre-concentrate can be determined. The main vaporous component (target analyte) in the headspace of fentanyl and fentanyl analogs has been identified as NPPA in previous research and NPPA is a potential target for IMS detection of fentanyl/fentanyl analogs by pre-concentration.

Several potential acrylate-based coatings from the library were screened to determine the ideal coating for NPPA. The phenyl-acrylate-coated sensor has an immediate significant adsorption of vapor that steadies out within a short time and is able to completely desorb in a short period of time. Ethylene Glycol Monomethyl Ether (EGMEA) -coated sensor uptakes significantly high masses of vapor through several hours and desorbs through an equally long period of time. Results depict that each coating has a different behavior with NPPA. The activity of the sensors shows that rapid significant uptake of vapor with equally rapid desorption are the ideal features necessary to be seen in a pre-concentrator coating for NPPA. Once the ideal coating is determined for NPPA, it can be applied to an array of Silicon Nanowires (SiNW) (developed by NRL) that is embedded in a micro-chip, fixable in the commercial handheld IMS.[1-5]

The outcome of this research caters the urgent need of improving sensitivity for non-contact detection, together with presumptive identification of trace-level fentanyl. This approach can further be generalized to detect other dangerous drug classes as well.

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Fentanyl; Polymer Coatings; QCM

B56   **Toxic Adulterant Prevalence in the United States Illicit Drug Supply: Geographical Trends and Raising Awareness Regarding Their Dangers to Promote Public Health**

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Learning Overview: After attending this presentation, attendees will be able to assess the emergence and proliferation of toxic adulterants throughout the United States.

Impact Statement: This presentation will impact the forensic science community by raising awareness of toxic adulterant substances in the United States drug supply. Attendees will also be able to describe the toxic effects of the most commonly encountered toxic adulterants.

Adulterants are pharmacologically active substances that can readily be obtained and added to drug street samples to increase bulk. The addition of these agents can have dangerous and toxic effects to the consumer through additive or synergistic effects with the principal drug. Adulterants change all the time based on their availability and changes in market preference. Comprehensive testing of all substances in each sample allows monitoring of emerging drug trends that ultimately impact the treatment and outcomes of acutely intoxicated patients.

Deidentified seized drug samples (n=1,448) were received from eight forensic laboratories across the United States between March 2022 and January 2023. Sites included the Northeast (NH, PA, VT), Midwest (IL, IN, OH), and Southeast (GA, WV). All samples were shipped as dried extracts and reconstituted with 90:10 5mM ammonium formate in H_2O: 0.1% formic acid in acetonitrile and analyzed using a Xevo® G2-S QTOF mass spectrometer coupled to an ACQUITY® UPLC® I-Class system. Data was processed using UNIFI® Scientific Information System against an in-house library containing >1,000 drugs, including a wide range of adulterants, precursors, and by-products along with controlled substances and a broad range of Novel Psychoactive Substances (NPS).

Of the 1,448 samples, adulterants were identified in 1,203 samples (83.1%). Six samples from Vermont were negative for any drugs or adulterants. The peak with the greatest abundance was considered the main component, while all other constituents identified were considered to be adulterants. Data related to the total number of constituents identified and number of adulterants by state is provided in Table 1. Throughout this study, 16 adulterants were identified across the samples including: acetaminophen, aminopyrine, benzocaine, caffeine, diphenhydramine, gabapentin, hydroquinidine, hydroxyzine, levamisole, lidocaine, phenacetin, procaine, quetiapine, quinine/quinidine, tramadol, and xylazine. Of the 1,436 drug positive samples, 7% contained diphenhydramine, 9% levamisole, 11% contained lidocaine, 12% contained quinine/quinidine, 14% contained caffeine, and 19% contained xylazine. All other adulterants were found at less than 7%. Results for individual states for the top six adulterants are shown in Table 2.

Table 1. Complexity of Drug Exhibits by State for Drug Constituents and Adulterants

State	1-4 Constituents (%)	5-8 Constituents (%)	9+ Constituents (%)	1-4 Toxic Adulterants (%)	5-8 Toxic Adulterants (%)	9+ Toxic Adulterants (%)
Pennsylvania	91	9	0	60	1	0
Ohio	84	11	5	51	6	3
West Virginia	81	13	6	28	10	5
Illinois	77	3	20	39	2	20
Georgia	73	19	8	45	16	6
New Hampshire	70	21	9	70	21	9
Indiana	60	35	5	61	24	3
Vermont	50	22	22	36	19	20

Table 2. Adulterant Positivity by State

	Total (n=1436)	IN (n=200)	IL (n=204)	GA (n=98)	NH (n=200)	PA (n=146)	OH (n=200)	WV (n=200)	VT (n=188)
Xylazine	19%	22%	13%	9%	21%	24%	18%	7%	37%
Caffeine	14%	21%	5%	5%	27%	6%	3%	11%	30%
Quinine/Quinidine	12%	15%	18%	12%	8%	0%	6%	19%	19%
Lidocaine	11%	12%	5%	11%	16%	4%	6%	8%	24%
Levamisole	9%	4%	12%	12%	8%	18%	4%	1%	10%
Diphenhydramine	7%	20%	3%	6%	2%	0%	11%	12%	2%

The identification of toxic adulterants is becoming more important in the field of drug chemistry as typically only controlled substances are reported. Continued surveillance of these adulterants may lead to a better understanding of the illicit drug supply and can enable better information to be distributed to the public regarding the potential toxic effects. This can assist clinical health professionals to treat patients, as well as educate people who use drugs. Toxic adulterant testing results give an important additional perspective for understanding the toxic effects of recreational drug use and emerging trends.

Adulterants; Drug Chemistry; Surveillance

B57   An Analysis of Plant Fragments Using X-Ray Fluorescence Spectrometry and X-Ray Absorption Fine Structure Technique Utilizing Synchrotron Radiation

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Learning Overview: This presentation deals with plant fragments that may be left at a crime scene as evidence, using synchrotron X-Ray Fluorescence (XRF) analysis and X-Ray Absorption Fine Structure (XAFS) measurements, in addition to DNA identification to determine the plant species. This will provide information on the environment in which the plants were grown. After attending this presentation, attendees will understand the importance of studying the distribution of elements in plant fragments and their chemical morphology.

Impact Statement: Currently, morphological observation and DNA analysis are used for plant analysis. In this presentation, we will show that the distribution and chemical state of elements in plants by synchrotron radiation X-ray analysis and XAFS measurements are useful for identifying plant varieties and growing environments. This allows the use of previously unobtainable information in criminal investigations. This presentation will impact the forensic science community by introducing this method that will be a new tool for connoisseurs who analyze plant fragments.

Plant fragments are important evidence samples left at crime scenes. Plant roots are often found with bodies buried in the soil. Analysis of plant fragments makes it possible to identify the person, alibi, time of the crime, and location of the crime.[1] DNA analysis of plant fragments can be used to identify plant species. However, it is difficult to know the plant's growing environment by DNA analysis. Trace element analysis by Inductively Coupled Plasma (ICP) has been used to reveal the disguised origin of crops.[2] It is known that some plant species concentrate different minerals at specific sites depending on their growing environment.[3] Mineral distribution in plant tissues is important information on plant characteristics.[4] However, ICP analysis can only provide averaged information on minerals in plants. Synchrotron radiation X-rays are highly brilliant, and X-ray nanobeams of several hundred nm can be used for analysis. Therefore, XRF imaging of small areas in plant tissue is possible. The chemical state of the elements can be determined by XAFS measurement in the same condition after XRF analysis. The distribution of elements in plant root cross-sections and the chemical state of detected elements can provide information on plant species and their growth environment.[5]

In this study, the roots of Chinese chives (*Allium tuberosum*), a familiar plant in Japan, were used for the measurements. This study aims to obtain the basic information for the discrimination of different plant roots based on the Chinese chive varieties and growing environments using synchrotron radiation nanobeam XRF imaging and XAFS measurements. Two types of Chinese chives were used in the experiment: Tough Boy and Miracle Green Belt. They were grown in soils with about 0.5ppm and 25ppm of manganese. Chinese chive root slices were prepared using a Leica® Cryostat (CM3050S). Synchrotron radiation XRF imaging was performed at BL36XU of SPring-8. XAFS measurements were performed at BL9A of the Photon Factory of the High Energy Accelerator Research Organization (KEK). As a result of Synchrotron radiation XRF imaging, the root cross-sections obtained from soils with low concentrations of manganese were lower X-ray intensities of Ca, K, Mn, and Zn than the roots obtained from soils with high concentrations of manganese. The elements of Mn and Zn were concentrated in the outer bark of Chinese chive's (Tough Boy) roots obtained from the soil with a high manganese concentration. In Chinese chive's (Miracle Green Belt), K, Ca, and Zn were concentrated in the inner sheath. XAFS measurements showed that the valence of manganese in the roots of Chinese chives (Miracle Green Belt) obtained from the soil with low concentrations of manganese was divalent. On the other hand, the valence of manganese in the roots of Chinese chives (Miracle Green Belt) obtained from soil with high manganese concentration was trivalent. In Chinese chives (Tough Boy), the valence of manganese in the roots from both soils was divalent. These results indicate that the distribution and the chemical states of elements in the root cross section of Chinese chives differed depending on the variety and growing environment. Therefore, Synchrotron radiation XRF imaging and XAFS measurements are useful for characterizing Chinese chives' roots. This approach can be applied to other plants. This method could be used as a new forensic tool for plant fragment analysis that is effective in combination with DNA analysis.

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Synchrotron Radiation X-ray Fluorescence Analysis (SR-XRF); X-Ray Absorption Fine Structure Analysis (XAFS); Plant Fragment

B58   **An Analysis of Single Synthetic Fibers by Metallic Components in Dyes by Nanobeam X-Ray Fluorescence Imaging and X-Ray Absorption Fine Structure Technique Utilizing Synchrotron Radiation**

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Learning Overview: In this presentation, we demonstrate the use of synchrotron radiation X-rays to show metallic elemental information of dyes in a single synthetic fiber, which could not be determined by conventional fiber analysis. After attending this presentation, attendees will better understand the importance of collecting these evidence samples, the utility of metallic elements in dyes for discrimination, and the importance of analyzing dyes in single fibers.

Impact Statement: As this presentation demonstrates the usefulness of information on the distribution of metallic elements in dyes and their chemical states, which has yet to be focused on in conventional fiber analysis, fiber analysts in forensic science will be impacted by having an opportunity to recognize the importance of this information.

The analysis of synthetic fibers is important in forensic investigation. Conventional methods of fiber analysis focus primarily on the shape, material, color tone, and organic components of dyes.[1] On the other hand, it is known that synthetic fiber dyes contain many metallic elements such as Al, Co, Cr, and Zn to bind the polymer.[2,3] In this study, we used nanobeam X-Ray Fluorescence (XRF) imaging and X-ray Absorption Fine Structure (XAFS) techniques utilizing synchrotron radiation for analyses of metallic elements contained in dyes of single fibers. The advantage of nanobeam XRF imaging is that it provides sensitive and high-spatial resolution imaging of trace elements in small samples. The advantage of XAFS technique is that the X-ray Absorption Near-Edge Structure (XANES) spectra allow valence and chemical compound estimation of trace elements.[4]

The purpose of this study is to improve the ability to discriminate single fibers by combining the metallic element information obtained from this nanobeam XRF imaging and XAFS technique with conventional analytical methods. Nanobeam XRF imaging was conducted at SPring-8, BL29XU. The measurements were performed with a beam diameter of 200nm (H) × 200nm (V), 500nm per step, exposure time of 2sec, and under a helium atmosphere. Thin section samples of single fiber cross sections used in nanobeam XRF imaging were prepared using a microtome. XAFS technique was performed at a high-energy accelerator research organization, Photon Factory, BL9A. The incident X-ray energy was varied from 9.155keV to 10.760keV because the K-edge of zinc is 9.661keV. As a result of nanobeam XRF imaging, chlorine, titanium, and zinc were successfully visualized in polyester fibers, and phosphorus, sulfur, and zinc were successfully visualized in acrylic fibers, respectively. The elements of chlorine derived from the dyes in the polyester single fibers were visualized as permeating the interior while mainly being present outside the cross-section. Micro titanium dioxide particles, the matting agent in the single fibers, were visualized in the cross-sectional samples. Since the phosphorus and sulfur were uniformly present throughout the acrylic fiber cross-section, they were of polymer origin. The elements of zinc were present in the outline part of the cross-section in both the acrylic and polyester fibers, indicating that the zinc is of dye derivation. As a result of the comparison of the XANES spectra of the zinc detected, they were similar to those of zinc chloride for acrylic fibers and to those of zinc acetate for polyester fibers. The chemical state of zinc differed depending on the type of polymer.

The results show the importance of analyzing the metallic elements in the dyes of single synthetic fibers. Combining the distribution of metallic elements in single fibers, information on their chemical states, and conventional fiber analysis methods would allow for more detailed discrimination of single fibers.

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Single Synthetic Fibers; Dyes; Synchrotron Radiation X-Rays

B59   The Application of the Expert Algorithm for Substance Identification (EASI) to the Mass Spectra of Various Bath Salts (Synthetic Cathinones)

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Learning Overview: At the end of this presentation, attendees will understand that replicate mass spectra of known standards can be used to improve the confidence in compound identification for synthetic cathinones that have very similar structures and spectra.

Impact Statement: This presentation will impact the forensic community by providing a flexible, robust, and transparent algorithm that can minimize the risk of false positive and false negative identifications in their seized drug analyses.

Current mass spectral algorithms use an approach in which normalized query spectra are compared to normalized known (reference) spectra. For our purposes, the average of many reference spectra for a substance is termed the exemplar or consensus spectrum. For this presentation, at least 13 synthetic cathinone isomers were analyzed in replicate using Gas Chromatography/Electron-Ionization/Mass Spectrometry (GC/EI/MS) on at least five different instruments in two different laboratories. The database included dozens of cathinones and other drugs collected from several publicly available databases. The final database contained more than 3,000 replicate spectra of cathinones. For each compound as the model, the spectra were randomly divided into a training and a test set. The ion abundances for each spectrum were first normalized to the base peak, then the 20 most abundant ions for the training set were used to build 20 General Linear regression Models (GLM) by sequentially using the abundance of each ion as the dependent variable and the abundance of the 19 remaining ions as the covariates.[1,2]

The models for each compound were used to predict the ion abundances for all the Known Positives (KPs) and Known Negatives (KNs) in the database. The predicted abundances were compared to the measured abundances using various similarity and dissimilarity metrics, like the National Institute of Standards and Technology (NIST) match scores and the mean absolute residual. Each metric was then used as a binary classifier to determine the true-positive and false-positive rates over a range of threshold values. These classifications were used to plot a Receiver Operating Characteristic (ROC) curve from which we could calculate the Area Under the Curve (AUC) to determine the accuracy of each model.

For larger cathinones with a molecular mass of 249 Da and a base peak at *m/z* 100, like N-ethyl pentylone, most of the KNs had smaller NIST scores than the smallest NIST score of all the KPs, thus enabling successful classification. EASI consistently outperformed the consensus approach with AUCs of 1.000 in the ROC plots for binary classification (i.e., no errors). The newest cathinone data includes several analogs of smaller cathinones like eutylone, dibutylone, and pentylone, which all share a molecular mass of 235 Da and a base peak at *m/z* 86. For the smaller cathinones, Expert Algorithm for Substance Identification (EASI) using NIST scores had AUCs greater than 0.99 whereas the conventional approach had AUCs of ~0.94.

Ion abundances predicted with EASI models were typically 3–4 times closer to the measured values than the consensus spectrum for a given cathinone. Regardless of the spectral comparison method, EASI outperforms approaches that employ an exemplar or consensus approach to library identifications. In short, the new algorithm outperforms existing algorithms for accurately identifying cathinone analogs and a wide variety of other drugs, from their mass spectra, even in the absence of chromatographic retention times.

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Mass Spectrometry; Seized Drugs; Novel Psychoactive Substances

B60   An Assessment of a Combined Field-Portable Raman Spectroscopy and Gas Chromatography/Mass Spectrometry Approach for the Analysis of Seized Drugs

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Learning Overview: After attending this presentation, attendees will have learned about the strengths and weaknesses of field-portable Raman spectroscopy and Gas Chromatography/Mass Spectrometry (GC/MS) instrumentation for the analysis of seized drug mixtures. Attendees will learn about the performance of each individual technique for both pure samples and seized drug mixtures, the improved identification rate when the two techniques are combined, and the resulting performance when the combined analytical scheme is applied to authentic seized drug mixtures.

Impact Statement: This presentation will impact the forensic science community by providing an assessment of the capabilities and limitations of three field-portable Raman and GC/MS instruments for the identification of seized drug mixtures across a range of drug to adulterant ratios. Assessing the performance of these three field-portable instruments will help inform policy decisions regarding the appropriate implementation of field-portable instrumentation for the analysis of seized drug mixtures.

Growing backlogs and lengthy turnaround times for seized drug analysis are causing significant strain on the judicial system. One potential solution is to improve the quality of field screening through on-site detection methods using field-portable instrumentation to reduce the amount of evidence submissions to forensic laboratories. Another benefit of field-portable instrumentation is that some methods, such as Raman spectroscopy, enable the identification of substances through transparent containers, minimizing contact between personnel and hazardous substances. In comparison, GC/MS analysis requires interaction between the sample and personnel but provides the ability to separate controlled substances from the presence of adulterants or diluents in the sample. However, the incorporation of field-portable instrumentation into routine on-site detection requires extensive empirical data to establish the capabilities, limitations, and performance characteristics for each instrument. This study involves evaluating the of use of field-portable instrumentation for the detection of seized drugs and adulterants in increasingly dilute mixtures to assess the instrument-specific performance. The central hypothesis of this research is that the combination of field-portable Raman and GC/MS instrumentation will enable the detection of controlled substances even in increasingly dilute mixtures.

In this study, a Rigaku ResQ CQL Raman spectrometer with a 1,064nm laser, a Field Forensics HandyRam™ spectrometer with a 785nm laser, and a FLIR® Griffin G510 GC-MS were used to analyze the seized drug mixtures. The mixtures analyzed were cocaine with levamisole, procaine, caffeine, and phenacetin, and methamphetamine with levamisole, caffeine, and phenacetin at 1:1, 1:4, 1:10, and 1:20 ratios. An extra mixture of phentermine and methamphetamine was analyzed using GC/MS instrumentation only to demonstrate the benefits of chromatographic separation for isobaric compounds that produce similar Electron Ionization (EI) mass spectra. The target compounds were chosen based on substances commonly found in seized drug casework to assess instrument performance before the analysis of the authentic adjudicated casework samples. The internal libraries for the ResQ CQL and the FLIR® Griffin G510 were used for compound identification, whereas external Raman libraries were used for identification of Raman spectra collected with the HandyRam™ instrument. Method development involved designing reproducible sample introduction and analysis schemes for all instrumental techniques. The figures of merit assessed prior to authentic adjudicated casework analysis were the selectivity, limit of detection, repeatability, reproducibility, and robustness.

The results showed that both hand-held Raman spectrometers were able to reliably identify the pure compounds and that both constituents could be seen in the spectra obtained from the 1:1 mixtures. As the mixtures became more dilute, the characteristic peaks corresponding to the cocaine and methamphetamine ($1,712cm^{-1}$ and $1,003cm^{-1}$, respectively) were less prominent or hidden by the adulterant entirely. In addition, higher levels of fluorescence were observed when using the HandyRam™ than the ResQ CQL, likely due to the shorter laser wavelength. In contrast, the FLIR® Griffin G510 GC/MS demonstrated superior sensitivity and the ability to identify both the controlled substance and adulterant in the more dilute mixtures. For example, even the isobaric phentermine and methamphetamine could be separated and identified based on their retention times and EI mass spectra. Even though there were issues with the identification of the controlled substance in the more dilute seized drug mixtures, when the analytical data from the individual hand-held Raman spectrometers and the portable GC/MS were considered together, all controlled substances were identified for each seized drug mixture.

Drug Analysis; Raman Spectroscopy; Mass Spectrometry

B61   An Assessment of Odorant Permeability for Canine Training Aid Mimics in Different Containers Utilizing HS/SPME-GC/MS

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Learning Overview: After attending this presentation, attendees will have a better understanding of the suitability of various containers to determine an optimal storage system for reducing permeation and cross-contamination among canine training aids stored in the same case.

Impact Statement: This presentation will impact the forensic science community by showing how certain containers are more desirable in reducing cross-contamination from others stored in the same case, thus providing optimal information on the selection of containers for canine training aid storage, which could result in improving a canine's training ability to detect specific odorants.

Training canines to detect specific odorants corresponding to drugs, explosives, or human remains is beneficial due to their highly sensitive sense of smell. Unfortunately, when training aids are stored in non-optimal containers, such as plastic bags, odorants will tend to cross-contaminate.[1] If canines are trained on cross-contaminated samples, then this would hinder the canine's detection ability to an odorant, causing them to provide a false positive or false negative response. While eliminating all types of cross-contamination in canine training aids is difficult and nearly impossible to achieve in specific environments, limiting and reducing contamination by significant amounts is pivotal to help improve their detection ability. A recent study discussed the permeability and ab/adsorption of certain types of containers using an explosive odorant, Triacetone Triperoxide (TATP) using Direct Analysis in Real-Time Mass Spectrometry (DART®-MS).[2] Mylar® and Opask® bags, primary containers, were ideal for 24-hour short-term storage; however, a longer study depicted Mylar® bags in a mason jar, secondary containment systems, showed TATP permeated out after one week.[2] This presentation includes research utilizing HS/SPME-GC/MS to assess the effectiveness and permeability of odorants in drug training aid mimics at certain time intervals stored in various containers.

To examine the prevalence of cross-contamination in actual canine training aids, used aids containing cocaine, cocaine base, methamphetamine, MDMA, heroin, and black tar heroin were donated by a federal agency and analyzed using HS/SPME-GC/MS to determine if cross-contamination occurred. The used training aids were indeed cross-contaminated with odorants from co-located aids. After confirming the presence of cross-contamination on actual used aids, methods to mitigate cross-contamination by utilizing optimal storage containment was explored. Mimic training aids for heroin, with acetic acid as the major odorant, and MDMA, with dimethyl sulfone as the major odorant, were created by pipetting and weighing, respectively, small quantities of the pure odorant onto a substrate, cellulose or alumina. A vapochromic cross-contamination sensor was implemented to provide a visual color change cue from green to blue to assess if cross-contamination was possibly present. The containers assessed within this study included plastic Ziploc®-type bags, Mylar® bags, Bitran® bags, mason jars, and glass Training Aid Delivery Devices (TADDs). The mimic training aid for heroin and MDMA, and a vapochromic cross-contamination sensor were packaged separately in the same type of container and stored together in a case.

Optimal equilibrium and extraction times were optimized using HS/SPME-GC/MS. Calibration curves for each odorant were assessed to determine limits of detection and quantification. Samples were collected the initial day each mimic was placed within the container and then again over time to monitor for evidence of cross-contamination. For this purpose, a small amount of sample was placed into a 20mL vial to assess if the cross-contaminated odorant was present. Samples were collected and analyzed, at time intervals, 1, 4, 7, 14, 21, 28 days, etc. Additionally, the color change from green to blue in the vapochromic cross-contamination sensor, indicating potential cross-contamination, was analyzed with a digital microscope at each time interval.

The samples analyzed deemed the cross-contaminated odorant permeated at shorter time intervals from certain containers than others. The cross-contamination sensor results were in agreement with these findings. This study provides canine trainers and handlers, as well as other individuals, with a better sense of understanding optimal containers and their length of suitability before cross-contamination of odorants occurs within them, potentially causing skewed results or affecting the accuracy and reliability of a canine's search.

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Training Aid Mimics; Containers; Cross-Contamination

B62   Color, Monogram, and Physical Measurements of Illicitly Manufactured Fentanyl Tablet Exhibits Seized Along the United States Southwestern Border

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Learning Overview: After attending this presentation, attendees will be able to assess the utility of various methods of physical characterization and the ability of these characteristics to differentiate between batches of illicit tablets. This presentation will highlight the various physical metrics by which illicit tablets can be measured and compared, including colorimetric, microscopy, gravimetric, and caliper measurements.

Impact Statement: This presentation will impact the forensic science community by contributing to the overall understanding of batches of fentanyl tablets entering the United States from the Southwestern border. Further, this presentation will highlight the advantages and limitations associated with using physical measurements to differentiate fentanyl batches seized at various ports of entry.

Fentanyl is increasingly infiltrating the United States via the Southwest border with federal agencies and independent crime watch organizations reporting Mexico has surpassed China as the primary exporter of fentanyl to the United States. Seizures of fentanyl along the United States-Mexico border are often disguised as oxycodone pharmaceuticals, appearing as counterfeit round blue tablets monogrammed "M 30."

This study aims to evaluate whether physical measurements, such as size, monograming, and color could be used as a diagnostic tool to differentiate exhibits of fentanyl-containing tablets monogrammed "M 30" seized by Customs and Border Protection along the United States-Mexico border.

Over 90 exhibits containing 30–50 tablets per exhibit were received from three ports of entry. Up to 30 total tablets per exhibit were characterized using the following methods of analysis: photographs were taken using an iPhone® 13, with Red-Green-Blue (RGB) color code measurements recorded using ImageJ software; pill width and diameter were taken using General® UltraTech® No.147 calipers; weight was recorded using a Mettler Toledo® XPR105 microbalance; and monogram measurements of the "M" diagonal length and "0" height were recorded using a Zarbeco MiScope®-MP3 microscope.

A total of 540 intact tablets from 23 exhibits were statistically analyzed to assess the discriminative potential of the above methods. Descriptive statistics, including the Coefficient of Variation (%CV), were generated for each parameter, and normality tests were performed on each exhibit. Shapiro-Wilk normality tests showed the data sets were not normally distributed, prompting a non-parametric Kruskal-Wallis Analysis of Variance (ANOVA) test to determine whether significant differences existed between exhibits. When the Kruskal-Wallis null hypothesis was rejected (meaning significant differences of the median existed between at least two exhibits), a Dunn's test, which is a post-hoc analysis for non-parametric hypothesis testing, determined whether all possible inter-exhibit pairwise comparisons were statistically different from one another at a 95% confidence interval. To compare the utility of the tested parameters in future multivariate modeling, the percentage of distinguishable exhibits from the population by each test was calculated. The proportion of differences to total pairwise comparisons are shown in Table 1. This serves as a direct indicator for how discriminatory a method of analysis is for the population of tested tablets.

Table 1. Summary of descriptive statistics and Kruskal-Wallis/Dunn's test pairwise comparisons for each parameter

Parameter	N	CV (%)	Total pairwise comparisons	Number of pairwise comparisons significantly different at 95% confidence	Proportion of total
Diameter	466	0.3%	231	49	21.2%
Width	466	4.3%	231	39	16.9%
Weight	525	7.1%	253	23	9.1%
R	525	7.7%	253	100	39.5%
G	525	4.7%	253	93	36.8%
B	525	4.9%	253	88	34.8%
"0" Height	297	5.6%	136	51	37.5%
"M" Diagonal Length	297	7.1%	136	33	24.3%

Measurements of tablet diameter had a %CV less than 1% for the entire population, indicating little difference in the sample population. Tablet width and weight appear to be more varied than diameter but still hold the two lowest proportion of differences. RGB color code measurements boast a collection of relatively high proportion of differences and from data visualization showed better association within exhibits and discrimination between exhibits. Monogram measurements had a combination of high proportion of differences and %CV, indicating these measurements hold some discriminatory power.

In conclusion, this study suggests that color and monogram measurements may serve as effective diagnostic tools to differentiate exhibits of fentanyl tablets, whereas physical measurements such as diameter, width, and weight did not provide as much discriminatory/associative power. Based on these findings, original workflows were optimized to prioritize methods that provide the most discriminatory data. Additional tablets from new and previously analyzed ports of entry should be analyzed to confirm these findings.

Fentanyl; Statistics; Opioid Epidemic

B63　The Detection of Airborne Methamphetamine in Contaminated Properties and Vehicles Using Active Air Sampling With Liquid Chromatography/Mass Spectrometry (LC/MS)

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Learning Overview: After attending this presentation, attendees will better understand the importance of using supplementary methods of surface wipe sampling and air sampling to detect methamphetamine in contaminated sites.

Impact Statement: This presentation will impact the forensic science community by demonstrating the importance of using air sampling as a supplementary method to surface wipe sampling for determining overall methamphetamine contamination within properties and vehicles. This is important to help limit methamphetamine exposure to innocent individuals.

Clandestine manufacture and smoking of methamphetamine results in contamination of properties and vehicles. Individuals are then at risk of Third-Hand Exposure to Methamphetamine (THEM) through dermal adsorption, ingestion, and inhalation contributing to associated adverse health effects, including headaches, respiratory problems, skin irritations, and behavioral effects.[1,2] Additionally, the detection of methamphetamine in a property could lead to eviction of the innocent renter or significant costs and anxiety for innocent buyers of such a property. This presentation will illustrate the importance of using additional techniques to assess the environmental risks to people from previously contaminated places to ensure justice for all.

Currently, contaminated sites are quantitatively tested for methamphetamine using a surface wipe sampling method coupled with analysis commonly by Gas or Liquid Chromatography/Mass Spectrometry (GC or LC/MS).[3] Even after remediation or discarding contents, the property may still be contaminated with methamphetamine and other hazardous chemicals.[4,5] In particular, airborne methamphetamine that has sorbed and deposited onto and adsorbed into surfaces could be released in the air in the particulate or vapor phase, leading to inhalation exposure.[6,7]

Active air monitoring of properties and vehicles with suspected or known methamphetamine contamination was undertaken using air sampling pumps (1/min air flow rate) connected to sorbent tubes with either XAD®-2 sorbent or activated coconut charcoal. After sampling, the sorbent tubes were liquid extracted with 0.1M sulfuric acid with methamphetamine-d$_5$ deuterated internal standard. Blank and field-blank sorbent tubes were also analyzed. LC/MS analysis was performed using a single quadrupole mass spectrometer with positive Ion Electrospray Ionization (ESI) at a limit of detection of 0.02μg/sample methamphetamine. Higher methamphetamine concentrations were detected using the XAD®-2 sorbent tubes compared with activated charcoal sorbent tubes from the same sampling environment, either indicating more absorption onto XAD®-2 or more retention onto the charcoal. Comparisons between surface wipe samples and air samples will be presented. Results indicated that methamphetamine was detected from properties and from vehicles in the range of 0.3 to 2μg/m³. An adult breathing 2μg/m³ at a normal breath rate of 6L/min would lead to an exposure of 0.72μg/hour.

Active air monitoring with sorbent tubes can be used to extract airborne methamphetamine to consider inhalation hazards that are overlooked when only surface wipe sampling contaminated media. It is recommended that active air sampling is coupled with surface wipe sampling to provide information on the extent of overall methamphetamine contamination in areas where exposure occurs and to assess the extent of third-hand exposure to innocent individuals.

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Drug Analysis; Liquid Chromatography; Mass Spectrometry

B64   **The Development of a Gas Chromatography/Mass Spectrometry/Selected Ion Monitoring (GC/MS/SIM) Method for Analyzing Adulterated Nitazene Samples**

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WITHDRAWN

B65   **The Development of a Novel Nanocomposite Using Polypyrrole and Single-Walled Carbon Nanotubes for Headspace Solid Phase Microextraction of Alkanes in Forensic Ignitable Liquid Analysis**

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Learning Overview: After attending this presentation, attendees will understand how to perform electrochemical preparation of nanomaterial-based Solid Phase Microextraction (SPME) fibers. The attendees will also learn about Ignitable Liquids (ILs).

Impact Statement: While Carbon Nanotubes (CNTs) have been widely tested, this study will impact the forensic science community by presenting the potential of microscale solid phase concentration combining CNTs and conductive polymers for forensic IL analysis. The new coating provides forensic analysts with an alternative opportunity in the SPME process.

In incendiary structure fires, ILs have been identified for more than 35% of cases involving civilian deaths.[1] The identification of the presence of an IL at a fire scene provides important investigative intelligence. Prior to Gas Chromatography/Mass Spectrometry (GC/MS) analysis, it is essential to pre-concentrate traces of an IL recovered from the scene. Headspace SPME is one of the standards for sample extraction in forensic IL analysis, as SPME gives simple and speedy extraction of volatile organic compounds from IL samples.[2] The performance of SPME relies on the distribution equilibrium between the analytes and the fiber coating. The fiber coating also decides the extraction capacity, stability, and affinity of SPME toward the target compounds in ILs.[3]

Carbon Nanotubes (CNTs) have gained increasing attention from the scientific community since their discovery in 1991.[4] Except for their thermal stability, the CNTs have a large surface area that improves extraction efficiency. The CNT's unique structure, including π–π stacking, van der Waals forces, and hydrogen bonding, assists hydrophobic interactions with organic molecules.[4] Those characteristics make CNTs potential SPME sorbent materials for extracting IL samples.

This work aimed to prepare a CNTs-based nanocomposite for SPME using an electropolymerization technique and evaluate its characteristics in extracting saturated alkanes, which are commonly found compounds in ILs via GC/MS. The pre-polymerization solution was comprised of single-walled CNTs as adsorbents, pyrrole as a monomer, 0.2M Sodium Dodecyl Sulfate (SDS) as a surfactant, and 1M sodium chloride (NaCl) as electrolytes. The SPME nanocomposite was synthesized electrochemically via *in situ* polymerization from the solution and directly deposited on the stainless-steel wire using cyclic voltammetry. Parameters influencing the formation of the coating, including the amount of CNTs, pyrrole, and the bath voltage, were optimized. C8-C22 saturated alkanes reference material was utilized to test the proposed CNT-SPME fiber. Diesel fuel, one of the most-used ILs, was chosen as a real sample to validate the extraction capability of the CNT-SPME fiber.[5]

Results demonstrated the optimized coating procedure for the CNT-SPME fiber as follows: 8mg CNTs were ultrasonically dispersed in 6mL SDS for 5min, then 200μL pyrrole in 6mL NaCl was added and sonicated for 30min. The bath voltage applied to coat the stainless-steel wire with the sorbent composite was determined as 0.8V. The Relative Standard Deviation (RSD) of the yielded chromatographic peak areas of the analytes in the reference material was under 1%. The CNT-SPME fiber was applied to extract neat diesel fuel samples. The characteristic pattern of distillates and major ions in the extracted ion profiles for the target compounds in the diesel fuel could all be identified. The detection limit was 0.01μg mL^{-1}, and the RSDs were under 6%.

To conclude, the CNTs could be electrochemically deposited onto a SPME fiber and ensure the sampling and analysis of alkane compounds in IL samples. The significance of this study was to demonstrate an easily made and sensitive SPME sorbent coating to facilitate ILs analysis. The selectivity offered by the CNT-SPME fibers could also improve the extraction efficiency of the SPME process.

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Headspace Solid Phase Microextraction; Electropolymerization; Carbon Nanotube

B66   Enhanced Ignitable Liquid and Substrate Database Functionality for Improved Casework and Research

Michael E. Sigman*, University of Central Florida, Orlando, FL; Nikhil Prakash*, University of Central Florida, Orlando, FL; Mary R. Williams, University of Central Florida, Orlando, FL; Larry Tang, University of Central Florida, Orlando, FL

Learning Overview: This presentation will cover enhancements to the Ignitable Liquid and Substrate Databases that will facilitate casework. After attending this presentation, attendees will understand enhancements to these databases and how to utilize the databases to better determine the presence of ignitable liquid residues in fire debris samples.

Impact Statement: This presentation will impact the forensic science community by providing insight into new features of these two heavily used databases, which will make the analysis of casework data easier and more visual.

Fire debris analysis relies heavily on visual pattern recognition.[1] Current implementation of the Ignitable Liquids Reference Collection (ILRC) and Substrate Database does not promote direct comparisons of ignitable liquid and substrate pyrolysis chromatograms. In the current implementation, records must be viewed independently in separate browsers or within two tabs of the same browser. The new implementation of the databases will allow for side-by-side visualization of returns from independent searches of the two databases. All the current search capabilities will remain in the enhanced website. The returns from the two searches will be independently scrollable to allow side-by-side comparison of the ignitable liquid and substrate pyrolysis chromatograms.

An additional database will be created based on random in-silico creation of computational fire debris samples. The in-silico samples will contain a single ignitable liquid mixed with contributions from up to three pyrolyzed substrates randomly selected from the Substrate Database. Ignitable liquid weathering will utilize numerical kinetic-based evaporation models from Smith's laboratory, rather than relying on the limited number of weathered samples in the ILRC.[2] The new database will be substantially larger than the existing databases, with a current target of 100,000 entries. The database will be searchable based on products, carbon range, ratio of ignitable liquid to substrate, ignitable liquid American Society for Testing and Materials (ASTM) class, fraction of the ignitable liquid lost to weathering, and other parameters. The in-silico database will provide direct visualization of computational fire debris and eliminate the need for analysts to mentally merge two chromatograms to imagine the resulting chromatographic profile. The in-silico data will also be available for download as a standard set of total ion chromatograms and total ion spectra for machine learning research.

This presentation will provide an overview of the envisioned implementation of the databases as a web site plugin with improved security to serve the community into the future with minimal interruptions in service.

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Data; Casework; Arson

B67   **An Estimation of Dyeing Methods From Wool Single Fibers by Nanobeam X-Ray Fluorescence Imaging Utilizing Synchrotron Radiation**

Yoshinori Nishiwaki*, Kochi University, Kochi, Japan

Learning Overview: The goal of this presentation is to demonstrate the use of synchrotron radiation X-rays to show metallic elemental information of dyes in a single wool fiber, which could not be determined by conventional fiber analysis. After attending this presentation, attendees will better understand the importance of collecting these evidence samples, the utility of metallic elements in dyes for dissimilarity identification, and the importance of analyzing dyes in single fibers.

Impact Statement: This presentation will impact the forensic science community by demonstrating the usefulness of information on the distribution of metal elements in dyes, which has yet to be focused on in conventional fiber analysis. Fiber analysts in forensic science will have an opportunity to recognize the importance of this information.

Single wool fiber is an important evidence sample in forensic investigations. Differentiating single wool fibers is complex, and there are reports of color analysis by microspectroscopy and analysis of organic components by Thin-Layer Chromatography (TLC) and Liquid Chromatography/Mass Spectrometry (LC/MS).[1-3] Wool fibers have a bilateral structure consisting of a paracortex with a high cystine concentration and high hydrophobic group content and an ortho cortex with a low cystine concentration and high hydrophilic group content attached together. Two dyeing methods are used to dye wool fibers for clothing: acid-mordant dyeing and metal-containing dyeing, using chromium or cobalt. If the distribution of dye-derived elements and wool's intrinsic elements in a single fiber could be measured, it would be useful for discriminating different wool fibers. Furthermore, forensic purposes would be beneficial if the dyeing method could be inferred from a single wool fiber.

In this study, nanobeam X-Ray Fluorescence (XRF) imaging and X-ray Absorption Fine Structure (XAFS) analysis using high-brilliance synchrotron radiation were performed on cross-sectional samples of single wool fibers prepared with a microtome to estimate the dyeing method from single wool fibers. The samples were wool fibers supplied by the fiber manufacturer with clear information on the dyeing method. Synchrotron radiation measurements were performed at SPring-8, a large synchrotron radiation facility where synchrotron radiation X-rays with a beam diameter of 100nm are available. As a result of XRF measurement, S, Ca, Cr, Co, and Zn were detected. Based on sulfur concentration differences, imaging measurements visualized the paracortex and ortho cortex areas. In chromium-containing dyeing, Cr was concentrated in the ortho cortex and cuticle but not in the central and paracortex. In chromium mordant dyeing, Cr was distributed throughout the wool cross-section but was concentrated in the paracortex and cell nucleus residue. The elemental distribution corresponding to the bilateral structure was successfully visualized from a cross-sectional section of a single wool fiber. The dyeing method may be estimated from the distribution of elements in the single fibers.

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Fibers; Dyes; X-Ray

B68   How to Minimize the Presence of Drug Residues on the Exterior of Drug Evidence Packaging After Analysis

Elizabeth Robinson*, National Institute of Standards and Technology, Gaithersburg, MD; Edward Sisco, National Institute of Standards and Technology, Gaithersburg, MD; Rebecca Mead, Forensic Quality Manager, Vermont Forensic Laboratory, Waterbury, VT

Learning Overview: After attending this presentation, attendees will understand how drug residues can be transferred onto evidence packaging as a result of common analysis protocols and what steps can be taken to minimize this phenomenon. Minimizing drug residues can be important in reducing potential cross-contamination and minimizing exposure risks for personnel handling sealed evidence.

Impact Statement: This presentation will impact the forensic science community by providing key information on how minor adjustments to the repackaging process, after drug evidence analysis, can reduce the spread of drug residue to the exterior of the packaging. Reducing the residue transfer will result in safer handing of drug evidence across the entire evidence handling process, from evidence handling technicians to crime scene technicians to submitting officers.

With the emergence of more potent drugs and novel psychoactive substances, the potential exposure risk to personnel who handle drug evidence is also increasing.[1] These exposure concerns are typically targeted at forensic chemists, due to their need to handle the actual evidence. However, there are a number of other personnel who handle the packaged drug evidence, typically without any protective equipment, and could possibly be exposed if the exterior of the packaging contained sufficient residue. Previous studies have shown that drug residues can be found on nearly every surface in a drug chemistry laboratory and can frequently be found in evidence receiving areas and police stations.[2-4] It has been demonstrated that the process of opening and analyzing drug evidence of a case in a forensic laboratory increases the level of drug particulate on the exterior of the drug evidence packing, especially when repackaging.[5] These observations prompted the current effort to identify ways to reduce the spread of drug particulate during the evidence repackaging process.

This work investigated how minor adjustments to the evidence repackaging process could affect the level of drug particulate on the exterior of evidence packaging. Prior work showed higher residue levels resulted from reusing the original evidence packaging, therefore repackaging into a new Kapak® bag was examined. Four repackaging processes were investigated using real casework (25 cases each), including: repackaging drug evidence into a new Kapak® bag (normal), changing gloves prior to repackaging evidence into a new Kapak® bag, repackaging into a new Kapak® bag on a freshly laid underlying surface, such as butcher paper, and changing both the gloves and the surface prior to repackaging into a new Kapak® bag. Both the original packaging (pre-analysis) and the new packaging (post-analysis) were sampled using wipe sampling to understand, qualitatively and quantitatively, how the drug residue profile changed.

Qualitative analysis was completed by Direct Analysis in Real Time Mass Spectrometry (DART®-MS) on the wipe extract and screened against an in-house built library of over 1,200 compounds. This data was used to identify which drug residues were present on the original packaging, to determine if those identifications were consistent with the drug contents, and to evaluate if the same, or different, drugs were detected on the exterior of the new Kapak® after repackaging.

Quantitative analysis was completed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Measurements for 30 drugs were obtained. The quantitative data was used to determine the effect the different repackaging processes had on the amount of drug residue present on the packaging relative to other processes and relative to the amount present on the original packaging.

Quantitative data shows that, after repackaging, single to tens of micrograms of drug residue is present on their exterior surface, consistent with previous studies. The data also suggests that changing gloves prior to repackaging evidence can result in lowering the amount of drug residue that is transferred. For example, changing gloves reduced the likelihood of transferring drug residue onto the repackaging from 83% to 50%. This information is also being combined with qualitative visualization data using mock case samples that can better convey these results. A discussion on how the results of this work can be combined with previous efforts to understand particulate transfer during the opening and handling of drug evidence to reduce drug background and minimize exposure or cross-contamination concerns will also be provided.

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Seized Drugs; Mass Spectrometry; Exposure Mitigation

B69   **The Identification and Classification of Major Cannabinoids Using Raman Microscopy, Chemometrics, and a Novel Artificial Intelligence Approach**

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Learning Overview: After attending this presentation, attendees will gain an understanding of the capability of Raman microscopy supported by traditional chemometrics and a novel Artificial Intelligence (AI) approach using the transfer learning technique to identify major cannabinoids.

Impact Statement: This presentation will impact the forensic science community by demonstrating an accurate, fast, simple, and automated AI approach to classify cannabinoids collected with Raman spectroscopy and a comparison to the results with a more traditional chemometric approach.

With the rise in the prominence of cannabis products and due to their widespread availability and varying legal status, there is an increased emphasis on the differentiation of cannabinoids present within cannabis. Various analytical techniques, including micro- Raman spectroscopy, have been applied to the detection of common cannabinoids. While chemometrics, such as Principal Component Analysis (PCA) followed by Linear Discriminant Analysis (LDA), have traditionally been used to classify forensic samples following their acquisition with Raman spectroscopy, AI approaches have become a more popular research topic. In this study, PCA-LDA and a novel AI approach were both applied to differentiate cannabinoids collected with Raman microscopy.

Seven of the most abundant cannabinoids naturally found in cannabis were selected for analysis, including Δ^9-Tetrahydrocannabinol (Δ^9-THC), its acidic precursor Tetrahydrocannabinolic Acid (THCA), Cannabidiol (CBD), its acidic precursor Cannabidiolic Acid (CBDA), Cannabigerol (CBG), Cannabichromene (CBC), and Cannabinol (CBN). The cannabinoids were analyzed as crystallized residue following the deposition and evaporation of each cannabinoid onto a microscope slide covered with aluminum foil to avoid interference. The solvent was evaporated at room temperature, and a 785nm laser was focused on the solid, crystallized residue to collect 34 spectra for each cannabinoid. The Raman microscope was used to determine the optimal sample area to collect Raman spectra based on the drying patterns of the cannabinoids. For the chemometrics, the raw data were baseline corrected. PCA was implemented to visualize and evaluate the variation of the data. The chemometric model was developed using LDA. In comparison, 238 Raman spectra were used to create the AI model using the transfer learning technique. Each Raman spectrum was converted into a two-dimensional image (i.e., a scalogram, a time-frequency representation produced from Continuous Wavelet Transform [CWT]). To evaluate the result of the training set, a new data set containing 70 Raman spectra (10 spectra per cannabinoid) was collected and analyzed separately.

The microscopy showed that the optimal sample area was located on the center of the dried residue for the nonacidic cannabinoids and along the edges of the dried residue for the acidic cannabinoids. The spectra exhibited distinct spectral patterns and peaks corroborated by vibrational bands in published literature for all cannabinoids. 93% of the variation across all seven cannabinoids was accounted for with the first principal component. The LDA model was 96% accurate in classifying the standard cannabinoids to their respective grouping. The developed model experienced the most difficulty with classifying THCA; the model misclassified three THCA spectra as CBC, CBG, and CBN. For the AI, the model training was conducted in less than 10min in a Graphical Processing Unit (GPU) environment. All scalograms were successfully classified into respective cannabinoid classes, with 100% classification accuracy. The average prediction probability for correct classification was 0.9931. The classification outcome provided by the AI model included both prediction labels and probability, which provided a comprehensive evaluation of the samples. The accurate, fast, simple, and automated AI model shows promise for cannabinoid differentiation.

Raman Microscopy; Artificial Intelligence; Cannabis

B70   One Gunshot Is Not Enough: A Case of Suicide by Double Gunshot Wound to the Head and a Literature Review

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Learning Overview: Suicidal gunshots involving the use of firearms are not an uncommon occurrence. Typically, the preferred locations include the head, particularly the temples, or the oral cavity, leading to an outcome that is usually fatal. Despite that, descriptions of cases involving subjects committing suicide by inflicting multiple gunshots to the head have been reported in the scientific literature. The goal of this presentation is to present an interesting case of suicide involving two gunshot wounds to the head in which the trajectories were reconstructed with the help of radiological techniques.

Impact Statement: This presentation will impact the forensic science community by sharing an intriguing forensic case, along with a concise literature review, that will demonstrate to attendees that death may not be instant, even in instances of gunshot wounds to the head. Such a scenario poses a challenge in differentiating between suicide and homicide. In this context, the elaboration of autoptic, radiological, and circumstantial data becomes crucial for reconstructing the dynamics of the events.

An 81-year-old man was found dead, lying on his bedroom bed, holding a Smith & Wesson® revolver. That night, the man's neighbors reported hearing multiple gunshots from his home. During the forensic examination, two gunshot wounds were documented: the first at the chin, and the second in the palate, without any other injury; however, at the crime scene examination, three bullets for five shells were found. The police found out that the weapon was regularly possessed.

Because of the peculiarity of the case, further investigations were ordered. The X-ray examination confirmed the presence of two metallic objects at the cranial level: the first in the nasal cavities and the second in the occipital fossa. Autopsy investigations confirmed a near-contact gunshot wound (entry hole) on the chin and identified a second entry hole in the hard palate. The black powder soot on the upper lip indicated that the second shot had been fired near the oral cavity. No signs attributable to a struggle were documented. The trajectories of the two shots were thus reconstructed: the first, a near-contact gunshot, was deflagrated close to the chin, crossed the palate, and ended in the nasal cavity; the second gunshot, which proved fatal, was deflagrated near the oral cavity, crossing the posterior portion of the palate, and ending in the posterior cranial fossa.

The case's successful resolution was only made possible by meticulously analyzing all the collected elements, concluding that the man had first shot himself at the chin level without hitting vital areas. Subsequently, he deflated a second shot inside the oral cavity.

Multiple self-inflicted gunshot wounds to the head or other sites are an uncommon occurrence and a real challenge to the forensic pathologist in determining the manner of death. The scientific literature offers various examples of subjects who committed suicide by multiple gunshots—for example, Jacob et al. exposed two cases of suicide by multiple gunshots to the head: the first victim exploded the first shot at the oral cavity, causing modest damage to his left optic nerve and frontal lobe.[1] Despite his injuries, the man could still drive his car home, where he deflagrated a second shot at the level of his right temple, only to die two days later. The second victim was a 58-year-old man found dead on his bed. Reconstruction of the case revealed that the first shot had gone through the tongue and slightly damaged the second cervical vertebral body.

In contrast, the second shot, fired at the left temple level and proving fatal, had damaged the temporal lobe. Also, Introna et al. described nine cases of multiple-shot suicides, showing that in these cases, the most common site of firing was the left hemithorax or precordial region.[2] Many other authors collected numerous cases of gunshots to the head without immediate incapacitation.[3,4]

In essence, a "lucid interval" following a deflagrated gunshot wound to the cranio-cephalic district is a possible occurrence, especially when combined with the lack of impairment of vital areas and the low caliber of the weapon. In the present case, the low caliber of the weapon and the fact that the first shot did not hit the cranial encephalic district allowed the man to deflagrate the second shot. In these cases, however, integration with the circumstantial data remains crucial as a decisive factor for accurately interpreting the dynamics.

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Gun Shot Residue; Suicide; Forensic Analysis

B71   **The Optimization of Sample Preparation Procedures for the Determination of Δ9-THC and Its Isomers in Seized Cannabis Oils, Concentrates, and Vapes by LC-PDA**

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Learning Overview: After attending this presentation, attendees will have learned about the sample preparation procedures, a Liquid Chromatography-Photodiode Array (LC-PDA) method at the National Institute of Standards and Technology (NIST), and inaccuracies of product labels for Δ9-Tetrahydrocannabinol (Δ9-THC), Δ8-Tetrahydrocannabinol (Δ8-THC), and Δ10-Tetrahydrocannabinol (Δ10-THC) in seized cannabis vapes, oils, and concentrates.

Impact Statement: This presentation will impact the forensic science community by providing a summary of sample preparation, cleanup, and analytical methods that NIST has developed for transfer to forensic laboratories in distinguishing seized cannabis vapes, oils, and concentrates as either legal hemp or illegal marijuana. This presentation will help inform the forensic science community of how product labels are inaccurate for Δ9-THC, Δ8-THC, and Δ10-THC in these type of cannabis products, while also gaining knowledge of some challenges potentially encountered in there laboratories.

In the 2018 Farm Bill, hemp was defined as cannabis containing 0.3 % or less of decarboxylated Δ^9-THC and removed hemp from the United States Drug Enforcement Agency controlled substances list. As a result, the need for forensic laboratories to have access to reliable analytical methods for differentiation between hemp and marijuana in seized cannabis samples increased significantly. Before the new legislation, forensic laboratories were dependent on qualitative measurements from Gas Chromatography with Mass Spectrometry (GC/MS) for Δ^9-THC identification in cannabis oils, concentrates, and vapes. Forensic laboratories have been switching from GC/MS to LC-PDA because of incomplete degradation of acidic cannabinoids to neutral cannabinoids in the GC inlet and LC-PDA permits them to be measured separately. In response, NIST developed a *Cannabis* Research Program to help provide forensic laboratories the necessary tools to quantitatively measure Δ^9-THC, its isomeric compounds (e.g., Δ^8-THC and Δ^{10}-THC), and its acidic precursor Tetrahydrocannabinolic Acid (THCA) permitting the calculation of a total Δ^9-THC mass fractions (%).

This presentation will focus on an optimization of a sample preparation procedure previously published at NIST for the determination of Δ^9-THC, THCA, and total Δ^9-THC in commercial hemp oil samples by LC-PDA.[1] Approximately 0.5g of hemp oil samples were mixed with 25mL of methanol and shaken for 15min. The methanolic extract sample was further diluted with methanol through a 10-fold and 100-fold sample dilutions prior to LC-PDA analysis. The goal of the new research was to minimize the sample preparation time to make it more desirable to forensic laboratories and expand to include cannabis concentrates and vape solutions/cartridges. Data will be provided showing how the original sample preparation procedure was modified to include only 0.1g of sample, 5mL of methanol, and 1min of shaking. The new sample procedure was validated by a single laboratory at NIST for approximately 60 commercial and seized cannabis products. Product labels for the seized cannabis samples often included a THC mass fraction of 80% to 90%; however, in many cases the actual mass fractions of Δ^9-THC were much less and its isomers (e.g., Δ^8-THC and Δ^{10}-THC) were present at high levels. Additionally, significantly co-elution issues were observed at these high levels that will affect the accuracy of Δ^9-THC measurements by forensic scientists.

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Cannabis; Vaping; Delta-9-Tetrahydrocannabinol

B73   Standards Development Activities in Human Forensic Biology

Jillian Conte*, Peckville, PA

Learning Overview: After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of human forensic biology testing.

Impact Statement: This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to human forensic biology, including serological and DNA testing. This presentation will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards and best practice recommendations that are forwarded to Standards-Developing Organizations (SDOs), such as the Academy Standards Board (ASB), that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards. The ASB approves consensus standards and best practice recommendations, including the revision, reaffirmation, or withdrawal of a document. This is done through consideration of comments, views, and objections collected during public comment periods.

During this presentation, updates related to standards development in human forensic biology testing will be discussed. These include: (1) recent standards that have been added to the OSAC Registry, (2) ASB New Work Products (NWP) that have been received from OSAC, (3) published standards from the ASB pending Registry approval process, and (4) documents currently being drafted by the Human Forensic Biology subcommittee of OSAC and those under development by the ASB.

Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts. Information on implementation may also be shared with the attendees.

Standards; Forensic DNA; Serology

B74   Human Factors in Forensic DNA Handbook Recommendations in Practice

Tiffany A. Roy*, ForensicAid, LLC, West Palm Beach, FL; Sasha B. Colvin*, ForensicAid, LLC, Riviera Beach, FL

Learning Overview: After this presentation, attendees will understand the application of reporting and testimony recommendations in independent practice. They will also understand the rationale behind these recommendations and how their application intends to mitigate the possible harm from error and bias.

Impact Statement: This presentation will impact the forensic science community by not only serving to promote awareness of the recommendations, but also will serve to notify the DNA community of how current and past laboratory practice will be compared with the best practices described in the Human Factors in Forensic DNA Interpretation guidebook with a specific focus on forensic DNA testimony and reporting.

DNA analysis is critical to the success of the nation's criminal justice system. In fact, DNA left at a crime scene—referred to as trace DNA—is the most highly regarded type of forensic science evidence.

The examination of DNA evidence consists of a series of steps involving the comparison of a trace evidence DNA profile to a known DNA profile. During this process, DNA examiners must reach <u>scientifically sound</u> conclusions; they are also expected to produce records of the examination and, in some cases, present their conclusions—and the reasoning behind them—in court.

In recent years, the accuracy of DNA interpretation, especially as to mixtures of DNA, has been the subject of increased scrutiny, including from the President's Council of Advisors on Science and Technology Report on forensic sciences in 2015 and 2016. To help address this issue, the National Institute of Justice and the United States National Institute of Standards and Technology (NIST) convened an expert working group to do a scientific assessment of the effects of human factors on DNA analysis and interpretation to develop recommendations to reduce the risk of error.

The working group addressed issues ranging from the collection and preservation of DNA to courtroom testimony, and from laboratory design and equipment to emerging methods for assessing questions regarding how DNA came to be present on an item. In addition to an overview discussion of how human factors relate to all aspects of DNA examinations, this presentation will focus on human factors involved with conclusions expressed through reporting and testimony.

This presentation will include a practical demonstration of how these recommendations can be compared against casework documentation and testimony produced across the country by demonstrating actual testimony transcripts and reports that would flag against best practice. Forensic DNA reporting and testimony can be inspected by private consultants to determine compliance with and adherence to these recommendations to mitigate bias and error in forensic DNA testimony and reporting.

Human Factors; Bias; Forensic DNA

B75   Speak Now: To Be a Part of the People That Make Meaning

Nancy Dinh*, Forensic Analytical Crime Lab, Hayward, CA

WITHDRAWN

B76 **Processing Gun Crimes in NYC: How the Department of Forensic Biology Was Able to Meet a 30-Day Turnaround Time**

Craig O. O'Connor*, New York City Office of Chief Medical Examiner, New York, NY; Katey Nori, New York City Office of Chief Medical Examiner, New York, NY

Learning Overview: This presentation will walk attendees through the approach employed at the New York City Office of Chief Medical Examiner's (OCME) Department of Forensic Biology for processing touch DNA from cases associated with gun crimes. Due to a city hall initiative to process gun crimes in an accelerated fashion from arrest to prosecution, the OCME's Forensic Biology laboratory needed to adjust its workflow to meet the testing time necessary to get results to the attorneys while keeping quality at the highest level. After attending this presentation, attendees will see the pre-testing protocols put in place as well as the adjustments to workflow introduced in the process, which may be of use in their own laboratories.

Impact Statement: This presentation will impact the forensic science community by showing how a large public laboratory is able to meet the demands of the criminal justice system, provide results to meet the accelerated time frame for bringing these cases through the court system, and lowering turnaround time from 60 days to 30 days in less than a year.

Timely reporting of DNA gun swab results is paramount to a robust criminal justice system. Criminal possession of a weapon has been a crime type that has been highlighted in New York City, and the submission of samples from guns has been on the rise over the past decade. As part of a 2018 City Hall initiative, "Project Fast Track," the criminal justice players have been tasked with bringing these cases from arrest to prosecution in an expedited fashion to help curb gun violence in New York City.[1] Law enforcement and the court system, with the help of crime labs, joined together to target the individuals driving gun violence. More recently, the Department of Forensic Biology was awarded $2.5 million to establish the nation's first DNA gun crimes unit of its kind. The unit focused heavily on streamlining the workflow to establish a turnaround time (measured from receipt of evidence to distribution of the report) of under 30 days. Dedicated staff whose sole responsibility would be to process gun crime evidence were assigned to the unit as well as the hiring of 24 new staff. Additional upgrades were made to instrumentation, including more automation for DNA extractions and purifications. The workflow of these cases was adjusted to interpret and apply statistical analysis of complex mixtures only when a comparison sample was received. Other areas of the workflow were also adjusted to increase efficiency to meet this initiative.

This presentation will highlight the different modifications to workflow and challenges that had to be overcome to meet the needs of the criminal justice system in New York City. The presentation will also showcase how the unit was able to reduce the turnaround time processing gun cases from 60 days to 30 days in under a year without lowering quality or affecting other crime types.

Reference:
1. New York City Office of the Mayor. (2016) Mayor de Blasio and State Courts Announce "Project Fast Track" to Ensure Shooters are Quickly Apprehended and Remain off the Streets.

Forensic DNA; Touch Sample; Casework

B77   A Global Survey on Activity-Level DNA Evaluative Reporting

Devyn Pirtle*, John Jay College of Criminal Justice, Long Island City, NY; Mechthild Prinz, John Jay College of Criminal Justice, New York, NY; Fabio Oldoni, Arcadia University, Glenside, PA

Learning Overview: By attending this presentation, attendees will gain an overview about the opinions and knowledge of global forensic practitioners regarding activity-level DNA evaluative reporting.

Impact Statement: This presentation will impact the forensic science community by providing an opportunity to discuss the current status of Activity-Level DNA evaluative Reporting (ALR).

For many criminal cases, the source of who deposited the DNA is not what the prosecutor and the defense are trying to dispute. In court, the question may be how the DNA was deposited at the crime scene and in response, Activity-Level Propositions (ALPs) can be used to evaluate the "How?"[1] Although some laboratories have begun to address ALP's following guidelines, there is still a gap on the knowledge and opinions of activity-level reporting within forensic practitioners globally.[2] This study builds upon our previous study, which surveyed and gathered the opinions on ALP reporting from forensic DNA practitioners in the United States.[3]

To gather information about ALP reporting on a global perspective, a 21-question Qualtrics™ survey was sent out to 20 international forensic science organizations such as the Asian Forensic Science Network (AFSN), International Society of Forensic Genetics (ISFG), European Network of Forensic Sciences Institutes (ENFSI), and the Canadian Society of Forensic Science (CSFS) across Canada, South America, Europe, Asia, Africa, and Australia to disperse to their members via email. The survey reached thousands of members and contained open response and multiple-choice questions.

In total, there were 148 global respondents, of whom greater than 75% had over ten years of forensic scientist experience and the majority came from small labs. When asked about their understanding of ALR, over 80% of the respondents had basic knowledge about ALR and how it can be applied within the legal system. Another question asked who should provide input on possible activities leading to certain DNA results, and 56% of respondents answered that forensic expert witnesses should be providing input, 19% thought this should be the judge, 15% the prosecution, 9% the defense. Moreover, 88% of participants agree that having a formalized objective approach would help testimonies in court. Participants mentioned many obstacles to ALR implementation (e.g., lack of training and resources for the courts, or lack of pertinent case and scientific information), but 48% believe that their countries would be moderately to very likely to legally accept ALR. Twenty-eight percent considered this moderately to very unlikely. When asked about what is needed to introduce ALR in a crime laboratory, the three main points were: (1) training of laboratory scientists and the courts, (2) more research on realistic activity scenarios, and (3) established protocols with standardized criteria.

The survey clearly points to the need for a formalized and universal approach to ALR. Future research should also focus on how to properly educate and effectively communicate the information required for DNA ALP reporting between forensic laboratories and the legal system.

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3. Yang YJ, Prinz M, McKiernan H, Oldoni F. American forensic DNA practitioners' opinion on activity level evaluative reporting. *J Forensic Sci*. 2022;67(4):1357-1369.

Forensic DNA Analysis; Expert Testimony; Activity-Level Reporting

B78 An Investigation of Handling Time on the Deposition and Persistence of Trace DNA in Pickpocket Scenarios

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Learning Overview: After attending this presentation, attendees will have a better understanding of how multiple individuals that handled/wore the same object for different durations of time impact the relative percent DNA contribution to the overall Short Tandem Repeat (STR) profile.

Impact Statement: This presentation will impact the forensic science community by investigating the deposition and persistence of object users' DNA on items commonly encountered in pickpocketing crimes. This presentation will provide insight to aid investigators in determining the potential of detecting an individual's DNA profile when trace DNA is collected from evidentiary items with different substrate types that may have been handled by multiple individuals.

Trace DNA, also known as "touch" DNA, is a crucial piece of evidence found at crime scenes and is latent DNA traces that are found on an object or individual through direct or indirect contact.[1] Trace DNA samples are challenging due to the low amount of DNA present in the sample and the presence of multiple donors. Evidentiary items are collected from the scene of a pickpocketing crime and are submitted to crime laboratories for DNA testing, where Short Tandem Repeat (STR) profiles are uploaded to the Combined DNA Index System (CODIS) database to potentially match an individual's profile. This study was designed to simulate pickpocket crimes and investigate the relative contribution of DNA recovered from objects handled by a primary and secondary user for varying time durations.

A total of three simulations were designed, and nine pairs of participants (4 males and 11 females) were recruited to act as the primary or secondary handler of the objects with different substrate types. The objects used in each of the three simulations included a pair of sunglasses (plastic), a credit card (plastic), a money clip (metal), and a wallet (synthetic leather). In each of the three simulations, the primary user handled/wore the object for 40 minutes each day for four consecutive days. The second user handled/wore the object within 24 hours after the primary handler for 1, 3, and 20 minute(s) in the three separate simulations. A reference buccal sample was collected from all participants and lab personnel. Trace samples were collected using the double swab method, extracted using the QIAshredder®/QIAamp®, quantified using the Quantifiler® Trio Quantification Kit™ on a QuantStudio Real-Time PCR system, and amplified using the GlobalFiler® Amplification Kit. The STR fragments were separated using the SeqStudio™ Genetic Analyzer and the STR profiles were analyzed using GeneMapper® ID-X Software. Data analysis was performed to determine the relative percent DNA contribution of the primary handler, secondary handler, and extraneous DNA to the overall STR profile.

Overall, 72 trace DNA samples collected from the sunglasses, wallet, money clip, and credit card from the three simulations were analyzed. The major contributor in the mixed STR profiles in the first simulation was the primary user with an average of 67% relative percent DNA contribution for both the sunglasses and wallet and the secondary user with an average of 55% and 50% for the credit cards and money clips, respectively. The major contributor in the mixed STR profiles for the second simulation was the secondary handler with an average of 49%, 51%, 68%, and 74% relative percent DNA contribution for the wallet, sunglasses, money clip, and credit card, respectively. In the third simulation, the major contributor in the STR profiles for all objects was the primary handler with an average of 53% relative percent contribution for the wallet and the secondary handler with an average of 71%, 81%, and 89% for the sunglasses, money clip, and credit card, respectively. Extraneous DNA was detected on all trace samples analyzed with an average relative percent contribution ranging from 2–8% for all objects.

In conclusion, the relative percent DNA contribution of the secondary handler increases as the handling time increases, as reported in previous studies.[1,2] It was also found that substrate type of the object handled by two individuals affects the DNA persistence on an object. The major contributor for porous substrates tends to be the primary user, and the major contributor for non-porous substrates tends to be the secondary user.

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Trace DNA; Mock Pickpocket Scenario; STR Analysis

B79   An Evaluation of Transfer, Persistence, and Recovery of Touch DNA From Mobile Phones

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Learning Overview: This presentation will demonstrate empirically generated data regarding DNA transfer and persistence involving mobile phones. After attending this presentation, attendees will understand how information on DNA quality and quantity as a result of specific activities can be used to provide statistical inferences for propositions in forensic casework.

Impact Statement: This presentation will impact the forensic science community by demonstrating the expansion of available literature in Transfer, Persistence, Prevalence, and Recovery (TPPR) of DNA for the purposes of commenting on activity level likelihood. Further, we seek to aid understanding of forensic biologists' capacity to comment on activity level propositions using published data.

Activity level evaluative reporting has become increasingly prevalent in scientific literature. While subsource level evaluation remains common, referring to propositions regarding who did or did not contribute to a DNA sample, assessment of propositions of how the DNA sample was deposited are being requested of forensic biologists.[1] Namely, researchers are now striving to expand the limited body of literature regarding activities for the purpose of making inferences regarding likelihood of propositions.[2] Studies involving TPPR of DNA in defined scenarios using specific items are essential to accurately assigning likelihood to the relevant hypotheses.[3] Our study presents an initial evaluation of quantifying DNA on mobile phones in scenarios of primary and secondary transfer. Herein, we define primary transfer as the direct transfer of DNA from one individual to a surface, whereas secondary transfer involves an intermediate medium between the individual and final sampled surface. Mobile phones, while commonly involved in crimes such as robbery and assault, have not been extensively studied in the scientific literature regarding DNA transfer and persistence. This study measured TPPR using participants (n=7) who handled either another participant's mobile phone or their own mobile phone following a handshake with another participant.

Using the resultant DNA profiles of 41 transfer and persistence mobile phone swabs, we assigned a minimum number of contributors to each profile and performed a comparison to reference genotypes from study participants. Trends regarding DNA deposition comparisons between primary and secondary transfer were consistent with expectations. In greater than 80% of samples, sufficient DNA was deposited from the study participants to yield a partial or complete DNA profile. Further, approximately 93% of sample profiles yielded at least one allele that could not be attributed to any study participant. The presence of non-participant DNA, or foreign DNA, was expected due to touch samples often containing DNA from multiple sources.[4] Obligate alleles were observed in multiple transfer scenarios in which the major contributor was not the mobile phone owner. Empirical study results were demonstrated to have value in modeling likelihood determinations using Bayesian statistics. Our study supports the potential for mobile phone swabbing to comment on activity level propositions involving mobile phone contact.

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Forensic DNA; Score Likelihood Ratios; Statistics

B80   The Design of a Protocol for Detecting Trace DNA From Skin-to-Skin Contact in a Mock Physical Assault Scenario

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Learning Overview: After attending this presentation, attendees will have a better understanding of how the detection and persistence of DNA deposited on human skin changes over time.

Impact Statement: This presentation will impact the forensic science community by exploring the persistence of DNA on human skin in mock physical assault scenarios. This presentation will expand our current knowledge on the detection of an offender's DNA on a victim's skin.

Trace DNA can be left behind at a crime scene due to a person coming into direct or indirect contact with another person or an object.[1] The detection of trace DNA from an offender on the victim's skin can provide forensically relevant information, which can be used to support the proposition that places the offender at the scene in the investigations of physical or sexual assaults. Although there is growing knowledge on trace DNA deposited on objects, there is a paucity of data on how long DNA evidence can persist on human skin.[2] This study was designed to investigate the persistence of trace DNA on the skin of individuals simulating a physical assault scenario.

Simulations of physical assaults were conducted with a male participant grasping the wrists of a female participant. Fourteen pairings of volunteers (12 females and 12 males) were recruited in which the male "offender" firmly grasped both wrists of the female "victim" for either 1 minute or 3 minutes. Both wrists of the "victim" and the hand palms of the "offender" were sampled after 1 hour, 2 hours, or 4 hours in distinct simulations. Every pair of participants was involved in every simulation combination for a total of 196 samples from the palms and 196 from the wrists. Reference samples from all volunteers were collected from buccal swabs and extracted using Chelex® resin. Trace samples were collected using a double swab technique with water as the wetting medium, extracted using the QIAshredder®/QIAamp®, quantified using the Quantifiler®Trio Quantification kit on a QuantStudio™ Real-Time PCR system. Trace and reference samples were amplified using the GlobalFiler® Amplification kit. The STR fragments were then separated using the SeqStudio™ Genetic Analyzerand STR profiles analyzed with the GeneMapper® ID-X software.

Overall, preliminary results on an initial set of 62 trace samples showed that the quantification values of samples collected from the victim's wrists ranged between 0.002ng/μl to and 0.011ng/μl, and those collected on the offender's palms ranged between 0.007 and 0.289ng/μl. A mixed STR profile was observed in all trace samples analyzed. A general decrease in the proportion of offender's DNA in relation to the victim's DNA was observed over time from trace samples recovered from the victim's wrists, which is in-line with a previous work.[3] Moreover, a decrease in the proportion of victim's DNA recovered from the offender's palms was observed over time in addition to the detection of a greater percentage of extraneous DNA that did not belong to either of the individual pairs.

In conclusion, a comprehensive investigation of the full set of trace samples is in progress to reveal the persistence and two-way transfer of DNA from the mock offender to mock victim's wrists and from the mock victim to mock offender's hand palms.

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Forensic Science; Touch Sample; STR Profiles

B81   Direct PCR Amplification for Forensic DNA Profiling From Adhesive Tape-Lift Samples

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Learning Overview: After attending this presentation, attendees will have learned a new way of performing direct Polymerase Chain Reaction (PCR) amplification from adhesive tape-lift samples.

Impact Statement: This presentation will impact the forensic science community by discussing how this alternative method of directly processing adhesive tape-lift samples for DNA will not only shorten work processes but also provide better DNA profiles for low-level DNA.

Tape-lifting is a collection method where the sticky side of an adhesive tape is repeatedly pasted onto and peeled from an item, thereby sequestering any biomaterials from the item onto the tape. This alternative to the conventional swabbing method in biomaterial collection for forensic DNA profiling is recommended for sampling fabrics and ammunition cartridge cases.[1,2] Traditionally, the tape-lift sample is processed through the standard multi-step DNA profiling method: DNA extraction, purification, and quantification, followed by PCR and capillary electrophoresis of the PCR products. As DNA loss in each step is inevitable, the negative impact of loss is compounded for low levels of starting biomaterial.[3]

It is hypothesized that direct PCR amplification of tape-lift samples bypassing the DNA extraction, purification, and quantification steps could potentially reduce DNA loss, profiling time, and physical footprints. As such, our laboratory set out to devise a simple method to perform direct PCR amplification of tape-lift samples. A small manual punch tool was used to excise discs from the tape-lift that were small enough to fit into a PCR tube. PCR products obtained using the GlobalFiler® PCR amplification kit were subsequently electrophoresed in a 3500xL genetic analyzer.

Two commercial adhesive tapes from different suppliers and different disc diameters were evaluated in this study. The adhesive tape from Lovell Surgical and a disc diameter of 6mm were found to be optimal, as complete DNA profiles from blood stains were obtained. These optimized conditions were adopted for the comparative evaluation of direct PCR amplification and the standard method of profiling low starting biomaterial samples (50nL of dried human blood). Compared to the standard method, the direct PCR method achieved a consistently significant ($p<0.05$) higher number of complete loci and yielded three to six times higher allelic peak heights. Better DNA profile quality from the direct PCR method would translate into greater potential for database hits and reference matches.

This proof-of-concept study demonstrated that forensic samples with low-starting biomaterials, such as fabrics or porous materials, will reap the greatest benefit from employing tape-lift collection combined with the direct PCR amplification method. Moreover, the adhesive tape-lift remaining after punching out the discs can be conveniently kept for re-examination or further analysis in jurisdictions that have this requirement.

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Low DNA; Direct PCR Amplification; Tape

B82   The Success Rate of Touch DNA

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Learning Overview: After attending this presentation, attendees will have learned which types of evidence offer the best and worst success rates of touch DNA using data collected from evidence analyzed in the four Indiana State Police regional labs. This information could be useful in updating guidelines for which items are accepted for DNA analysis in other labs.

Impact Statement: This presentation will impact the forensic science community by providing information regarding what types of evidence develop the highest success rates for obtaining a useful DNA profile. This can be used to update guidelines for what items of evidence undergo DNA analysis.

Law enforcement agencies typically submit items of evidence that have been touched by an individual, but do not have body fluids present. At the Indiana State Police Laboratory, over half of the current case work that the lab receives is property crime, which may only have touch DNA evidence present. These items may require time and resources that could result in limited to no valuable information for the case.

To determine the success rates of items of evidence, a total of 2,070 cases from the Indiana State Police Laboratory were collected and analyzed. The information collected from these cases included the offense type, each item of evidence, the evidence category, how many swabs were made of the item, the amount of DNA present, and the conclusions drawn from the DNA profile if one was developed. A positive result was considered if a deconvolution was run, and the number of contributors was determined. A negative result was considered if there was not enough DNA present to continue analysis (less than 0.01ng/µL) or an inconclusive result was drawn. Inconclusive results occurred if there were more than four contributors, an uncertain number of contributors, inconsistent quantification results, or results not qualifying for calculation. Items with more than 20 data points (swabs made) were quantified based on positive and negative results to determine the success rate. Forty-nine items and item categories had over 20 data points. Containers had the highest success rate (72.73%). Matches were the only item with a 0% success rate, but as all of the data points came from one case, this information is not significant enough to draw any conclusion. The items that were at or under 10% included wrenches (7.14%), windows (8.85%), locks and deadbolts (9.43%), safes and cash registers (10%), and smudges (10%).

By observing the success rates of evidence, laboratories may be able to update their guidelines as to which types of evidence are accepted for DNA analysis.

Touch DNA; Casework; Evidence Evaluation

B83 Total Human DNA Sampling

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Learning Overview: In this presentation, attendees will gain insights into an innovative and efficient DNA sampling technique that focuses on human skin cells found on the ground, specifically applicable to large areas.

Impact Statement: This presentation will impact the forensic science community by, for the first time, showcasing a method that presents the possibility of using sampled skin cells from a large area for forensic purposes. It has the potential to significantly enhance the identification and prosecution of criminals.

Introduced in 1986, DNA profiling is now a widely adopted forensic technique with global recognition. Until today, no efficient sampling technique existed to collect DNA from human skin cells from a large area, not to say from the floor of an entire room. However, there is enormous forensic potential in these DNA traces from the ground to provide clues as to who has been present at a particular location (i.e., at the crime scene). Humans shed several millions of skin cells per day, everywhere they stand, sit, or walk; and there is little they can do about it.

We present here a method by which we can make use of all those lost skin cells. We use a vacuum cleaner equipped with a specialized filter cartridge to sample the ground. The complexity of the dust mixture is significantly reduced by fragmentation of the filter membrane and subsequent parallel processing of the resulting fragments, using a modified Chelex® 100 extraction protocol. Our method is simple and fast and comes with reasonable costs. Overall, at least around 40% of the DNA profiles generated from the various tested surfaces fulfilled the submission criteria for single major contributor profiles to the Swiss DNA database. As demonstrated through a mock crime scene scenario set up in a private apartment, the perpetrator's DNA could be found on the floor even after a very short stay in the room of less than one minute. Testing the method in real forensic casework, already the first application of the method led to relevant case information for the police. Given its large investigative potential, we recommend Total Human DNA Sampling as a helpful complementary forensic tool to traditional DNA trace collection in all major crimes. Adopting this innovative approach has the potential to further improve crime scene investigations and significantly assist law enforcement in solving crimes.

DNA Profiling; Total Human DNA Sampling; Skin Cells

B84   A Combined Method to Locate and Improve DNA Recovery From Fabrics

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Learning Overview: This presentation will utilize a multi-method approach to demonstrate the possible alternatives to locating and collecting touch DNA from fabrics at higher concentrations than current collection methods. After attending this presentation, attendees will better understand the importance of surface type in regard to DNA collection methods, vacuum metal deposition's effects on touch DNA samples, and the M-Vac® wet vacuum's capability in obtaining DNA from different types of fabrics.

Impact Statement: This presentation will impact the forensic science community by showing that the use of Vacuum Metal Deposition (VMD) and the M-Vac® wet vacuum can be a possible combined collection method for touch DNA retained on a fabric's surface. DNA and latent prints evidence are known to overlap in cases; however, it is hard to know which takes precedence over the other at times. The acknowledgment and development of collection procedures for touch DNA found in latent prints on fabrics could be essential to the prosecution of a case.

The first step to collecting touch DNA is determining the location to target as touch DNA on surfaces is not readily identifiable.[1] Utilizing the VMD's ability to produce a visual negative print on an evidence item, analysts can determine the location of possible DNA present. VMD is a technique used to detect latent fingerprints of non-porous surfaces.[2] Once the touch DNA is located on the substrate, analysts must determine the best collection method to obtain the highest concentration of DNA available. Touch DNA samples do not contain large amounts of biological material and must be carefully collected to prevent the risk of degradation or contamination.[3] Currently, analysts employ swabbing techniques to collect touch DNA from clothing from crime scenes. The M-Vac® wet vacuum system could be a possible alternative to current collection methods. The Microbial wet-vacuum instrument (M-Vac® Systems Inc.) was developed for microbial sampling of large surfaces, using a chemical-based buffer for collection and stabilization.[4] The stabilization of the DNA sample allows for the creation of a more complete DNA profile. Nine fabrics with duplicates, 18 in total, are collected and cut into hand-sized squares for processing. Dark-colored fabrics are processed with 0.002g of silver and 1.0g of zinc, and the light-colored fabric samples are processed with 0.002g of gold and 1.0g of zinc. The samples in this study compare silica-membrane based (QIAmp®) and paramagnetic silica bead resin-based (DNA IQ™) extractions to determine the most efficient method to receive higher DNA yields. Each sample fabric type underwent each step, extraction to genetic analysis, in triplicates for statistical analysis. Samples also undergo size exclusion chromatography (Micro Bio-spin® column with Bio-Gel® P-30) to remove any metal ions that might be present that could inhibit the Polymerase Chain Reaction (PCR) process. All samples are quantified using Quantifiler® DNA Quantification kit and amplified using the PowerPlex® Fusion amplification kit. Preliminary results indicate that the M-Vac® wet vacuum has a higher average concentration of DNA than the wet swab method for DN AIQ™ extraction samples. However, QIAmp® extractions indicated that wet swab method samples contained a higher concentration of DNA compared to the M-Vac® wet vacuum samples. The combined method to locate and improve touch DNA recovery on fabrics could be an essential tool in the forensic science field, especially in regard to assault cases.

Touch DNA analysis has become an integral part of the forensic science community when other biological evidence may not be available and serves as a tool for investigators. Surfaces retain touch DNA samples differently, and it is imperative that investigators consider the appropriate collection method for the types of surfaces they encounter. The purpose of this research project is to identify touch DNA on different clothing surfaces and improve DNA recovery and efficiency. The results obtained will direct the proper downstream processing to obtain the best quantity of DNA from items of evidence.

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Touch DNA; M-Vac®; VMD

B85   The Identification of MicroRNAs for Forensic Biofluid Detection Using a MicroRNA Expression Profiling Method

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Learning Overview: By attending this presentation, attendees will learn about differentially expressed microRNAs that can potentially be used to facilitate forensic biofluid detection.

Impact Statement: This presentation will impact the forensic science community by identifying additional microRNAs that show differential expression among forensically relevant biofluids to the current repertoire of microRNA markers.

Biofluid identification plays a pivotal role in forensic case investigation, as it assists in crime scene reconstruction and provides guidance for subsequent analysis. MicroRNAs are short, non-coding sequences that regulate gene expression.[1] MicroRNAs exhibit high stability during long-term storage and against environmental degradation due to their small sizes.[2] In addition, microRNAs demonstrate specific expression patterns in different biofluids and tissues, such as peripheral blood and semen.[2] The biofluid specificity and exceptional stability of microRNAs render them an optimal choice for biofluid identification assays. In this research, a next generation sequencing-based microRNA expression profiling method was used to determine potential microRNA markers for forensically relevant biofluids.

Human peripheral blood, menstrual blood, vaginal fluid, saliva, and semen were collected from various donors and analyzed with HTG EdgeSeq™ microRNA Whole Transcriptome Assay. This assay couples a proprietary quantitative nuclease protection assay and next generation sequencing for microRNA expression profiling. The sequencing data was investigated using computer software for principal component and differential expression analyses. A total of 2,083 human microRNAs were assessed to pinpoint those that offer the clearest differentiation among the biofluid types examined.

Principal component analysis was conducted using experimental data that showed distinctive microRNA expression profiles for different biofluid types. Following differential expression analysis, the top differentially expressed microRNAs based on p-values were assessed for observable trends in their fold changes. Several differentially expressed microRNAs aligned with some in the existing literature, including miR-451a and 486-5p for both kinds of blood, miR-185-5p for peripheral blood, and miR-888-5p and 891a-5p for semen. Previously unreported microRNAs with biofluid-specific expression patterns were also identified, such as miR-4306 for peripheral blood, miR-147a for vaginal fluid, and miR-184 for saliva. MicroRNAs such as miR-891a-3p and 891b demonstrated significant up-regulation in normal semen compared to azoospermic semen, suggesting that these microRNA markers are more closely associated with sperm cells rather than the fluid components of semen.

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MiRNA; Body Fluid Analysis; Next Generation Sequencing

B86   A Microfluidic Approach Toward Automated Forensic Differential Extraction of DNA-Based Sexual Assault Evidence

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Learning Overview: After attending this presentation, attendees will have been introduced to a microfluidic platform that achieves enclosed fluid fractionation and automation of the forensic Differential Extraction (DE) process for analysis of sexual assault evidence.

Impact Statement: This presentation will impact the forensic science community by proposing the advancement of novel technology that stands to streamline the analysis of DNA-based sexual assault evidence.

The unique nature of biological evidence collected from victims of sexual assault and rape contributes to an increasing need for forensic DNA services due to slow turnaround times and subsequent backlogs of evidence.[1] To ensure the judicious prosecution of sexual predators from the population, it is crucial that the processing of sexual assault evidence occurs in an effective but timely manner. The conventional and most widely utilized sample preparation technique for the processing of sexual assault evidence relies on the separation of the critical Sperm (SF) and Non-SSperm (NSF) Fractions prior to downstream genetic analysis.[2] This traditional DE process is manually intensive, prone to cross-contamination and DNA loss due to sequential washing steps, and occasionally fails to generate adequate sperm cell DNA recovery for proper perpetrator identification. To address the limitations associated with standard DE techniques, we propose an enzymatic, enclosed microfluidic approach that retains the substrate cutting throughout the extraction workflow and therefore reduces the risk of contamination and genetic material loss.

Among the many advantages of microfluidic systems is the ability to improve throughput of processes and, in turn, decrease turnaround times.[3] We report a microfluidic approach to differential cell lysis that emulates the workflow steps associated with traditional DE: pipetting, mixing, centrifuging, and fractionating; is amenable to automation and provides timed-release of on-board reagents as well as temperature control for sequential enzymatic unit operations. Furthermore, we describe a fully enzymatic extraction method that precludes the use of hazardous reducing agents and other known Polymerase Chain Reaction (PCR) inhibitors associated with conventional DE. Rapid prototyping and testing of the proposed microdevice is afforded by leveraging common office equipment and inexpensive plastic materials for device fabrication, and fluidic control needed for successful fractionation incorporates laser-based valving methods for channel opening and closure.[4,5]

We demonstrate substantial progress toward the development of a self-contained, rotationally driven platform for forensic DE. Compatibility of the microdevice with an entirely enzymatic extraction process will be demonstrated. Eluates recovered from on-disc extraction will be shown to be amplification-ready and compatible with distinct downstream analysis modalities, such as quantitative PCR (qPCR). In addition, our approach permits substrate retention within the microdevice, enabling lysis of sperm cells directly from the cutting, thus, ultimately, improving the DNA yield from the alleged offender contribution. Advancement of the proposed microfluidic approach stands to transform the analysis of DNA-based sexual assault evidence in the criminal justice system, thus addressing the current societal crisis of backlogs and helping victims get justice.

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Extraction; DNA; Automation

B87   A Comparative Analysis of Swabbing Solutions for Sexual Assault Evidence: The Direct Impact on Spermatozoa and DNA Recovery

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Learning Overview: This presentation compares common swab-wetting agents used to swab sexual assault evidentiary items, including acetone, due to its active use in casework. After attending this presentation, attendees will understand how different wetting agents may impact spermatozoa recovery directly and the quality of the DNA recovered when swabbing sexual assault evidence.

Impact Statement: This presentation will impact the forensic science community by exploring how different wetting agents may affect the integrity of the DNA recovered when swabbing sexual assault evidentiary items. These results will shed light on the best option to be used in a casework setting. Additionally, the forensic science community will gain insight into differing laboratory protocols and their connection to quality control and standardization.

Forensic laboratories often face a backlog of sexual assault cases to process due to a multitude of reasons, not limited to a lack of resources and funding or the lack of qualified analysts to process cases at the rate that crime occurs.[1] Therefore, research on the most effective laboratory techniques can be used to develop uniform protocols between laboratories to help address the backlog issue and identify the scientific gaps observed in practice.

Recovering optimal DNA from sexual assault evidentiary items has proven to be challenging due to different factors such as the time of collection after the assault. It has been found that biological evidence can successfully be recovered within 24-96 hours after the crime occurred.[2] Additionally, several variables exist in recovery due to location, type, and condition of sampling and can have a significant impact on recovery. Thus, validated protocols are crucial to ensuring the development of a successful Short Tandem Repeat (STR) profile and often start with the swabbing procedure used when collecting a stain. However, laboratories may not follow identical standard operating procedures to one another, resulting in minor variations during evidence processing that can potentially result in dramatic differences in DNA yields, differential extraction success, and STR profile success.

This was observed in a state lab when protocols were developed allowing the use of acetone to swab sexual assault evidence. The use of acetone originated due to their case-routing protocols in multi-section cases involving latent fingerprints. These cases are first routed to the Fingerprint section to undergo superglue-fuming prior to DNA processing. This process is performed to develop prints on non-porous substrates for examination before sending them to the DNA section. In the DNA section, acetone is then used to melt the superglue layer in order to collect touch DNA from the print. While not necessary for sexual assault and porous surfaces, the laboratory included swabbing with acetone as an option, and thus it is routinely used in sexual assault casework. Consequently, the goal of this project was to compare different wetting agents, including acetone, when swabbing sexual assault evidentiary items. By exploring this methodology, insight can be gained into the different wetting agents' interaction with the protective plasma membrane of spermatozoa to address potential recovery differences as well as the determination of possible DNA degradation.

Acetone, SDS, TX-100, and water-moistened swabs were used to recover 1:5 dilutions of semen deposited onto cotton underpants. Sperm recovery, DNA yield, and integrity were compared to both cuttings of the same volumes of a sample as well as the liquid control. The same study was repeated using a 1:1 vaginal:semen mixture to mimic a mixture of DNA profiles from the male and female contributors.

In both sets of experiments, taking cuttings of the underpants produced the highest number of spermatozoa present. The Kruskal-Wallis Analysis of Variance (ANOVA) test was performed to determine if there was a significant difference between treatment groups and found the distribution of cell yield across treatments was not the same. However, the only significant difference between treatments was observed when comparing TX-100 and acetone to cuttings. While no samples demonstrated signs of inhibition, when assessing the average DNA yields, cuttings outperformed each of the wetting agents with the highest DNA yields. With these results, conclusions can be made that analysts should choose to take cuttings of sexual assault evidentiary items when the evidence permits; additionally, acetone does not seem to interfere with sperm integrity or recovery when compared to other swabbing methods.

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Forensic Science; Sexual Assault; Swabbing

B88   **The Discovery of Additional Male-Specific Protein Markers in Semen: Potential Applications for the Processing of Sexual Assault Evidence**

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Learning Overview: After attending this presentation, attendees will have learned more about the composition of semen in Sexual Assault Evidence (SAE) and the discovery and validation of new SAE markers. Attendees will also learn about challenges in current SAE processing methods, and how these can be mitigated using new approaches.

Impact Statement: The detection of male-specific protein markers is essential in the processing of SAE. Investigators need to prioritize the testing of elements from SAE. They also need to reconstruct the context of the sexual assault and corroborate differing accounts. Most detection occurs through immunological lateral flow devices. These are affordable and easy to use, but they are not sensitive and are prone to false positive detections. There is a need for highly sensitive and specific testing that meets the standard of confirmatory evidence. This presentation will impact the forensic science community by introducing new approaches for the processing and detection of semen in SAE.

This project discovered additional semen-specific proteins that can enhance the specificity and sensitivity of body fluid identification in SAE. Three vaginal fluids and two semen preparations were purchased and applied to cotton swabs. These were then proteomically processed using liquid chromatography and shotgun mass spectrometry. The resulting spectra were matched to protein sequences using peptide spectra matching software. Proteins were measured semi-quantitatively and were compared between the vaginal fluid and semen groups. Proteins that were at least 64-fold more abundant in semen were identified and ranked in order of abundance.

The experiment detected and measured the abundance in 1,366 proteins. Of these, 868 were differently expressed proteins in vaginal fluid and semen. A statistical comparison of the two body fluids identified 59 proteins in semen that: (1) were at least 64-fold more abundant compared to vaginal fluid, and (2) passed the Benjamini-Hochberg correction for False Discovery Rate (FDR=1%). The proteins were then ranked in order of abundance in semen tissue and ranged over five orders of magnitude. The four most abundant are well known and have been the basis of semen detection assays: semelogelin-2, semenogelin-1, prostate-specific antigen, and prostatic acid phosphatase. An additional 55 proteins were detected as being male-specific. These proteins can form the basis of new and more specific assays for both body fluid detection and human identification. Interestingly, 15 proteins were also detected in vaginal fluid, including 7 of the most abundant semen-specific proteins. The average difference in abundance was 2.2×10^3. This indicates that these proteins are stable over the post-coital interval and that investigators need to be quantitative in order to establish activity levels.

New semen (male)-specific markers were identified using current shotgun proteomic methods. These can be the basis of new methods using new markers in SAE processing and development of accessible and confirmatory assays. These also demonstrate the need for quantitative approaches to establish activity levels in SAE processing.

Bodily Fluid; Proteomics; Sexual Crimes

B89   A Portable Microfluidic System for Rapid Serological Analysis of Forensically Relevant Body Fluids

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Learning Overview: After attending this presentation, attendees will have been introduced to a rotationally driven microdevice that integrates messenger RNA (mRNA) extraction, multiplexed Reverse Transcription Polymerase Chain Reaction (RT-PCR) amplification, electrophoretic separation, and fluorescence detection for rapid, field-forward Body Fluid Identification (BFID).

Impact Statement: This presentation will impact the forensic science community by introducing attendees to a rotationally driven microdevice that integrates mRNA extraction, multiplexed RT-PCR amplification, electrophoretic separation, and fluorescence detection for rapid, field-forward BFID.

BFID is a critical component of forensic science as it contextualizes a crime scene and is the central caveat for telling a narrative.[1] Standard identification techniques, while rapid and simple, are presumptive and can require days of laborious microscopic analysis or chemical, enzymatic, or immunological assays, many of which are limited in specificity and sensitivity, particularly when applied to mixed samples.[2] Further, samples collected from crime scenes are often limited in volume, making serological analysis, which consumes the scarce sample, incompatible with DNA profiling. Conversely, transcriptomics, or RNA analysis, is a promising alternative to conventional BFID as it does not compromise the compatibility of DNA for profiling.[3] Unfortunately, existing transcriptomic methods, while sensitive and specific, require time-consuming and labor-intensive steps that require a skilled analyst and cumbersome instrumentation in a centralized laboratory. These limitations can be overcome on a microfluidic platform that integrates multiple sample processes and leverages the physics associated with the microscale to perform standard reactions in a fraction of the time.[4]

Microfluidic systems offer many advantages over conventional benchtop instruments, including decreased reagent and sample requirements, rapid analysis times, an enclosed format that minimizes the risk of contamination, and the potential for automation which reduces required user intervention.[4] We describe a microfluidic system that integrates multiple steps of the conventional transcriptomic analysis workflow to rapidly perform BFID from mRNA targets.[3]

This system includes a microfluidic disc that integrates co-extraction of RNA and DNA, rapid RT-PCR amplification, electrophoretic separation, and fluorescence detection all on one portable *sample in-answer out* system. A validated panel of mRNA markers are used for the identification of saliva, menstrual blood, venous blood, sperm, seminal fluid, and vaginal fluid; these markers were selected based on sensitivity and specificity of the targets.[3] Progress has been made toward the validation of these processes at the microfluidic level, with enzymatic co-extraction, multiplexed RT-PCR, and electrophoresis completed in 15, 30, and 10 minutes, respectively, with successful identification of tissue specific allelic markers. All assays are carried out on a single microfluidic disc that is operated by a fully enclosed instrument that minimizes manual intervention. Ongoing efforts are focused on developing a single multiplex primer panel to mitigate workflow time, decrease reagent cost, and further aid integration onto the microfluidic disc. This microfluidic system conserves DNA for genetic analysis, mitigates risk of contamination, and integrates multiple laborious benchtop processes into a single, portable device for rapid body fluid identification at the point of need.

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Forensic Analysis; Serology; Genetics

B90   To Test or Not to Test for Body Fluids: The Integration of Body Fluid Identification and Direct PCR in One Workflow

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Learning Overview: After attending this presentation, attendees will consider the possibility of integrating body fluid identification through immunochromatographic tests and direct Polymerase Chain Reaction (PCR) in one workflow, leading them to fully characterize the biological evidence.

Impact Statement: This presentation will impact the forensic science community by presenting for the first time a validation of a forensic protocol, applicable to a small amount of biological evidence, that allows performing both tests without losing evidence.

Frequently at crime scenes, it is possible to encounter a small amount of biological evidence, preventing the ability to perform all the analyses to fully characterize the evidence: body fluid identification, DNA extraction, quantitation, and profiling. In these situations, DNA profiling is favored with respect to body fluid identification. With the current advancements in forensic genetics, particularly direct PCR, this is also more feasible as it allows for skipping the steps of DNA extraction and quantitation to avoid losing sample. Though, in certain cases, as important as DNA profiling, is the identification of the body fluid.

The present study analyzed the possibility of integrating both body fluid identification and direct PCR in one workflow without losing sample, being able to fully characterize the evidence.

Blood samples (20ul) were deposited on a plastic surface and allowed to dry for 48 hours. Three replicates per experiment were performed. In the first set of experiments, three blood samples were swabbed with a regular cotton swab and the other three blood samples with 4N6FLOQSwabs®, both moisturized with SERATEC® extraction buffer for the detection of blood samples. The swabs were incubated for 10 minutes at room temperature in 400ul SERATEC® extraction buffer. Three drops of this extraction buffer were deposited into the sample well of the HemDirect test for the identification of blood. While the HemDirect test was still wet, a microFLOQ® swab was used to touch the sample well of the test, and another microFLOQ® swab was used to touch the result window of the test. Then, when the HemDirect test dried, the dry sample well and the dry result window were touched with microFLOQ® swabs. In the second set of experiments, three blood samples were swabbed with a regular cotton swab and the other three blood samples with 4N6FLOQSwabs®, both moisturized with SERATEC® extraction buffer. After swabbing, a microFLOQ® swab was used to touch a small surface of both swabs to perform direct PCR. Then, cotton and 4N6FLOQSwabs® were incubated in 40 ul SERATEC® extraction buffer and carried out the identification of blood with the HemDirect tests. In the third set of experiments, the microFLOQ® swab was directly applied to the blood samples, moisturized with SERATEC® extraction buffer, and eluted in different volumes of the buffer to perform the body fluid identification. The microFLOQ® swab was used to carry out direct PCR with the PowerPlex® Fusion 6C System in the three experiments. It was possible to identify blood with SERATEC® HemDirect test in the three set of experiments.

Direct PCR, applying PowerPlex® Promega Fusion 6C on the microFLOQ® swabs, was successful in experiment 2, obtaining full DNA profiles, though the quality of the profiles was better on the samples retrieved from 4N6FLOQSwabs® than cotton swabs. In experiment 3, the microFLOQ® swab eluted on 60ul of extraction buffer also provided full, good-quality STR profiles. In contrast, in experiment 1, the PCR from dry samples (sample well and result window) produced partial profiles, while wet samples did not retrieve profiles.

In conclusion, this study demonstrated for the first time the possibility of integrating both body fluid identification and direct PCR in one workflow, leading to obtaining as much information as possible from the evidence. Future research would be able to expand these results, assessing this identification in other body fluids, and additionally trying to optimize the detection of these body fluids with the microFLOQ® swabs.

Bodily Fluid; LFI; Direct PCR Amplification

B91   The Development of a Novel Multiomic Assay for Biofluid Identification

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Learning Overview: By attending this presentation, attendees will learn about a novel multiplex assay for body fluid identification that targets protein and nucleic acid biomarkers in a single benchtop instrument.

Impact Statement: This presentation will impact the forensic science community by describing a sensitive, Polymerase Chain Reaction (PCR) -based biofluid identification multiplex assay that could be used in place of single target colorimetric and lateral flow chromatography assays.

Biofluid identification in forensic case work is important for providing context, demonstrating specific elements of a crime are present and helping to inform downstream analysis. Codetta™ Bio has developed a unique instrument and assay capability that enables multiomic, multiplex assays to be executed in a single benchtop instrument with minimal user hands-on time. The assay utilizes a proprietary strategy to develop sensitive and specific multiplex assays for both protein and nucleic acid biomarkers. The Codetta™ platform integrates elements of known methods, specifically immunoassay-based molecular recognition and Polymerase Chain Reaction (PCR) amplification. Target biomarkers are captured from 50mL samples using bead-based antibody capture and the bead mixture is introduced (pipetted) into the instrument by the user. The Codetta™ platform performs immuno-PCR for protein analysis and PCR for nucleic acid targets. Protein targets are captured using a sandwich immunoassay in which the detection antibody is labeled with a proprietary nucleic acid tag. Assay read-out is based on PCR amplification of the tag molecule, increasing assay sensitivity and specificity as compared with traditional sandwich immunoassays. Nucleic acid assays, such as those for X and Y chromosome targets, are performed via PCR. The instrument incorporates a proprietary microchip and detection strategy that enables combination digital and quantitative PCR (qPCR) readout for simultaneous multiplex, multiomic analyses with high specificity and sensitivity.

As an initial proof of concept to demonstrate the utility of a multiplex assay for forensic biofluid identification, Codetta™ developed an 11-plex assay for the detection of nine protein markers and two internal PCR controls. The multiplex assay includes select biomarkers for five specimen types: blood (glycophorin A, hemoglobin), menstrual blood (D-dimer, MMP7, hemoglobin), semen (KLK3, semenogelin 2), saliva (statherin, MMP7, cystatin SA), and urine (uromodulin). Liquid samples of human blood, saliva, semen, urine, and menstrual blood were purchased from a commercial vendor or collected from volunteers in accordance with an approved Institutional Review Board protocol. Initial studies were performed using a broad range of specimen dilutions in preparation for future studies using dried samples.

Preliminary results from ten biofluid specimens showed that the Codetta™ 11-plex assay was able to successfully differentiate the fluid types, demonstrating that biofluids can be identified based on detection of protein biomarkers present in each specimen, even from dilute samples. For example, a 1:10 semen sample showed a 95-fold and a 27-fold increase in signal over a blank sample for Semenogelin 2 and KLK3, respectively; the other seven biomarkers demonstrated statistically insignificant increases over the blank. Due to the high sensitivity of the assays, low concentration biomarkers can be detected. For instance, a 1:1,000 blood sample showed a 15-fold increase in the hemoglobin biomarker over a blank sample while other biomarkers remained at similar signals to the blank sample. This approach streamlines laboratory workflow to allow scientists to obtain more information from limited samples. The Codetta™ protein assay will be expanded to include genetic markers for X and Y chromosome identification, and additional sample testing on dried stains and mixed biofluids is ongoing to further demonstrate the utility of a multiomic, multiplex assay for biofluid identification.

Bodily Fluid; Proteins; Criminalistics

B92   A Comparison of Traditional Serological Techniques and DNA Methylation for the Identification of Body Fluids

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Learning Overview: After attending this presentation, attendees will have learned about DNA methylation techniques and how this emerging technology can be utilized by the forensic science community. Attendees will also learn about pyrosequencing, a cost-effective sequencing-by-synthesis methodology.

Impact Statement: This presentation will impact the forensic science community by highlighting how using pyrosequencing-based DNA methylation for the identification of body fluids can be a strong alternative to traditional immunoassay serological tests.

Immunoassay-based serological tests are commonly used assays in laboratories to presumptively identify fluids such as semen, blood, and saliva. These tests are quick and easy to use; however, they come with their share of limitations, including specificity and the lack of an assay to test for the presence of vaginal fluid and nasal fluid[1] These disadvantages have led some crime laboratories to bypass serological testing and encourage researchers to explore new techniques.

One of the emerging technologies is DNA methylation-based assays detecting epigenetic modifications that occur within the genome at CpG sites, which are locations in the genome where a cytosine is followed directly by a guanine. These sites can undergo methylation of the cytosine based on various environmental factors that can affect gene expression. There can also be methylation at tissue-specific methylation sites that were exploited as epigenetic markers to differentiate body fluids.[2] Bisulfite conversion is performed prior to target amplification to determine which CpG sites in the genome are methylated or are unmethylated. During bisulfite conversion, methylated cytosines are protected from the conversion, will remain cytosines throughout the conversion, and are read as cytosines in the final sequence. Unmethylated cytosines will be converted into uracil, which during the sequencing will be read as thymine. This percentage of cytosine and thymine at specific CpG sites is the percent methylation.

Pyrosequencing is a sequencing-by-synthesis method that measures chemiluminescent inorganic phosphate detection.[2] Newly developed DNA methylation assays that make use of single-amplicon-based pyrosequencing can serve as alternative tools to traditional serological assays to detect semen, vaginal fluid, peripheral blood, menstrual blood, saliva, and nasal fluid.[3-5] Percent methylation of the CpG sites within these amplicons is measured and is what determines the body fluid that is present in the sample.

In this study, two sets of 58 samples that include various types of single source and mixtures of body fluids were created, including off-targets (non-human) such as monkey blood. Prepared body fluids were deposited on a variety of substrates, including denim, leather, and cotton. Additionally, some samples were exposed to humic acid at several concentrations to simulate varying levels of inhibition. All samples were blind tested with both traditional immunoassay tests and DNA methylation assays. Immunoassay tests utilized include RSID™ Blood, RSID™ Semen, RSID™ Saliva, and Seratec® PMB. The DNA methylation assays were performed via the QIAGEN® PyroMark® Q48 Autoprep benchtop instrument to sequence ZC3H12D (semen), VE8 (vaginal fluid), CG06379435, MDFI (peripheral blood), SLC26A10 (menstrual blood), FAM43A (saliva), and SOX2OT (nasal secretions).[3-5] Results from each of the serological methods were compared and the body fluids present on each sample were determined. Specificity and sensitivity were computed as part of the concordance assessment of the two methods. Using the immunoassays, vaginal fluid was falsely identified as saliva and/or blood when in a mixture. Twelve of the 58 samples (20.7%) were false positives in the immunoassays, compared to only two menstrual blood (3.45%) false positives as peripheral blood using the DNA methylation assay. Interestingly, one sample that contained primate blood was incorrectly identified as peripheral blood with the immunoassays but was correctly identified as primate by analyzing the quality of the pyrograms and confirming using the SpeID assay.[6] This study demonstrates that emerging technologies such as DNA methylation show promising results to identify a variety of body fluids that the conventional strip-based immunoassays may fail to detect.

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DNA Methylation; Serology; Immunoassays

B93   Alternative Direct-to-Amplification Cell Lysis Techniques for Forensically Relevant Non-Sperm Cells

Rhea Arya*, Fox Chase Cancer Center, Philadelphia, PA; Brittany Hudson, Fox Chase Cancer Center, Philadelphia, PA; Tracey Dawson Green, Fox Chase Cancer Center, Philadelphia, PA

Learning Overview: After attending this presentation, attendees will be informed about the following: (1) the significance of DNA testing in sexual assaults and the pervasive backlog of sexual assault evidence collection kits in the laboratories; (2) the traditional differential cell lysis process and its limitations; (3) the goal and approach toward developing an alternative differential extraction technique that would decrease the processing time, limit tube-to-tube transfers, minimize sample loss, and perform comparably, if not better, for comprehensively lysing the cells that are observed in sexual assault samples; and (4) the evaluation and comparison of different methods to identify the best non-sperm cell lysis method. This method, when combined with an already-developed alternative direct sperm cell lysis method (using 1M NaOH), would form a new differential extraction method that is quick, inexpensive, minimizes manual labor, and obviates the need for multiple purification steps.

Impact Statement: The forensic science community is looking for new ways to process sexual assault evidence more efficiently. The traditional differential extraction procedure is time-consuming, involves multiple purification steps, and is also labor-intensive, contributing significantly to the backlog of sexual assault kits. This presentation will share the results of their research on developing a new method for cell lysis and DNA liberation from forensically relevant non-sperm cells. The best method identified from the research could precede the current direct-to-amplification method employed for sperm cell lysis. This new alternative differential extraction method could replace the traditional differential extraction procedure and significantly reduce the time and labor required to process sexual assault samples. This presentation will impact the forensic science community by presenting a faster and more efficient method for processing sexual assault evidence. This method could potentially help to reduce the backlog of sexual assault kits and bring justice to more victims.

While efforts have been made to reduce the pervasive backlog of sexual assault evidence collection kits, the actual laboratory process remains very time-consuming due to the requirement of a differential lysis step before DNA purification, as well as intricate mixture analysis toward the end of the DNA workflow.[1-5] Recently, an alternative, direct-to-amplification sperm lysis method (using 1M NaOH) was identified.[6] However, a direct cell lysis method for non-sperm cells has not been identified yet. Thus, the primary objective of this work was to find an alternative method that is quick, inexpensive, and does not require multiple purification steps for the lysis of non-sperm cells in sexual assault samples. Ideally, this new non-sperm cell lysis method, when combined with 1M NaOH for sperm lysis, would potentially separate DNA fractions from sperm and non-sperm cells more efficiently than current methods and would enable an alternative in-tube differential technique that would be quick, inexpensive, and obviate the need for multiple purification steps.

In this study, vaginal swab samples were lysed with the control method, *prep*GEM®, as well as six alternative reagents: alkaline buffer with 25-200mM NaOH, high salt stain extraction buffer, modified Radioimmunoprecipitation Assay (RIPA) buffer, mammalian protein extraction reagent (M-PER™), digitonin buffer, and urea/thiourea buffer. Quantification using Quantifiler® Trio of vaginal and semen lysates revealed that the alkaline (25mM NaOH) and M-PER™ methods were efficient for the lysis of vaginal epithelial cells without substantial sperm cell lysis. Following quantification, vaginal samples were amplified with the Promega® PowerPlex® Fusion 6C System and analysis of Short Tandem Repeat (STR) profiles from vaginal lysates revealed that the M-PER™ method showed promising results across all metrics examined, including the percentage of detected STR alleles, mean peak heights, peak height ratio, and interlocus balance. Thus, this method was recommended as an alternative to traditional differential lysis method for non-sperm cells given its ability to produce amplification-ready lysates without any DNA purification step. Going forward, testing with additional donors and replicates, including mock sexual assault mixture samples, would strengthen the implications of these findings.

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Sexual Assault; Direct PCR Amplification; Non-Sperm Cells

B94   Decoding the Ocular Region From DNA: Building Predictive Models in Forensic Contexts

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Learning Overview: The goal of this presentation is to highlight the importance of developing molecular morphofacial prediction algorithms. The ocular region is essential for the appearance of an individual's face. This region is often the only one that can be used for identification as perpetrators tend to keep their faces covered, leaving only their eyes exposed. Decoding the information contained in DNA to obtain ocular biometry dimensions is a very valuable source for generating useful information to guide human identification in situations where there are no viable alternatives, as well as providing additional evidence that can reduce uncertainty in identification work.

Impact Statement: This presentation will impact the forensic scientific community by showing the breakthrough of obtaining predictive models of facial morphology from the analysis of a DNA sample. Therefore, understanding the relationship between the genome and externally visible traits could greatly contribute to the identification of potential suspects as well as missing people. In addition, this encourages forensic collaboration and the creation of a stronger cooperative consortium to address the challenges posed by genetic identification.

Genetic identification currently requires the comparison of genetic data from remains against reference profiles of indubitable individuals or stored in forensic databases. However, lack of a matching genetic profile or an individual with whom to match the DNA profile found on a trace means that some cases remain unsolved. In such circumstances, advances in Forensic DNA Phenotyping (FDP) that seeks to determine Externally Visible Characteristics (EVCs) from the DNA could assist in guiding the search of suspects in criminal cases or the identification of missing persons in civil cases.[1] Although EVCs are multigenic and multifactorial traits, as well as being influenced by environmental factors, making it difficult to identify the genes involved, different assays have been developed and validated over the past decade in which **Single Nucleotide Polymorphisms (**SNPs) associated with appearance are analyzed simultaneously. One of the most recent is the VISAGE Enhanced Tool for Appearance and Ancestry system, which includes, in addition to the HIrisPlex-S SNPs for predicting hair, iris and skin color, SNPs related to eyebrow color, presence of freckles, hair shape, male pattern baldness and biogeographic ancestry.[2-9]

When studying the major discriminating facial traits, the ocular region has been found to be one of the most striking and informative. It has been shown that this region is where we tend to look initially and more frequently than other features when considering a particular face.[10] Moreover, the ocular region is often the only one that can be used for identification as perpetrators tend to keep their faces covered, leaving only their eyes exposed.

Considering the interest of developing predictive models and that ocular region is critical for facial appearance, the possible association of the palpebral fissure length as well as the inner and outer canthal distance with 117 candidate SNPs has been studied in a sample of 413 individuals of both sexes and from the Iberian Peninsula.[11-21] Additionally, 37 SNPs of the HIrisPlex-S system have also been studied to obtain information on eye color.[3] The anthropological study was carried out using the Skeleton-ID™ software while the study of genetic variability was conducted using a combination of Fluidigm and SNaPshot technologies. Based on all data obtained, association studies using Analysis Of Variance (ANOVA), Multivariate Analysis of Variance (MANOVA), and logistic regression analysis (categorizing the measures into smaller, normal, and increased) have been performed to obtain predictive models for the ocular region.

In view of the results obtained in this work, the presence of the genotypes rs62578082 AA/CC, rs1454072 AA/AT, rs10175706 CC/CT, rs9456748 AA, and rs6129564 AA/GG has shown a significant association with the studied measures. Combining this morphological information, which takes into account both the size and distance between the eyes, with data obtained from the analysis of iris pigmentation markers, which classifies the eyes into blue, intermediate and brown, a more comprehensive predictive model can be provided.

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Facial Identification; DNA; Modeling

B95   Targeted SNP Typing for Forensic Genetic Genealogy Using Hybridization Capture

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Learning Overview: After attending this presentation, attendees will understand how hybridization capture can be utilized to generate extensive, yet targeted, Single Nucleotide Polymorphism (SNP) profiles for forensic genetic genealogy applications.

Impact Statement: This presentation will impact the forensic science community by providing a method for developing forensic genetic genealogy profiles from challenging sample types without whole genome sequencing.

An alternative to Polymerase Chain Reaction (PCR) -based targeting, hybridization capture methods use long, biotinylated oligonucleotide probes to enrich a sample for DNA sequences of interest. The use of hybridization capture for enrichment is particularly beneficial when the DNA is too degraded for PCR, as is typically the case with nuclear DNA in hair shafts. and highly compromised samples, or in instances in which environmental DNA is present in far greater abundance than the human DNA of interest.[1] In addition, hybridization capture has utility when the number of genomic targets is large and multiplex PCR would be untenable, but targeted sequencing is still desired.

To enable targeted typing of more than 1.3 million SNPs for Forensic Genetic Genealogy (FGG) applications from challenging sample types, we developed a workflow that combines a custom hybridization capture probe set with single-stranded DNA library preparation for Illumina® sequencing and a custom informatics pipeline for data analysis. In this presentation, we will detail the workflows and results obtained from high and low DNA quantity samples and from both fresh and aged rootless hairs. Testing of the probe set and analysis workflow demonstrated that an average of 15x high-quality read coverage could be obtained for approximately 95% of the target SNPs from 500pg of pristine DNA, with higher read depth and greater than 99% SNP recovery obtained from increased DNA input amounts. Though multiple library preparation methods may be suitable for high-quality DNA samples, our testing indicated that with the degraded nuclear DNA present in hairs, a higher proportion of the target SNPs could be recovered when using the SRSLY Pico Plus Kit (Claret Biosciences) as compared to two other library preparation methods that are not specifically designed for damaged and degraded DNA templates. Using this single-stranded method, more than one million target SNPs with high-quality base calls were typed from a 40-year-old cut hair, and a genealogical database matching of the SNP profile produced the expected outcome. The results presented will demonstrate the ability to use the approach to develop an FGG SNP profile without whole genome sequencing from single hairs and other challenging sample types.

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FGG; Hybridization Capture; Hair

B96   Genetic Ancestry of the Unidentified Human Remains From the East Marshall Street Well

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Learning Overview: After attending this presentation, attendees will be informed about the usefulness of biparental and uniparental ancestry predictions applied in commingled human remains contexts. By illustrating the ancestry investigations performed for the East Marshall Street Well project, it will be highlighted how ancestry investigations can help to clarify historical records.

Impact Statement: This presentation will impact the forensic science community by providing information on the methodologies, analysis, and applicability of genetic ancestry prediction to commingled human remains forensic contexts.

The East Marshall Street Well was uncovered in 1994 during building construction at the Virginia Commonwealth University in Richmond, VA. Within the well, dated to the mid-19th century, human remains from over 50 individuals were found. Archaeological analysis suggested that the human remains were discarded following their use as cadavers for training in dissection and amputation. Historical records indicate that the early decades of medical practice in Virginia heavily relied on grave robbing.

Aiming for future genetic identification of the human remains, analysis of uniparental and biparental ancestries is being performed to shed new light on the history surrounding the well. Crania and/or mandibles belonging to 23 individuals were genotyped for either 46 AIM-InDels by capillary electrophoresis or for 56 autosomal SNPs, using Primer B of ForenSeq™ DNA Signature Prep Kit on the MiSeq® FGx sequencing system. Continental ancestry proportions were calculated using STRUCTURE software, for K=3 (African, European, and Native American continental contributions). Paternal haplogroups were predicted with NevGen software using data for 24 Y-chromosomal Short Tandem Repeats (Y-STRs), for those samples typed with Primer B of ForenSeq™ DNA Signature Prep Kit. Mitochondrial DNA typing is ongoing.

Although most individuals showed predominantly African autosomal ancestry, European and Native American input was also detected for some samples. Five out of the six Y-STR profiles obtained were predicted as sub-Saharan haplogroup E1b1a, while the remaining profile was classified as European. Principle Component Analysis (PCA) performed with Y-STRs from previously published African populations, which provided joint information for Y-chromosome Single Nucleotide Polymorphisms (Y-SNPs), helped to classify two samples within branch E1b1a–L485 and another within branch E1b1a–U175.

The results so far corroborate historic records suggesting a network of medical schools engaged in grave robbing and cadaver trading that disproportionately targeted African Americans. For those samples for which it was possible to obtain a more detailed phylogeography, the Central-West African region was pinpointed as the most probable origin of the paternal lineages. This is also supported by historical records indicating the regional origin of enslaved African peoples in Virginia. Ongoing analysis of the remaining skulls in addition to mitochondrial DNA (mtDNA) genotyping will deepen our understanding of enslaved and free African Americans in 19th-century Richmond, VA.

Genetic Ancestry; Commingling; Human Remains

B97   Estimating the Return on Investment on Forensic Investigative Genetic Genealogy: Using the Golden State Killer and the University of Idaho Cases as Case Models

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Learning Overview: After attending this presentation, attendees will understand the basic principles of the Forensic Investigative Genetic Genealogy (FIGG) process and the value of investigative leads provided by FIGG across the lifecycle of a case. Six cases cleared with FIGG provide data to assess the cost savings associated with a historical case and an active case under investigation to inform a business case model that demonstrates the Return on Investment (ROI) of this technique. Case triaging strategies and recommendations for future application of Next Generation Sequencing (NGS) technology will be provided.

Impact Statement: This presentation will impact the forensic community by providing a business case model and recommendations to improve the implementation of FIGG to provide investigative leads. Forensic cases that have gone cold and current cases benefit from leveraging new information derived from NGS and genealogical research earlier in investigations. This builds upon the positive impact of objective DNA-based evidence provided by forensic science. This model will assist forensic science service providers to justify investments and implement FIGG, supported by the cost savings demonstrated by these case examples.

A presentation will demonstrate the costs and benefits of FIGG, including time to provide investigative leads, and its ROI. The most common application of FIGG technology has focused on solving cold cases, such as the Golden State Serial Killer. Investigative leads provided by FIGG supported the apprehension and conviction of Joseph James DeAngelo, which had gone unsolved for over four decades. Similarly, on November 13, 2022, FIGG was applied to the quadruple homicide at the University of Idaho in Moscow, ID, leading to the arrest of Bryan Christopher Kohberger, who currently stands trial on four counts of first-degree murder and one count of felony burglary. Data from this historic case of Joseph James DeAngelo, an estimation for the University of Idaho quadruple homicide case, and six recently completed FIGG cases will provide the foundation for a business case model. The six cases were selected on the basis of covering a range of time required to result in identification as well as covering multiple technologies utilized for **Single Nucleotide Polymorphism** (SNP) profile development: SNP array, whole genome sequencing, and targeted SNP sequencing with the ForenSeq™ Kintelligence system. Recommendations will be provided for future application of FIGG in active cases earlier in the investigative process where DNA is found at the crime scene and there is no Combined DNA Index System (CODIS) hit.

A business case using data from the multiple homicide and sexual assault cases of Joseph James DeAngelo, the University of Idaho homicides, and six current cases demonstrate an extremely large ROI, ranging in savings from $8,314.53 to $30,375.21 for every $1 spent for the model established for the eight cases examined. ROI was calculated using published peer-reviewed costs of various crime types, which includes victim costs, criminal justice system costs, lost productivity estimates for both the victim and the criminal, and estimates on the public's resulting willingness to pay to prevent future violence.[1,2] These costs of crime are in turn divided by the average expense from six FIGG cases to generate an ROI for each dollar spent on generating investigative leads that lead to solving a case. Even applied to a single case of homicide or sexual assault, the business case is compelling, saving $2,078.63 and $54.04 per $1 spent, respectively. Strategies for triaging casework based upon the results of the database searches to efficiently allocate resources, considering the ongoing growth of genealogical research databases accessible to law enforcement, will be presented. Recommendations for FIGG implementation to enable forensic scientists to apply this technology to their outstanding cases will be discussed.

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2. DeLisi, M. et al., Murder by numbers: monetary costs imposed by a sample of homicide offenders. *Journal of Forensic Psychiatry & Psychology*, 2010. 21(4): p. 501-513.

Forensic Investigative Genetic Genealogy; Joseph James DeAngelo; University of Idaho Homicides

B98   **A Comparative Evaluation of Illumina® Global Screening Array BeadChip, Whole Genome Sequencing, and Verogen™ ForenSeq™ Kintelligence to Analyze Degraded and Low Template DNA Extracted From Semen**

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Learning Overview: After attending the presentation, attendees will understand how the quantity and quality of DNA affects the analysis of high-density Single Nucleotide Polymorphism (SNP) genotypes developed by Global Screening Array (GSA) BeadChip, Whole Genome Sequence (WGS) analysis, and targeted sequence analysis. Additionally, attendees will learn how the quantity and quality of DNA affect the ability to search and match high-density SNP genotype kits curated in GEDmatch, a third-party genealogical database accessible for criminal justice investigations and identification of missing persons.

Impact Statement: This presentation will impact the forensic science community by providing forensic scientists with technical data regarding the effect of DNA quality and quantity on the accuracy and call rate of high-density SNP genotype profiles produced by the three genotyping technologies currently used for forensic genealogical applications. Additionally, the data will allow practitioners to make more informed decisions when working with limited resources in forensic investigations.

Forensic Investigative Genetic Genealogy (FIGG) is a powerful investigative technique to aid in the identification of victims and suspects of unsolved crimes or missing persons. By establishing the capabilities and limitations of the three genotyping technologies, the forensic community will be able to address the inevitable court challenges and more effectively provide best practices to provide investigative leads associated with unsolved murders, sexual assaults, and missing person cases. Previous FIGG foundational studies have utilized DNA extracted from either blood or bone; this research is based on DNA extracted from semen.

This study evaluated the three technologies currently available for developing high-density SNP genotypes from human DNA samples and compared their abilities to generate profiles from semen samples. Using both fresh collections of semen and a well characterized human reference material extract, genotyping by GSA BeadChip, WGS on NovaSeq 6000, and targeted sequencing with Verogen™ ForenSeq™ Kintelligence Kit were compared for sensitivity to low-level DNA input concentrations and specificity for artificially degraded DNA. For sensitivity comparisons, GSA v2 BeadChips and WGS were tested with inputs ranging from 50ng to 250pg, and Kintelligence was tested with inputs from 2ng to 25pg, in accordance with recommended functional ranges of the various technologies. In line with other reports, Kintelligence is demonstrating sensitivity to DNA inputs as low as 25pg while WGS and GSA analyses are demonstrating high sensitivity to inputs of at least 25 pg when using semen extracts.[1-3] For specificity, three methods of chemical degradation were employed to produce damaged semen DNA extracts: Ultraviolet (UV) irradiation, oxidative damage via Fenton reaction, and depurination. Sample degradation was confirmed using Quantifiler® Trio Degradation Index values and Short Tandem Repeat (STR) profile quality metrics. The damaged samples were genotyped with all three methods using a DNA input of ~1ng. This presentation will provide cross-technology comparisons of the resultant SNP call rates, heterozygosity, and concordance with reference genotypes for each sample donor. We will also discuss the degradation effects observed, their impacts on each sample processing workflow, and the resultant call rates and genotype concordance to know references.

Additionally, the resulting SNP genotype profiles were uploaded to GEDmatch to assess limitations in matching kits from known relatives of varying genetic distances. High density genotypes developed with GSA or WGS consistently enable detection of known relatives past 2nd cousin (5th Degree relation) with the low input sensitivity data. GEDmatch comparisons with Kintelligence do show inconsistencies in distant relationship matches as the DNA input drops below 100pg, but 5th degree relationships are identified with inputs of >100pg.

References:
1. Peck MA, Koeppel AF, Gorden EM, Bouchet JL, et al. (2023) Internal validation of the ForenSeq Kintelligence Kit for application to forensic genetic genealogy. *Forensic Genomics* 2(4):103-114. DOI:10.1089/forensic.2022.0014.
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3. Russell DA, Gorden EM, Peck MA, Neal CM, et al. (2023) Developmental validation of Illumina Infinium Assay using Global Screening Array on the iScan system for use in forensic laboratories. *Forensic Genomics* 3(1):15-24. DOI:10.1089/forensic.2022.0013.

Forensic Genetic Genealogy; GEDmatch; SNP Genotyping

B99   Advances in Canine Science and the Implementation of National Standards

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Learning Overview: This presentation will allow attendees to gather more insight into the latest research involving Volatile Organic Compound (VOC) analysis and field testing of canine targets of interest.

Impact Statement: The presentation will impact the forensic community by providing recommendations for improved training protocols and explaining why the understanding of scientific advances is critical in fostering better detection canines.

Canines are well known for their odor detection capabilities due to a highly developed olfactory system. As a result, they have found regular use within law enforcement and private agencies for the successful detection of targets of interest such as explosives, narcotics, firearms, human remains, mass storage devices, etc. Analysis of the VOCs associated with these targets has become a key component of canine research as it allows for a better understanding of the odors that canines are responding to.[1] Research has in fact shown that canines often respond to the VOCs rather than the parent material of targets.[2] In cocaine, for example, dogs primarily respond to the VOC bi-product methyl benzoate instead of the cocaine molecule itself. Similarly, with the explosive C-4, dogs recognize 2-ethyl-1hexanol, a VOC associated with plasticizers rather than RDX. Knowledge of these compounds has aided in improved training regimens, the development of safe, reliable training mimics that can closely replicate the odors of target materials, and consensus-based national standards for training and certifying canines for their respective disciplines.

This presentation will allow attendees to gather more insight into the latest research involving VOC analysis and field testing of targets. Specific examples that will be discussed, including differentiating hemp versus marijuana odors, mass storage devices versus electronic controls, and the detection of corrosion in structures. These topics will benefit the forensic community by providing recommendations for improved training protocols and explaining why the understanding of scientific advances is critical in fostering better detection canines. Additionally, the implementation of national standards in the United States through the International Commission on Detector Dogs (ICODD) will be discussed. ICODD's mission is to globally improve the performance of detector dog teams via information sharing and implementation of standards through expanded adoption of scientifically based national guidelines.

References:
1.   Harper, Ross J., José R. Almirall, and Kenneth G. Furton. Identification of dominant odor chemicals emanating from explosives for use in developing optimal training aid combinations and mimics for canine detection. *Talanta* 67.2 (2005): 313-327.
2.   Macias, Michael S., Ross J. Harper, and Kenneth G. Furton. A comparison of real versus simulated contraband VOCs for reliable detector dog training utilizing SPME-GC-MS. *American laboratory* 40.1 (2008): 16.

Canines; VOCS; ICODD

B100   Current and Future Standards Development Activities Related to the Analysis of Seized Drugs

Agnes D. Winokur*, Drug Enforcement Agency, Miami, FL

Learning Overview: After attending this presentation, attendees will have a better understanding of the current status and future path of discipline-specific standards and activities pertinent to the analysis of seized drugs. Attendees will gain awareness of what challenges and obstacles are encountered in the development of standards for seized drugs as well as the opportunities presented to overcome those challenges and help prioritize the specific areas for the standard development process.

Impact Statement: This presentation will impact the forensic science community by increasing awareness of current efforts in standard development activities pertinent to the examination of seized drugs. This presentation will also increase understanding of current and evolving training, tools, and resources that support implementation and compliance monitoring.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. The OSAC Seized Drugs Subcommittee has been drafting standards and forwarding them to the standards developing organization, ASTM International, to further develop and publish. Once published, the OSAC reviews the standard documents for inclusion in the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to the standards being developed at both the OSAC Seized Drugs Subcommittee and the American Society for Testing and Materials (ASTM) E30 Forensic Sciences Committee will be discussed. These include: (1) newest standards added or in the process of being added to the OSAC Registry; (2) documents currently at ASTM E30 for further development; (3) new and on-going documents currently at the OSAC Seized Drugs Subcommittee or ASTM E30; and (4) other highlights of activities related to standards and the analysis of seized drugs.

In particular, the status and updates for the following standard documents will be presented: Standard Practice for the Differentiation of Marijuana and Hemp in Seized Drug Analysis, Standard Guide for Court Testimony in Seized-Drug Analysis, Standard Practice for Reporting Results and Opinions in the Analysis of Seized Drugs, Standard Practice for Evidence Handling of Seized Drug Evidence, and many more standards related to training and education, validation and verification, chromatography, gas chromatography/mass spectrometry, gas chromatography/infrared spectroscopy, Fourier-transform infrared spectroscopy, colorimetric methods, spectral libraries, quantitative measurement uncertainty, assessment of structural similarity of substances, and others. The process for prioritization of specific areas for standard development will also be discussed as well as the tools to facilitate gap analysis of discipline-specific standards. Highlights of how standards progress through the ASTM process after they move from the OSAC Seized Drugs Subcommittee will also be shown, with specific emphasis on the challenges and successes encountered. The process for the revisions of standards once they are added to the OSAC Registry and how those revisions are balanced with the need for new standards is handled by the Seized Drugs Subcommittee.

Standards; Seized Drugs; Forensic Analysis

B101 Firearms and Toolmarks Standards and Best Practices: A Collaboration of the NIST OSAC and AAFS Standards Board

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Learning Overview: After attending this presentation, attendees will have a better understanding of the work of the National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees (OSAC) Subcommittee and the American Academy of Forensic Sciences (AAFS) Academy Standards Board (ASB) Consensus Body for Firearms and Toolmarks and updating the status of those discipline-specific standards.

Impact Statement: This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to firearms and toolmarks focusing on the collaborative efforts of the NIST OSAC and the AAFS ASB on those standards.

The OSAC for Forensic Science was created by the NIST to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards. The OSAC Firearms and Toolmarks Subcommittee is comprised of over 20 experts in the field from crime laboratories, academia, and government agencies. Its work includes the comparison of microscopic toolmarks on bullets, cartridge cases, and other ammunition components as well as firearm function testing, serial number restoration, muzzle-to-object distance determination, and forensic examination of tools and toolmarks.

The ASB was formed by the AAFS and is recognized as an SDO to accomplish the task of completing and publishing Standards, Best Practice Recommendations, and Technical Notes. The Firearms and Toolmarks Consensus Body (CB) was created by the ASB. The Firearms and Toolmarks Consensus Body is comprised of representatives of the various stakeholders in the firearms and toolmarks forensic discipline. It includes analysts, academics, prosecution and defense lawyers, industry representatives, and related forensic scientists. The Firearms and Toolmarks Consensus Body meets regularly and has done so over the past several years. The CB does most of its work through committees that meet on specific work proposals. Those proposals generally are originated by the Firearms OSAC, which promulgates draft standards. The Consensus Body reviews the proposed draft standards, then submits them for rigorous discussion and review by the relevant committee members and by the Consensus Body itself. The proposed standards are then circulated for public comment and often return for several rounds of discussion and revision.

During this presentation, updates related to firearms and toolmark evidence will be discussed. These include: (1) recent standards that have been added to the OSAC Registry; (2) OSAC-proposed standards; (3) published standards from the ASB) that have yet to go through the Registry approval process; (4) documents currently in development at the OSAC or by the ASB; and (5) priorities for new documents or work proposals, and other highlights.

Firearms; Standards; OSAC

B102   OSAC Is Ten Years Old—What Will the Next Decade Bring?

Mark D. Stolorow*, Associate Guest Researcher, National Institute of Standards and Technology, Gaithersburg, MD; John Paul Jones II*, National Institute of Standards and Technology, Gaithersburg, MD

Learning Overview: After attending this presentation, attendees will have learned about the Organization of Scientific Area Committees (OSAC) for Forensic Science and its impact over the past ten years. This presentation will describe OSAC's purpose and how the implementation of standards will become expected in the future. In addition to reinforcing this knowledge for established examiners, this will provide an excellent foundation to help prepare new examiners for future testimony.

Impact Statement: The United States federal government is investing resources to facilitate the development of forensic science standards for use in the criminal justice system, and there are active efforts to encourage Forensic Science Service Providers (FSSPs) to implement these standards in their operations. This presentation will impact the forensic science community by informing new forensic scientists entering the field how the forensic science standards enterprise evolved over the past ten years and how it will impact their careers for many years to come.

The OSAC for Forensic Science is celebrating its tenth anniversary in February 2024. OSAC was first introduced to the American Academy of Forensic Sciences (AAFS) at the 2014 annual conference in Atlanta, GA, that featured a special session with the OSAC developing committee from the National Institute of Standards and Technology (NIST). OSAC has achieved significant milestones in its ten-year life. We would like to share some of the salient history of OSAC and explore what the next ten years may bring.

Published in 2009, the National Research Council (NRC) Report, *Strengthening Forensic Science in the United States: A Path Forward*, criticized the practice of forensic science in America for (among other things) its failure to have in place a network of nationally recognized, consensus-based standards with scientific merit.[1] In 2014, NIST and the United States Department of Justice (DOJ) responded by creating OSAC to facilitate the development and implementation of high-quality standards for the forensic science community.

OSAC is an organization consisting of more than two dozen forensic science disciplines. There are more than 400 members and 300 affiliates in OSAC. As of August 2023, more than 150 standards have been posted on the OSAC Registry, and more than 130 FSSPs have been recognized as implementers of these standards.

OSAC continues to evolve in its organizational structure, expanding from five Scientific Area Committees (SACs) to seven and consolidating its subcommittees from 25 to 22. Over that time, OSAC recognized additional forensic science disciplines, including digital evidence and forensic nursing. OSAC introduced the Scientific and Technical Review (STR) process in 2020 to provide an independent subject matter expert and peer review to all relevant drafted standards.

OSAC has entered into a cooperative agreement with the AAFS to develop an array of tools to help FSSPs learn about and implement standards on the OSAC Registry. These tools include fact sheets, checklists, and training modules to guide practitioners in the process of standards implementation.

What will the next ten years bring to the world of forensic science standards development and implementation? OSAC has established a cohort of FSSPs who have successfully implemented standards on the OSAC Registry. Their mission will be to help other FSSPs initiate standards implementation and address current and future implementation needs.

OSAC's efforts to improve the practice of forensic science through standards depend on the collaboration and dedication of more than 800 volunteers from all 50 states. It is a testament to the success of all our stakeholders that OSAC has achieved so many valuable milestones, and we look forward to many more in the next ten years.

Reference:
1.   National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward.* Washington, DC: The National Academies Press. https://doi.org/10.17226/12589.

OSAC; Standards Development; Standards Implementation

B103   **Results From an Interlaboratory Study to Evaluate Analytical Schemes in ASTM E2329—Standard Practice for Identification of Seized Drugs**

Jeremy S. Triplett*, Kentucky State Police Central Forensic Laboratory, Frankfort, KY; Michael J. Salyards, Compass Scientific Consulting LLC, Tucson, AZ; Sandra E. Rodriguez-Cruz, Drug Enforcement Administration, Dulles, VA; Jeremiah Morris, Johnson County Sheriff's Office Criminalistics Laboratory, Olathe, KS; Megan Grabenauer, RTI International, Research Triangle Park, NC; Darryl Creel, RTI International, Research Triangle Park, NC

Learning Overview: This presentation uses data from over 1,300 analytical tests performed by 71 forensic laboratories to evaluate the performance of analytical techniques categorized in the American Society for Testing and Materials (ASTM) E2329-17. After attending this presentation, attendees will know the relative sensitivity and specificity of the included techniques and technique combinations in identifying methamphetamine or cocaine in solid dose samples.

Impact Statement: This presentation will impact the forensic science community by providing evidence upon which to base decisions about the suitability of analytical techniques for testing seized drug samples. The study's findings confirm the importance of using multiple techniques to test suspected methamphetamine seized drug samples. The results obtained in this study apply only to methamphetamine and, to a limited degree, cocaine.

The Organization of Scientific Area Committees for Forensic Science (OSAC), sponsored by the National Institute of Standards and Technology (NIST), recently joined the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) effort to vet existing standards and promote the development of additional consensus-based documentary standards for seized drugs. An important recommendation developed by SWGDRUG is the minimum standard for identifying commonly seized drugs related to the selection of analytical technique(s), which was formally developed into ASTM E2329.[1,2]

The technique classifications in ASTM E2329-17 have been well vetted, and there is little objection to the rationale behind the technique classification. However, there is a lack of empirical data demonstrating that one technique or combination of techniques is better (e.g., is more accurate or has a lower false-positive or false-negative error rate) than others.[3] There have been previous efforts to determine error rates for seized drug analysis processes, and the National Institute of Justice Technology Working Group for Seized Drugs has highlighted the need for additional error rate studies in forensic drug chemistry.[4-6] The study presented here uses known ground-truth samples to compare the accuracy of various single analytical techniques versus technique combinations for samples containing methamphetamine or cocaine.

This study involved 71 forensic seized drug laboratories performing over 1,300 tests analyzing 65 total samples; 17 were ground-truth positive (i.e., they contained methamphetamine or cocaine); 48 were ground-truth negative (i.e., they did not contain methamphetamine or cocaine). The positive samples were prepared at several target-analyte concentrations and included common cutting agents. The negative samples were designed to be challenging and prepared to contain positional isomers of methamphetamine. Participants were sent one sample set and directed to only use a single, pre-selected analytical technique. They were sent a separate second sample set and directed to use a specific analytical scheme consisting of multiple techniques in compliance with ASTM E2329-17. The results of the study showed good accuracy; sensitivity was 1.000 for all analytical schemes with 1-specificity (the false-positive rate) ranging from 0.000 to 0.250 when ASTM E2329-17-compliant analytical schemes were used. When only a single technique was used, accuracy was generally not as good; sensitivity ranged from 1.000 to 0.091, and 1-specificity ranged from 0.000 to 0.245.

References:
1. Scientific Working Group for the Analysis of Seized Drugs, *SWGDRUG Recommendations Edition 8.1* https://www.swgdrug.org/Documents/SWGDRUG%20Recommendations%20Version%208.1_FINAL_ForPosting_Rev%201-23-23.pdf, 2022 (accessed July 2023).
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Error Rate; Seized Drugs; ASTM 2329

B104   Standards Development Activities in Trace Materials

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Learning Overview: After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the examination and interpretation of trace materials in development by the Organization of Scientific Area Committees (OSAC) for Forensic Science.

Impact Statement: The presentation will impact the forensic science community by creating greater awareness of standards development activities related to trace material examination and interpretation. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

OSAC was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Development Organizations (SDOs) that ballot and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

As of July 2023, the OSAC Trace Materials Subcommittee has 16 published and 4 proposed standards on the OSAC Registry. Work is on-going for over 25 additional documents making their way through the OSAC Registry and American National Standards Institute/ American Society for Testing and Materials (ANSI/ASTM) processes as well as continued partnership with RTI on the development of the Trace Evidence Collection App.

During this presentation, updates related to standards development activities in Trace Materials will be discussed. (Standards were placed in categories based on their status projected for February 2024. Thus, the status of a given document may differ at the time of presentation.) These include:

1. Recent Standards That Have Been Added to the OSAC Registry (published)a. E2224 Standard Guide for Forensic Analysis of Fibers by IR
b. E2225 Standard Guide for Forensic Examination of Fabrics and Cordage
c. E2227 Standard Guide for Forensic Examination of Non-reactive Dyes in Textile Fibers by Thin Layer Chromatography
d. E2228 Standard Guide for Microscopic Examination of Textile Fibers
e. E3254 Standard Practice for Determination and Comparison of Color by Visual Observation in Forensic Soil Examination

2. Recent Standards That Have Been Added to the OSAC Registry (proposed—pending SDO approval)
a. WK78748 Standard Practice for a Forensic Fiber Analysis Training Program
b. WK78747 Standard Guide for Forensic Fiber Analysis and Comparison
c. WK78749 Standard Guide for Forensic Analysis of Fibers by Microspectrophotometry
d. WK84047 Standard Guide for Forensic Physical Fit Examinations
e. WK86723 Standard Guide for Interpretation and Reporting in Forensic Comparisons of Trace Materials
f. Standard Guide for Physical Fit Training Program

3. Published ASTM Standards Going Through the Registry Approval Process
a. WK72932 Standard Guide for Forensic Glass Analysis and Comparison
b. E3175 Standard Practice for Training in the Forensic Examination of Hair by Microscopy
c. E3295 Standard Guide for Using X-ray Fluorescence in Forensic Polymer Examinations

4. Standards Going Through the ASTM Process (5-year review)
a. E2926 Standard Test Method for Forensic Comparison of Glass Using Micro X-ray Fluorescence (u-XRF) Spectrometry
b. E2927 Standard Test Method for Determination of Trace Elements in Soda-Lime Glass Samples Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry for Forensic Comparisons
c. WK84554/E3085 Standard Guide for Fourier Transform Infrared Spectroscopy in Forensic Tape Examinations
d. E2937 Standard Guide for Infrared Spectroscopy in Forensic Paint Examinations
e. E1610 Standard Guide for the Forensic Analysis of Paint

5. Standards Going Through STRP/Non-STRP Process
a. Standard Practice for a Forensic Glass Analysis Training Program
b. Standard Guide for the Recovery of Forensic Trace Evidence

6. Documents Currently in Development With the Subcommittee
a. Standard Guide for Using Scanning Electron Microscopy for Morphology and Energy Dispersive Spectroscopy in Forensic Soil and Geological Materials Analysis
b. Standard Guide for the Use of Polarized Light Microscopy in the Forensic Examination and Comparison of Sands and Soils
c. Standard Guide for Using X-ray Diffraction in Forensic Polymer Examinations

d. Standard Guide to Raman Spectroscopy in Forensic Polymer Examinations
e. Standard Guide for Assessing Physical Characteristics and Using Light Microscopy in Forensic Tape Examinations
f. Standard Guide for Forensic Examination of Textile Damage and Textile Impressions
g. Standard Guide for Forensic Examination of Fibers and Textiles Using Fluorescence
h. Standard Terminology Relating to Forensic Analysis of Trace Evidence Materials

7. Priorities for New Documents or Work Products
a. Interdisciplinary development of technical guidance related to the examination of textile-related impressions
b. Interdisciplinary development of technical guidance related to the examination of General Unknowns

Trace Analysis; Standards; Accreditation

B105   Standards Development Activities Related to Friction Ridge Examination

Heidi Eldridge*, The George Washington University, Washington, DC; Henry Swofford*, National Institute of Standards and Technology, Gaithersburg, MD

Learning Overview: After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards related to the examination of friction ridge detail from the hands and feet.

Impact Statement: This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to the examination of friction ridge detail.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was initially created in 2014 through a cooperative agreement between the National Institute of Standards and Technology (NIST) and the Department of Justice (DOJ) to address the lack of discipline-specific standards guiding forensic science practices. To this end, the OSAC facilitates the development of discipline-specific forensic science standards and guidelines that can be further developed and published by Standards Developing Organizations (SDOs). The OSAC also evaluates standards for placement on the OSAC Registry, which serves as a central repository of consensus-based standards that have been deemed high quality and technically sound. The Academy Standards Board (ASB) was established by the AAFS in 2015 as an American National Standards Institute (ANSI) -Accredited SDO. Both organizations are comprised of volunteer practitioners and other stakeholders, and they have a collaborative relationship in which documents created by the OSAC Friction Ridge Subcommittee (FRS) are passed to the ASB Friction Ridge Consensus Body (FRCB) for the standards development process, after which they are considered for inclusion on the OSAC Registry.

During this presentation, updates related to friction ridge standards development activities will be presented. These include: (1) standards and guidelines that have been proposed by the OSAC FRS and are currently being evaluated and further developed through the ASB FRCB; (2) standards and guidelines that have been published by the ASB and are being considered for the OSAC Registry; (3) standards and guidelines published by the ASB that have been placed on the OSAC Registry; (4) documents currently in development by the OSAC or by the ASB; and (5) priorities for new documents or work products by the OSAC FRS or by the ASB FRCB. This presentation will also highlight how the process of moving documents back and forth between the OSAC and ASB works for the friction ridge discipline, challenges and successes that process has encountered, and what the near future looks like for the promulgation of friction ridge standards and guidelines.

Standards; Latent Prints; Professional Responsibility

B106   Standards Development Activities Related to Ignitable Liquids, Explosives, and Gunshot Residue

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Learning Overview: After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards related to Ignitable Liquids (IL), Explosives (E), and Gunshot Residue (GSR).

Impact Statement: The presentation will impact the forensic science community by creating greater awareness of standards development activities related to IL, E, and GSR.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high quality, consensus-based, technically sound standards.

During this presentation, ILs, E, and GSR standards development activities will be discussed. These include:

1. Recent standards that have been added to the OSAC Registry
a. ASTM E2451-21 *Standard Practice for Preserving Ignitable Liquids and Ignitable Liquid Residue Extracts from Fire Debris Samples*
b. ASTM E3196-21, Standard Terminology Relating to the Examination of Explosives
c. ASTM E3253-21, *Standard Practice for Establishing an Examination Scheme for Intact Explosives*
d. ASTM E3329-21, *Standard Practice for Establishing an Examination Scheme for Explosive Residues*
e. ASTM E3309-21, *Standard Guide for Reporting of Forensic Primer Gunshot Residue (pGSR) Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry (SEM/EDS)*

2. OSAC proposed standards
a. OSAC 2021-N-0009, *Standard Practice for the Collection and Preservation of Organic Gunshot Residues* (added December 7, 2021).
b. OSAC 2022-S-0002, *Standard Guide for the Identification of Compounds Related to Organic Gunshot Residue (oGSR) by Gas Chromatography-Mass Spectrometry (GS-MS)*
c. OSAC 2022-S-0003, *Standard Practice for the Identification of Compounds Related to Organic Gunshot Residue (oGSR) by Liquid Chromatography-Mass Spectrometry (LC-MS*)
d. OSAC 2022-S-0023, *Standard Practice for the Forensic Analysis of Explosives by Polarized Light Microscopy*

3. Published standards from the ASTM that have yet to go through the Registry approval process
a. ASTM E2881-18 *Standard Test Method for Extraction and Derivatization of Vegetable Oils and Fats from Fire Debris and Liquid Samples with Analysis by Gas Chromatography-Mass Spectrometry*.
b. ASTM E2998-16 *Standard Practice for Characterization and Classification of Smokeless Powder.*
c. ASTM E2999-17 *Test Method for Analysis of Smokeless Powder by Gas Chromatography-Mass Spectrometry and Fourier-Transform Infrared Spectroscopy*.
d. REVISION to ASTM E1386 *Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Solvent Extraction* (under revision as WK78319).
e. REVISION to ASTM E2997-16 *Standard Test Method for Analysis of Biodiesel Products by Gas Chromatography-Mass Spectrometry* (under revision as WK78732).
f. REVISION to ASTM E2998 *Standard Practice for Characterization and Classification of Smokeless Powder.*
g. REVISION to ASTM E2999 *Test Method for Analysis of Smokeless Powder by Gas Chromatography-Mass Spectrometry and Fourier-Transform Infrared Spectroscopy.*
h. REVISION to ASTM E3197 *Standard Terminology Relating to the Examination of Fire Debris.*
i. REVISION to ASTM E3255 *Standard Practice for Quality Assurance of Laboratories Performing Chemical Analysis of Ignitable Liquids and Ignitable Liquid Residues* (Annex).

4. Documents currently in development at the OSAC or by the SDO
a. Standard Practice for Training in the Forensic Examination of Fire Debris
b. Standard Practice for the Characterization of Solid Oxidizer/Fuel Explosive
c. Standard Practice for Collection and Preservation of oGSR
d. Standard Practice for Expert Opinions on the Interpretation of pGSR by SEM/EDS
e. Standard Practice for Training in the Forensic Examination of GSR Using SEM/EDS

5. Priorities for new documents or work products
a. Fire Debris and Explosives annexes for ASTM E2549
b. Standard Practice for Validation and Verification of Analytical Methods for Forensic Science
Service Providers Performing Forensic Chemistry Analysis
c. Standard Guide for the Performance of Automated pGSR Analysis by SEM/EDS

d. Standard Guide for Reacquisition of pGSR Particles by SEM/EDS
e. Standard Guide for the Classification of pGSR particles by SEM/EDS analysis and other highlights.

Standards; Explosion; Gun Shot Residue

B107　A 2024 Update From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

Jaclyn Iera*, Secretariat, SWGDRUG, Springfield, VA

Learning Overview: The goal of this presentation is to provide a summary of the most recent activities and work products developed by SWGDRUG.

Impact Statement: This presentation will impact the forensic science community by identifying how SWGDRUG supports the development of internationally accepted minimum standards for forensic examination, identifies best practices within the international community, and provides resources to help laboratories meet these standards.

SWGDRUG was formed in 1997 in a joint effort between the United States Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). SWGDRUG works to improve the quality of the forensic examination of seized drugs and to respond to the needs of the forensic community by supporting the development of internationally accepted minimum standards, identifying best practices within the international community, and providing resources to help laboratories meet these standards. This presentation will provide attendees with information on SWGDRUG activities during the past two years.

The SWGDRUG core committee members met in the summer of 2022 after a hiatus due to COVID-19 and approved the most recent version of the SWGDRUG Recommendations, version 8.1, which includes revisions to PART IVA (*Quality Assurance/General Practices*) and Supplemental Document SD-5 (*Reporting Examples*). During the summer of 2023, core committee members revised sections PART II (*Education and Training*), PART IIIA (*Methods of Analysis/Sampling Seized Drugs for Qualitative Analysis*), and PART IIIB (*Methods of Analysis/Analytical Scheme for Identification of Drugs or Chemicals*). Revisions in these sections address virtual training, options for sampling when negatives are encountered, and the use of validated methods in an analytical scheme.

Revisions were also made to PART IVB (*Quality Assurance/Validation of Analytical Methods*) and definitions in the Annex. Revisions include additional background information and clarifications on the performance characteristics to be evaluated during the validation of both qualitative and quantitative methods. Future work will involve the revision of the currently existing Supplemental Document SD-2 (*Validation of Analytical Methods*) to better assist seized-drug practitioners during method validation activities.

This presentation will also summarize recent updates on multiple SWGDRUG resources such as the MS library, IR library, and Drug Monographs.

The SWGDRUG core committee includes representatives from regional, national, and international forensic organizations; educators, practitioners, and scientists from the United States; and representatives from the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana de Criminalistica y Estudios Forenses (AICEF), the Asian Forensic Science Network (AFSN), and the United Nations Office on Drugs and Crime (UNODC).

SWGDRUG; Drug Analysis; Criminalistics

B108   The Forensic Science Initiative (FSI): A Pathway to College and STEM Education

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Learning Overview: After attending this presentation, attendees will be aware of the impact and success of the FSI in introducing underrepresented high school students to forensic science as a pathway to college and Science, Technology, Engineering, and Math (STEM) education, fostering increased interest in forensics, and preparing students for successful careers.

Impact Statement: This presentation will impact the forensic science community by showcasing the effective implementation of the FSI, demonstrating how targeted educational programs can enhance diversity, interest, and preparedness for STEM careers among underrepresented high school students.

Launched in 2022 through a collaboration among New Jersey public school districts, and New Jersey Institute of Technology's (NJIT's) College of Science & Liberal Arts (CSLA) and the Center for Pre-College Programs (CPCP), the FSI provides underrepresented high school students in New Jersey with a pathway to college by strengthening their skills in STEM through the gateway of forensic science. NJIT is committed to increasing the enrollment of underrepresented students from New Jersey public schools and feels an obligation to prepare students for admission, not just review their admission applications. Supported by a $1.4M grant from the United States Department of Higher Education's Governor's Emergency Education Relief (GEER) fund, FSI was a part of the state's "Opportunity Meets Innovation" challenge. The initiative, spearheaded by the NJIT, introduced high school students to forensic science as a pathway to higher education through an intensive summer experience featuring class work, lab work, field research, tutoring, and college preparation. In the fall, students took a college-level course "FRSC 201—Introduction to Forensic Science," and in the spring, they completed a capstone research project with a faculty mentor. By using forensic science as a stealth mechanism to help students develop competency and preparation for college-level STEM work, the FSI program achieved a 100% college acceptance rate for all students enrolled in the program's first year, with 62% electing to attend NJIT.

Underserved students are frequently under-equipped to gain admission to STEM programs at universities, at least in part due to a dearth of highly prepared STEM teachers in K-12 schools nation-wide. Alongside the student component, we also provided professional development for high school forensic science teachers in both lesson plan development and gap lesson design to create a successful dual enrollment course, "Introduction to Forensic Science," that students can take in future years at their high schools. Professional development consisted of lesson study, content development, and flipped classrooms. The projected registration for Fall 2023 is 240 high school seniors.

STEM; Education; College Acceptance

B109   What Is the Probability That a True Match Will Appear in the Top K Correlations After a Database Search?

Alicia Laura Carriquiry*, CSAFE, AMES, IA; Heike Hofmann, Iowa State University, Ames, IA; Blanca I. Parker, Binghamton University, Schenectady, NY; Cathryn Barbour, Mount Holyoke College, Lexington, MA

Learning Overview: After attending this presentation, attendees will understand that searches such as those conducted by firearms examiners using a system such as the National Integrated Ballistic Information Network (NIBIN) have a high probability of excluding a true match. The probability depends on the size of the local database that is searched, the number of potential candidate matches requested by the investigator, and the degree of overlap of the distributions of same-source and different-source similarity scores.

Impact Statement: This presentation will impact the forensic science community by informing attendees that some investigators may be inclined to heavily rely on the output of database searches that typically consist of a list of potential matches to the questioned item. Even though no practitioner would reach a conclusion without examination of the physical items, the fact that systems such as NIBIN produce lists of promising matches is suggestive and can introduce biases. Therefore, an important goal is estimating the reliability with which these systems include true matches among the candidate lists. If the probability that a true match is not included among the most promising candidates happens to be high, then the benefits of using a database search may be overshadowed by the introduction of bias.

A crime has been committed and firearms examiners have recovered spent bullets and cartridge cases from the scene. To generate investigative leads, examiners often rely on proprietary systems such as NIBIN. The NIBIN system was established over 25 years ago and includes a database of bullet and cartridge case images from real cases that have been uploaded by examiners in the course of their investigations. At present, the database includes 57+ million images that can, in principle, be searched by any NIBIN user to correlate a piece of evidence to potentially similar bullets or cartridge cases recovered from other crime scenes. Neither the database of images nor the correlation algorithm is publicly available, so they cannot be tested for accuracy or reliability.

Systems such as NIBIN work as follows. Investigators upload images of the crime scene item(s) onto the system and request a list of the top k most similar items from the database. Examiners then decide whether any of the k candidates are similar enough to the questioned items to warrant a physical comparison. Published statistics are impressive. According to data presented in the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) website, over 640,000 leads were generated nationwide since NIBIN was established, and 145,000 of them have resulted in a confirmed "match." Last year alone, examiners captured over 630,000 pieces of evidence and generated almost 200,000 leads.

But how reliable are NIBIN searches? Suppose that one or more true matches to a questioned piece of evidence is in the NIBIN database. How likely are those true matches to be included in the list of the top k correlations identified by the system? Because NIBIN is proprietary, there is no straightforward way to estimate how frequently a search may fail to include a true match among the k most promising candidates. The reason this is important has to do with contextual bias. If among the k items that are most similar to the questioned item there is one or more that are close non-matches, the investigator may be persuaded to find correlations that are not really there. In a similar situation, Federal Bureau of Investigation (FBI) latent print examiners incorrectly identified Mr. Mayfield as the perpetrator of the 2004 train bombing in Madrid.

To explore this question, we carried out a simulation where we varied the potential number of correlations (N), and the size of the list of candidates (k). In a small state such as Iowa, the size of the local NIBIN database is likely to be small, with N around 500 potential correlations. In other states such as Texas, California, or New York, the potential number of correlations may be in the order of a few hundred thousand. We used beta probability models to represent the distribution of Same Source (SS) and Different Source (DS) similarity scores, with parameters fixed at values that would reproduce the scores calculated from data obtained by Fadul and Weller, after rescaling their values to a (0, 1) interval.[1] We considered databases of size N=500, 5,000, 50,000, and 5,000,000, and for each, requested the top k=10, 20, 50, 100 most similar items when $N-1$ items were sampled from the DS distribution and one item was sampled from the SS distribution. Our findings suggest that at least three factors have a strong impact on the probability of inclusion of the one true match in the candidate list. Those factors are: the size of the search space N, the number of candidates k, and the degree of overlap between the DS and the SS distributions.

Reference:
[1]   Song, J., Chen, Z., Vorburger, T.V., Soons, J.A. 2019. Evaluation likelihood ratio (LR) for firearm evidence identifications in forensic science based on the congruent matching cells (CMC) method. *Forensic Science International.*

Firearms; Data; Accuracy

B110 **The European Network of Forensic Science Institutes (ENFSI) Guideline for the Calculation of Measurement Uncertainty in Quantitative Forensic Investigation**

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Learning Overview: After attending this presentation, attendees will have knowledge on the current version of the latest guideline of the ENFSI on how to determine measurement uncertainty when performing chemical or physical investigations.

Impact Statement: This presentation will impact the forensic community by illustrating solutions for typical issues that may arise when determining measurement uncertainty when conducting chemical or physical investigations.

The users of forensic expert reports have to interpret the contents correctly. This implies that the results and conclusions of quantitative measurements should also include the degree of reliability of measurement data provided, including the Measurement Uncertainty (MU) involved. The requirements of ISO 17025 underline the latter. Naturally, statistics and, as a derivative thereof, the performance of MU calculations have been part of the professional training of forensic experts. Nevertheless, forensic experts often experience the performance of MU calculations as a difficult necessity. To facilitate calculating of MU in quantitative forensic investigations, there was an existing guideline by the **Quality & Competence** Liaison Group-Quality and Competence standing Committee (QCLG-QCC) project of the ENFSI, which dated from 2006. In the period 2019–2022, a team of practitioners from several fields of expertise, combined with a team of forensic statisticians, wrote a revised version of this guideline.

The guideline aims to provide information and practical advice to anyone who is looking for applicable and understandable knowledge and worked out examples on how to apply MU in forensic technical methods. The scope of this guideline focuses on MU calculations as applied in quantitative chemical and physical forensic investigation methods. The manner in which information, originating from, for example, sampling aspects, results of validation studies, or Proficiency Tests (PT) can be used for calculations of MU, are present in the worked-out examples as included in this guideline.

The analysis consists of recommendations on how to calculate uncertainty in standard casework, where either measurements on certified reference material are present or results in proficiency testing, but also in complex casework, where typically small experiments have only a few measurements are present. The content of the guideline is structured along seven examples.

Examples of forensic chemistry include determination of ethanol in blood using headspace gas chromatography with flame ionization detector, besides determination of both cocaine and MDMA in powders/seizures by high performance liquid chromatography, and Tetrahydrocannabinol (THC) in blood. For this type of casework, the guideline describes several methods to determine MU, either based on measurements on certified reference materials or PT results, including a statistical background of the latter. Next to this, it is explained how free software may be used for this.

Examples of forensic physics include calibration of digital thermometers, determination of drug weight, excluding packing material, and velocity estimation of speeding cars in video footage. Here, typically MU is determined by devising statistical models like linear regression on a case-to-case basis.

The examples in the guideline are straightforward, even for the more complex examples. This allows a wide application of this guide. The examples are offering the reader, therefore, a framework to calculate MU, not a normative set of rules.

Statistics; Drug Analysis; Accuracy

B111   A Comparison of Computational and Laboratory-Generated Fire Debris Data

Larry Tang*, University of Central Florida, Orlando, FL; Mary R. Williams, University of Central Florida, Orlando, FL; Michael E. Sigman, University of Central Florida, Orlando, FL

Learning Overview: This presentation will cover machine learning aspects of enhanced ignitable liquid and substrate databases. After attending this presentation, attendees will understand how training data is generated computationally from the database records and how to implement machine learning methods on the training data.

Impact Statement: This presentation will impact the forensic science community by providing insight into how well calculated and laboratory-generated fire debris agree and the use of calculated fire debris to train methods for predicating the presence of ignitable liquid in casework samples.

In this study, we analyze: (Analysis 1) how computationally mixed fire debris data compares with laboratory-generated samples, and (Analysis 2) compare laboratory-generated samples and computationally mixed fire debris data utilizing the Ignitable Liquids Reference Collection (ILRC) with digital weathering and the Substrate Database. Statistical measures were used to assess how well the in-silico mixture data compared to the ground truth fire debris samples. The statistical measures utilized were the concordance and Pearson correlation coefficients. Concordance measures the agreement between samples and Pearson measures linear relationship. If two Total Ion Chromatograms (TIC) are in perfect agreement, the best fit line for an XY plot of the two TICs will fall completely on a 45-degree line from the origin, and the concordance Correlation Coefficient (CC) will equal 1. A high Pearson correlation between two TICs indicates a linear XY plot, but the slope may not fall on a 45-degree line. Preliminary results from Analysis 1 indicate almost perfect CC agreement between sets of TICs and a strong linear relationship. Preliminary results from Analysis 2 show a lower CC agreement and weaker linear relationship between sets of TICs compared to results from Analysis 1.

Under National Institute of Justice (NIJ) sponsorship, a large standard in-silico fire debris dataset will be generated and made publicly available for machine learning research. Examples of machine learning methods, including linear discriminant analysis, random forest, gradient boosting, and neural network for classification utilizing the standard dataset will be given. Classification accuracy measures, including false positive rate, false negative rate, and the area under the receiver operating characteristics curve, for the examples will be demonstrated. The accuracy measures will be plotted for various machine learning methods to visualize how the accuracy changes with different methods. The machine learning methods will also be applied to the laboratory-generated samples to evaluate the accuracy of these methods. The accuracy for laboratory-generated samples will be compared with the computationally mixed samples.

Fire Debris; Database; Casework

B112   A Fire Debris Interpretation Using Quantitative Measures of Chromatographic Features in Medium-Range Ignitable Liquids and the Use of Graphical Display to Demonstrate Data Sufficiency

Kelsey R. Winters*, Virginia Department of Forensic Science, Norfolk, VA; Brenda B. Christy, Virginia Department of Forensic Science, Norfolk, VA

Learning Overview: The goal of this presentation is to introduce a validated method for the evaluation of fire debris Gas Chromatography/Mass Spectrometry (GC/MS) data that quantifies the support for a medium petroleum distillate identification using a sufficiency chart.

Impact Statement: This presentation will impact the forensic science community by introducing a quantitative sufficiency chart that will increase the objectivity and transparency of the fire debris expert's inferential process.

This presentation will discuss a method for the evaluation of fire debris GC/MS data for the presence of medium petroleum distillates using quantitative measures to determine the sufficiency of the sample to support an identification. This presentation will highlight the use of key chromatographic features to create a graphical display of the sufficiency of the data from the sample. This approach to data interpretation is the first step toward a validated method that will be a part of an objective, increasingly transparent process for the identification of medium petroleum distillates in the presence of complex matrices.

Ignitable liquid identification is based on chromatographic pattern recognition. While the analytical processes associated with the analysis of fire debris for the presence of ignitable liquids are based on fundamental chemical properties, the interpretation process is subjective. The degree of subjectivity is directly related to the abundance and type of interference from volatile compounds produced by the matrix (inherent, pyrolysis, and combustion products) and the concentration of any ignitable liquid residues present in the sample. This can, and does, lead to a lack of consistency in interpretation and reporting within the field for complex samples.

Previously, a study was conducted and published showing the application of quantitative measures of chromatographic features and graphical display of data sufficiency for gasoline.[1] This study was similarly designed to establish quantitative measures of assessing the chromatographic features of medium petroleum distillates and to apply statistical measures to create sufficiency parameters for use in interpreting data and rendering conclusions. The study involved the evaluation of medium petroleum distillate samples evaporated up to 90%, negative matrix samples, and real-world samples composed of mixtures of matrix and medium petroleum distillates. Key chromatographic features in over 150 medium petroleum distillate samples were evaluated. Up to 192 potential chromatographic peak heights were collected per sample and up to 122 ratios were determined for comparison. Statistical analysis was conducted to determine the variation observed for each of these ratios in known medium petroleum distillate samples and to determine the frequency of the presence of these key chromatographic features in negative matrix samples. This information was evaluated to determine relative scores for each of these features. The scores were used to create a sufficiency chart, a graphical display of the amount of data supporting a potential medium petroleum distillate identification. This sufficiency chart allows the analyst to objectively evaluate the data. Statistically developed decision lines on the sufficiency chart also identify the "gray" area where analysts are more likely to form differing opinions.

Ultimately, this project will help standardize fire debris experts' inferential process (by establishing a fully validated method) and increase objectivity (by establishing quantitative measures) and transparency (by implementing graphical documentation), all of which will be statistically based.

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Fire Debris Analysis; Interpretation; Sufficiency

B113   An Evaluation of Headspace Solid Phase Microextraction of Diesel Fuel From Cotton Swabs for Forensic Analysis

Ting-Yu Huang*, Sam Houston State University, Huntsville, TX; Jorn Yu, Sam Houston State University, Huntsville, TX

Learning Overview: The presentation will demonstrate how the headspace Solid Phase Microextraction (SPME) procedure performs for extracting diesel fuel from cotton swabs. The attendees will understand that cotton swabs are promising for the collection of Ignitable Liquid (IL) residues in fire scenes. The attendees will also understand the performance between silicone-oxygen-based and conducting-based SPME polymer fiber for IL extraction.

Impact Statement: This presentation will impact the forensic science community by considering proper sample collection and extraction techniques to improve the recovery of trace ILs in fire scenes. Forensic analysts will learn the selection of different sorbent materials in achieving desirable affinity toward target compounds extraction in ILs.

The aim of sample collection at a fire scene is to provide forensic information on physical evidence critical to the fire investigation. Cotton swabs have been useful for collecting trace physical evidence, such as biological evidence, stains, gunshot residues, and explosive residues.[1-3] Cotton swabs are versatile to be adapted to different types of objects and surfaces, and they are relatively affordable compared with other sampling devices.[4] With their good absorption capability, cotton swabs are potential collecting media for ILs in fire cases. SPME offers a rapid, automated, and solvent-free approach for extracting volatile organic compounds from ILs samples.[5] The SPME fibers can be reused by a simple conditioning process at an autosampler. Those advantages have attracted increasing interest from researchers and made SPME one of the standardized test methods for fire debris analysis.[6] Therefore, this work aimed to investigate the feasibility of a headspace SPME method for determining an IL collected by cotton swabs using SPME fibers coated with different sorbent materials.

To test the research question, diesel fuel was chosen in this work. The SPME fibers used for the analysis included a commercial 100μm Polydimethylsiloxane (PDMS) fiber and a lab-built fiber made by electro-polymerization of pyrrole and Carbon Nanotubes (ppy-CNTs) on the surface of a stainless-steel wire. Eight levels of calibrator samples were prepared, and 5μl of each calibrator was spiked onto different swabs in a 20mL headspace vial. The SPME fibers were utilized to extract seven target compounds in the diesel fuel, followed by their identification and quantitation by Gas Chromatography/Mass Spectrometry (GC/MS). The effects of different collection time intervals and deposited time intervals on a glass surface for the diesel fuel were assessed.

The results indicated that the linear dynamic ranges were 0.01–.6μg mL^{-1} for PDMS fiber and 0.01–0.3μg mL^{-1} for ppy-CNTs fiber in neat samples, and 0.01–1.3μg mL^{-1} for both SPME fibers in spiked swabs, with the coefficients of determination all above 0.99. The relative recoveries were 24.2–49.2% for PDMS fiber and 24.2–39.1% for ppy-CNTs fiber, respectively. The proposed method could recover 1.3μg mL^{-1} of diesel fuel after one hour of being spiked on a swab. The same amounts of IL deposited on a glass surface for up to four hours of exposure to air could also be identified by GC/MS.

In conclusion, the proposed headspace SPME-GC/MS method was successfully applied to the determination of IL traces from spiked swabs and direct transfer of swabbed samples. The qualitative and quantitative results obtained by commercial and lab-built SPME fibers were both promising. The importance of the proposed work was to demonstrate a more flexible, low-cost, speedy, and environmental-friendly approach for sample collection and forensic analysis of ILs. It is expected that the method offers promising opportunities for sampling and identifying other ILs to support fire investigation.

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Solid Phase Microextraction; Swab; Ignitable Liquid

B114   An Evaluation of Tape for the Collection of Ignitable Liquid Residues From Non-Porous and Porous Surfaces

Cynthia J. Kaeser Tran, Eastern Kentucky University, Lexington, KY; Laird Jarrett*, Eastern Kentucky University, Richmond, KY

Learning Overview: After attending this presentation, attendees will better understand the current methods for collection of Ignitable Liquid Residue (ILR) from porous fire debris evidence and the potential for using tape to simplify and improve the collection process.

Impact Statement: This presentation will impact the forensic science community by introducing a new method of ILR collection using tape. This method could provide a more cost-effective and practical method for the recovery of the Ignitable Liquids (ILs).

ILs are flammable mixtures used to accelerate a fire. ILs are composed of a broad range of hydrocarbons and include commonly encountered materials such as gasoline, diesel, and kerosene. In the event of a suspected arson, ILR may be found within the pores of materials at the crime scene such as concrete. Traditionally, the whole material is collected and packaged into air-tight containers to prevent evaporation and allow transportation back to the lab. However, this may require the use of heavy machinery, such as a jack hammer, which takes a lot of manpower to operate and may increase the probability of loss of the ILR during collection. Recent research has investigated other options for direct collection of the ILR, including the use of absorbent materials as they are able to penetrate the pores of the fire debris evidence and achieve a higher collection efficiency. These materials have included granular minerals, hydrophobic absorbent pads, or everyday absorbers such as paper towels and microfiber cloths.[1-3] Each of these have had limitations stemming from the ability to penetrate and/or be removed from a porous material as well as in compatibility with the individual components of an IL.

In this work, tapes of a variety of compositions are investigated for direct ILR collection from non-porous and porous surfaces. Tape is a cost-effective absorbent material selected because of its sturdy but flexible backing. Additionally, the adhesive is typically composed of non-polar films and resins that are chemically compatible with the hydrocarbons in the ILs.

This presentation will discuss six different types of tape to classify which tape provides the highest collection efficiency of ILR and has the least amount of interference with a standard passive headspace collection using activated charcoal strips and analysis using Gas Chromatography/Mass Spectrometry (GC/MS). The tapes used came from a wide variety such as normal household repair tapes to stronger industrial and laboratory tapes. All six tapes were checked for interferences from the adhesive and backing. The effectiveness of the tape's collection is assessed by comparing the amount of standard homologous n-alkanes collected by the tape to the amount left on a small area of non-porous tile or porous concrete (a relative collection efficiency). Overall, the most common household tape demonstrated some interferences with the process and the more specialized tapes showed more promise. This presentation will outline the results for all tapes' validation and provide a recommendation for which tape provides the highest relative collection efficiency from non-porous and porous surfaces.

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Trace Analysis; Fire Investigation; Arson

B115   Expert and AI Opinions: Machine-Assisted Analysis of Fire Debris

Michael E. Sigman*, University of Central Florida, Orlando, FL; Mary R. Williams, University of Central Florida, Orlando, FL; Frances A. Whitehead, University of Central Florida, Orlando, FL

Learning Overview: This presentation will discuss the use of subjective logic to describe expert opinions and application of the logic to Artificial Intelligence (AI) results in fire debris analysis. After attending this presentation, attendees will understand how to represent expert and AI opinions with subjective logic and how to combine the two opinions to reach a consensus.

Impact Statement: This presentation will impact the forensic science community by providing attendees with the opportunity to understand how computers can generate a forensic opinion, rather than a single answer, and how AI can assist analysts in the data interpretation process.

Subjective logic provides a method for reasoning about data under uncertainty and allows analysts to express their opinions, which are influenced by uncertainty. Subjective binomial opinions about two exclusive and competing hypotheses are often encountered in forensic science. An example set of propositions regarding a fire debris sample could be H1: the sample contains Ignitable Liquid Residue (ILR) and, H2: the sample does not contain ILR. A subjective opinion about the sample is characterized by four parameters: the belief mass that the sample contains ILR, the disbelief mass, uncertainty mass, and base rate. The application of subjective logic to quantitatively characterize an analyst's opinion will be demonstrated for the analysis of known ground truth fire debris samples.[1]

Opinions can also be obtained from AI using the principles of subjective logic. An ensemble of AI models is each trained on different training data to generate a set of probabilities that a test sample contains ILR. The set of probabilities is used to formulate the AI opinion. If the probabilities from applying the ensemble of models to a test sample are widely distributed, the uncertainty of the AI opinion will be high. If the probabilities are more narrowly distributed, the uncertainty will be smaller. When the probabilities of ILR in the sample are high, the belief mass will be larger, and the disbelief mass will be smaller. If the probabilities of ILR in the sample are low, the belief mass will be lower and the disbelief mass will increase. The belief, disbelief, and uncertainty masses are calculated from the shape parameters obtained by fitting the probabilities for a single sample to a beta distribution. The process of generating AI opinions will be demonstrated for known ground truth fire debris samples.[1]

The AI opinions are more useful than a single numerical result in assisting an analyst in data interpretation.[2] The AI opinion can also be combined with the analyst's opinion to form a consensus opinion.[1]

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Statistics; Artificial Intelligence; Data

B116 Investigating the Capability of DART®-MS for the Analysis of Common Homemade Explosives

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Learning Overview: After attending this presentation, attendees will have a better understanding of the strengths and limitations of Direct Analysis in Real-Time Mass Spectrometry (DART®-MS) to maximize the detection and identification of intact and explosive residues.

Impact Statement: This presentation will impact the forensic science community by demonstrating a workflow involving minimal sample preparation to rapidly and accurately detect and identify intact explosives and explosive residues using DART®-MS.

During the investigation of an explosion, the rapid identification of the explosive compound(s) is key to provide investigative leads for homeland security purposes. Due to military-grade high explosives being more difficult to acquire, domestic attacks are commonly conducted using Homemade Explosives (HMEs) with reagents sourced from common household materials and legal precursors that can easily be obtained commercially. Whereas current laboratory techniques such as Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) are used to accurately identify explosive compounds, they also present several drawbacks, such as extensive sample preparation and long chromatographic runs. This study aims to explore the use of DART®-MS to provide a rapid and reliable analysis of HME precursors and explosive residues that do not require complex sample preparation. Furthermore, DART®-MS can also provide useful additional information, including the presence of oils, binders, plasticizers, and polymers.

A targeted list of commonly encountered energetic materials was constructed with the aid of the Montgomery County Fire Marshal's Office (MCFMO) to create a representative set of explosive compounds relevant to current casework, including Ammonium Nitrate and Fuel Oil (ANFO), nitromethane, and smokeless powders. The DART®-MS method development involved optimization of the DART® source gas temperature and mass spectral acquisition parameters for each intact compound, with additional modifications for explosive residues.

Several simulated Improvised Explosive Devices (IEDs) were constructed and detonated with assistance from the MCFMO to collect the explosive residues. The simulated IEDs consisted of several witness materials, including batteries, metal shrapnel, wires, and cell phone parts placed in a plastic toolbox. Post-blast fragments were then collected and analyzed using DART®-MS via an indirect sampling method.

Ammonium nitrate and other inorganic oxidizing salts are not traditionally amenable to DART® ionization processes; however, the use of an unconventional solvent, namely 50:50 Deionized (DI) water:Methanol (MeOH), demonstrated successful detection and characterization of non-dissociated ammonium nitrate ion clusters at nominal *m/z* 98, *m/z* 178, and *m/z* 258. A non-dissociated ammonium perchlorate ion cluster was also detected at nominal *m/z* 135. Nitromethane was not able to be detected using DART®-MS due to only minimal direct Penning ionization of nitromethane relative to alternative outcomes. Smokeless powders were shown to produce consistent ion signal with DART®-MS analysis, with some additional challenges related to explosive residues. Characteristic ions for ethyl centralite, methyl centralite, and diphenylamine were detected at nominal *m/z* 269, *m/z* 241, and *m/z* 170, respectively. Furthermore, 2,4–dinitrotoluene and dibutyl phthalate were also detected at nominal *m/z* 181 and *m/z* 279, respectively.

This study demonstrates the benefits and limitations of using DART®-MS for the analysis of intact and explosive residues from HMEs, as well as demonstrates the feasibility of using DART®-MS with only limited sample preparation using an indirect sampling method. Additionally, DART®-MS was able to detect challenging non-dissociated ammonium nitrate and ammonium perchlorate ion clusters using a custom 50:50 DI water: MeOH solvent enabling the wider applicability of DART®-MS analysis to ammonium-based inorganic oxidizing salts.

Direct Analysis in Real Time (DART®); Mass Spectrometry; Trace Analysis

B117   **Combining Surface-Enhanced Raman Spectroscopy (SERS) and Paper Spray-Mass Spectrometry (PS-MS) for Illicit Drug Detection and Identification**

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Learning Overview: After attending this presentation, attendees will be aware that many fentanyl analogs are developed and mixed with other illicit drugs such as cocaine and heroin. Detecting fentanyl and fentanyl analogs in these illicit drug mixtures gains more importance. Most confirmatory procedures require time-consuming and expensive, highly sophisticated laboratory equipment and experimental procedures, which can delay critical information that might save a victim or identify a suspect. In this project, we propose miniaturizing and accelerating this process by combining SERS analysis and mass spectrometry on a single microfluidic device. This procedure, known as PS-MS, can isolate fentanyl analogs from even complex drug mixtures. Thus, we propose to develop a novel device capable of simultaneously detecting fentanyl and fentanyl analogs in illicit drug mixtures.

Impact Statement: Coupling portable SERS and Paper Spray Ionization-Mass Spectrometry (PSI-MS) methods will provide strong confirmatory results for real-world samples and drug mixtures. The robustness, portability, and sensitivity of this study will provide important convenience for field applications, thereby impacting the forensic science community. The experimental results obtained in this project show that SERS analysis can be done in the field for identification followed by PS-MS in a laboratory setting for confirmation.

There is an ongoing effort in the black market to make new drugs that are more potent and addictive. Due to these continual modifications, many fentanyl analogs are developed and mixed with other illicit drugs such as cocaine and heroin. The presence of fentanyl analogs as mixtures in illicit drugs makes it hard to estimate their potencies. This makes the detection and differentiation of fentanyl analogs important. Most current screening methods have difficulty detecting the full range of opioid analogs due to a wide variety of structural variations. However, SERS can differentiate structurally similar fentanyl analogs due to its ability to yield spectroscopic fingerprints for the detected molecules. Once SERS has identified a fentanyl analog, an additional confirmatory technique is required to provide identification of the compound in a court of law. A second, more precise method, such as liquid chromatography/mass spectrometry, is necessary for typical mixtures, which include other drugs and diluents at very low concentrations. Most confirmatory procedures require time-consuming and expensive, highly sophisticated laboratory equipment and experimental procedures, which can delay critical information that might save a victim or identify a suspect.

To our knowledge, this is the first study to identify fentanyl analogs in real seized drug samples with SERS/PS-MS.

SERS; Mass Spectrometry; Fentanyl

B118   Evaluating the Sensitivity, Stability, and Cross-Reactivity of Commercial Fentanyl Immunoassay Test Strips

Sandra E. Rodriguez-Cruz*, Drug Enforcement Administration, Dulles, VA

Learning Overview: After attending this presentation, attendees will have a better understanding of the utility and limitations of using commercial Lateral-Flow Immunoassay (LFIA) test strips for the analysis of suspected fentanyl materials.

Impact Statement: This presentation will impact the forensic science community by providing an in-depth assessment of the performance of four commercially available Fentanyl Test Strips (FTS), under variable scenarios, at different target-analyte concentrations, and in the presence of other Fentanyl-Related Substances (FRS) and adulterants. This information will also influence how these commercial tools are used by seized-drug laboratory analysts, first responders, and harm-reduction organizations throughout federal, state, and local jurisdictions.

The United States of America has been fighting an unprecedented battle against opioids during the better part of the past ten years. According to the Centers for Disease Control and Prevention, the age-adjusted rate of overdose deaths increased by 31% from 2019 to 2022. In 2020, about 75% of the almost 92,000 drug overdose deaths in the United States involved an opioid, with the vast majority of those being synthetic opioids such as fentanyl or other FRS. Harm reduction centers funded by local governments or non-profit organizations have played an important part in this battle by reaching out and educating those at risk of overdose. In addition to providing educational and overdose-risk counseling, access to methadone or buprenorphine treatment, and a safe environment for drug use, many of these centers are equipped with opioid-antagonist nasal sprays such as NARCAN® and also distribute commercial LFIA strips so users can test their drugs prior to consumption. Recent studies have explored the impact that using FTS may have in the drug user's behavior, with some of them suggesting that, upon encountering a positive response using the FTS, drug users may discard their drug supply, avoid using the drugs alone, or alert others about the potential risks of using a particular drug batch.

Immunoassay tests are classified as Category C techniques by the Scientific Working Group for Analysis of Seized Drugs (SWGDRUG) and Drug Enforcement Administration (DEA) laboratories have implemented their use as part of their standardized analytical scheme for analyzing suspected fentanyl exhibits. FTS are designed to provide presumptive information about the presence of fentanyl in a suspected sample. However, their adoption by law enforcement personnel and seized-drug analysts throughout the general community has been limited because most products are advertised for urine testing, not for assays using water solutions. This study presents an evaluation of four commercial FTS: Rapid Response® from BTNX, Inc.; T-Dip® Fentanyl (FTY) Urine Dip Cards obtained from Amazon.com; Premier BioDip® FYL10 from Premier Biotech, Inc.; and MobileDetect® Fentanyl strips from DetectaChem, Inc.

Performance characteristics curves were used to compare the products' sensitivity, showing that all can reliably detect fentanyl in aqueous solutions at concentrations below 1µg/mL, with some of the tests able to reliably detect the drug at 200ng/mL. A stability study demonstrates the performance of all four FTS brands was only slightly affected after 30 days of storage at two extreme environmental conditions. FRS are also evaluated using the Rapid Response® FTS, which showed high cross-reactivity with para-fluorofentanyl and acetylfentanyl, but lower with ortho-chlorofentanyl, carfentanil, and 4-ANPP. Users should be aware that FTS may give false negative results even when potentially dangerous levels of carfentanil are present. When testing other common drugs, adulterants, and diluents frequently encountered in seized tablets, concentration-dependent results were obtained, and multiple instances of false positives were recorded.

Immunoassay; Fentanyl; Seized Drugs

B119   Exploring Evidence of Common Origin of Recreational Drug Exhibits by GC/MS

Joshua S. DeBord*, Center for Forensic Science Research and Education, Willow Grove, PA; Barry K. Logan, PhD, Center for Forensic Science Research and Education, Horsham, PA; Alex J. Krotulski, Center for Forensic Science Research and Education, Willow Grove, PA

Learning Overview: After attending this presentation, attendees will understand the chemical and data methodology used by the authors to profile drug samples. Attendees will also learn how the presented methods could be used as indicators that two or more exhibits of drug samples may have a common origin, based upon shared chemical signatures.

Impact Statement: This presentation will impact the forensic science community by providing deeper insights into the correlations among the physical and chemical properties of drug exhibits and improving the utility of drug evidence analysis in criminal investigations.

Across the landscape of recreational drug products, complex strategic intelligence efforts increasingly rely upon sophisticated analytical techniques and comprehensive chemical analysis. Beyond simply identifying controlled substances within a drug exhibit, identification of non-controlled substances and quantification of those components add informative points of comparison among samples. This presentation explores the application of Gas Chromatography/Mass Spectrometry (GC/MS) in addressing issues of possible common source determination for drugs and enhancing the utility of drug analysis as an investigative tool beyond its use simply for prosecution. The approach described involves the comparison of chemical signatures from recreational drug samples with the aim of determining if they share characteristics pointing to a common source.

Our laboratory performs drug markets' trend surveillance and confirmatory quantitative analysis for public health and safety agencies for the purposes of assessing drug safety and threats, monitoring drug market changes, and guiding public health harm reduction strategies. Our workflows include a qualitative analysis and an externally calibrated quantitative analysis, both performed by GC/MS. The data analysis of the qualitative results includes identification by retention time and mass spectral comparison to an in-house library, and a relative proportion analysis, determined for each compound in the sample by relating the peak area(s) to that of the major drug component. As an early step toward making common source determinations, this presentation will explore the relative proportion analysis and the quantitative data for their utility in creating comparable chemical profiles. A detailed explanation of the GC/MS methodology used to acquire chemical results and the data processing programs that were written in R, an open-source coding environment for statistical computation will be shared as part of the discussion.

Our GC/MS method includes a full-scan window acquisition method that is identical for both qualitative identification and quantitative measurement; however, samples are aliquoted and processed for both assays independently. All data is then manually reviewed by a trained analyst, then post-review data processing is performed by a custom R program, which automates the relative parts analysis, quantitative calculations, and data collection/reporting.

Analysis of the data identified some groups of exhibits with distinctive properties that were both internally consistent within the group, and distinct between groups, emphasizing the potential for linking exhibits with each other. For examples of such groups, one consisted of four independently submitted samples originating from Massachusetts having physical descriptions consistent with counterfeit oxycodone tablets. These exhibits each contained fentanyl, xylazine, para-fluorofentanyl, and 4-ANPP at concentrations of 1.0–1.2%, 2.7–2.9%, 0.40.5%, and 0.2%, respectively. A second group consisted of three powder opioid samples collected in Pennsylvania containing fentanyl, xylazine, and 4-ANPP at concentrations of 4.2%, 59.6–62.6%, and 0.7–1.0%, respectively. A third group consisted of two powder opioid samples collected in an undisclosed United States state, containing fentanyl, xylazine, heroin, 4-ANPP, and caffeine at concentrations of 15.9%, 9.4/9.5%, 9.1/9.3%, 1.6% and 0.5%, respectively. Apart from the close similarities in chemical composition, similarities with respect to physical morphology, packaging, date, and location of collection were also persuasive.

The traditional role of forensic drug chemistry has been to obtain analytical evidence of the presence of controlled substances in exhibits submitted for analysis. This exploratory work suggests that performing testing beyond the controlled substance identification, to quantitative analysis of the principal components, adulterants, and precursors/byproducts creates the possibility of linking exhibits between cases to provide further investigative leads and insights to investigators.

Drug Analysis; Fentanyl; Profiling

B120   GC Derivative VUV Spectroscopy of JWH-018 Positional Isomers and Select Synthetic Cannabinoid Diastereomers

Annika Dombrowski*, Arlington, VA; Ira Saul Lurie, The George Washington University, Washington, DC

Learning Overview: After attending this presentation, attendees will understand the capability of Gas Chromatography-Vacuum Ultraviolet (GC-VUV) derivative spectroscopy for the identification of positional isomers and diastereomers of select synthetic cannabinoids. Additionally, attendees will have an understanding of the specificity of this technique as compared to both underivatized VUV and more staple forensic techniques such as Gas Chromatography/Mass Spectrometry (GC/MS).

Impact Statement: This presentation will impact the forensic science community by providing evidence for the value of derivative analysis of VUV spectra in the identification of isomeric compounds that remain difficult to distinguish by GC/MS and/or GC-VUV. Analysis of the first and second derivatives of VUV spectra can be used to improve confidence in the accuracy of identification of positional isomers and diastereomers that are currently difficult or impossible to distinguish without spectral manipulation. This research aims to demonstrate the consistency and specificity provided by derivative analysis, compared to underivatized spectra and traditional MS.

With the recent rise in emerging drugs synthesized to circumvent existing drug laws, the identification of isomeric compounds has become of increasing concern to the forensic science community. Traditional and widely used techniques such as GC/MS have proven insufficient to distinguish these isomers due to the similar mass fragmentation patterns of certain positional isomers and diastereomers. As a result, new techniques are required in order to analyze and identify these drugs. One such emerging technique is GC-VUV, which has been demonstrated to accurately distinguish between many positional isomers.[1,2] However, despite this improvement over traditional techniques, GC-VUV has remained unable to distinguish between some positional isomers and diastereomers[2].

One technique with the potential to remedy this is the use of derivative analysis, which has previously been applied to Ultraviolet (UV) spectra of similar compounds to pull out greater spectral detail and allow for compounds with near identical spectra to be identified.[3] By applying first and second derivative manipulations to raw spectra, spectral details such as maxima, minima, and inflection points are highlighted, providing more points of comparison with library spectra, and highlighting small differences that may not be visible in raw spectra, such as potential differences in the spectra of diastereomers or indistinguishable positional isomers. As a result, derivative analysis has the potential to increase the specificity of existing spectroscopy-based methods.

The research presented here considers the application of derivative analysis to the VUV spectra of a select group of synthetic cannabinoids, including 16 positional isomers of JWH-018 and three pairs of diastereomers, that can be difficult to distinguish with raw VUV spectra.[2] For the positional isomers, derivative analysis of the spectra yielded greater consistency in accurate identification as demonstrated by library searches with repeatability analyzed for both day-to-day and run-to-run. Additionally, principal component analysis demonstrated that within groups of positional isomers and diastereomers, the first and second derivatives showed improved discrimination compared to raw data, demonstrating the power of derivative analysis for improving identification accuracy as well as specificity compared to non-derivative spectra. This research will also demonstrate comparisons between the discrimination power of this new seized drug analysis technique for VUV as compared to traditional electron ionization MS.

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Vacuum Ultraviolet Detection; Derivative Spectroscopy; Cannabinoid Isomers

B121   **The Effect of Spectral Intensity on Mass Spectral Discrimination of Fentanyl Positional Isomers**

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Learning Overview: After attending this presentation, attendees will have learned about a statistical method to distinguish spectra of positional isomers and identify reliable ions for discrimination.

Impact Statement: This presentation will impact the forensic science community by demonstrating the utility of a statistically objective method to differentiate mass spectra of positional isomers and will highlight the effect of spectral intensity on the ability to distinguish such isomers.

One of the most pressing concerns in seized drug analysis is the identification of novel synthetic opioids, primarily fentanyl analogs. Numerous analogs have surfaced within the past several years, and the proliferation of these analogs is only accelerating, with 73,000 opioid-related deaths being reported by the United States Centers for Disease Control and Prevention between July 2021 and June 2022.[1] These deaths are primarily attributed to fentanyl and its analogs and mark an increase of nearly 9,000 from the year prior.[1] Moreover, due to their structural similarity, the analogs are challenging to definitively identify based only on comparison of electron-ionization mass spectra.

To address this challenge, this presentation will discuss a method previously developed in our laboratory to statistically evaluate mass spectra. Briefly, the method relies on the unequal variance *t*-test to compare the intensity at each *m/z* value in two mass spectra. If the intensity of one or more ions is statistically different between the two spectra, then the spectra are statistically distinguishable, with any such ion being defined as a discriminating ion. Our previous work demonstrated the ability to associate and discriminate structurally similar compounds, including fentanyl analogs.

The present study will assess this ability with three sets of positional isomers of fentanyl analogs. As a further test of robustness, the effect of spectral intensity on the association and discrimination is also evaluated. The isomers selected for this evaluation are the following: *ortho-*, *meta-*, and *para-*fluorobutyryl fentanyl (*o-*, *m-*, and *p*-FBF); *ortho-*, *meta-*, and *para-*fluoro methoxyacetyl fentanyl (*o-*, *m-*, and *p*-FMAF); and *ortho-*, *meta-*, and *para-*methylfentanyl (*o-*, *m-*, and *p*-MF). The FBF and FMAF analogs have a fluorine substituent on the aniline ring, while MF has a methyl group. Similarly, FBF and MF have an alkyl-chain substituent on the amide group, while FMAF has a methoxyacetyl group.

Each set of isomers was prepared at three concentrations (1.0, 0.5, and 0.1mg/mL) and analyzed in replicate by Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS). Spectra within each collection were statistically compared to evaluate the effect of spectral intensity on the ability to associate and discriminate the positional isomers. Additional spectral collections were completed in a similar manner for a total of four times over an eight-month period to evaluate the effect of inherent instrument variation.

In the first collection, at 1.0mg/mL, *o*-FBF was discriminated from the corresponding positional isomers *m*-FBF and *p*-FBF with 12 and 8 ions, respectively, responsible for discrimination at the 99.9% confidence level. Under the same conditions, *o*-FMAF was also discriminated from its corresponding positional isomers *m*-FMAF and *p*-FMAF with 25 and 21 discriminating ions, respectively. Similarly, *o*-MF was discriminated from the corresponding positional isomers *m*-MF and *p*-MF with 8 and 12 discriminating ions, respectively. As concentration decreased, discrimination was retained at 0.5mg/mL, albeit with fewer discriminating ions, but was lost for all comparisons at 0.1mg/mL.

Similar trends were observed across the four spectral collections. However, the number of discriminating ions per comparison differed, which is expected due to differences in instrument tune conditions. To allow for the consideration of both time and concentration of the comparison, the calculated *t*-values were ranked to determine the most reliable discriminating ions. For example, for the comparison of *o*-FBF and *m*-FBF, *m/z* 164 was the most reliable discriminating ion while for the comparison of *o*-FBF and *p*-FBF, *m/z* 102, 164, and 234 were the most reliable discriminating ions.

In this presentation, the statistical comparison method will be described in detail, the effect of spectral intensity on the discrimination of positional isomers will be discussed, and reliable ions for discrimination within each set of isomers will be identified.

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Fentanyl; Statistics; Mass Spectrometry

B122   The Classification of Cannabinoids Using Mass Spectral Data for the Identification of Novel Synthetic Cannabinoids

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Learning Overview: The goal of this presentation is to demonstrate the development of a drug classification system to facilitate the identification of previously unknown synthetic cannabinoids.

Impact Statement: This presentation will impact the forensic community by demonstrating the effectiveness of the use of classification models that were developed using mass spectral data and chemometric techniques in determining the presence of newly synthesized cannabinoids.

Detection and characterization of newly synthesized cannabinoids is challenging due to the lack of availability of reference standards and chemical data.[1] Identification of these substances usually involves the use of intelligence data, prior knowledge on New Psychoactive Substances (NPSs), and structural elucidation of these new compounds using expensive and time-consuming analysis methods such as High-Resolution Mass Spectrometry (HRMS) and Nuclear Magnetic Resonance (NMR) spectroscopy.[2,3]

The focus of this study is to develop a proactive solution to identify newly synthesized psychoactive substances. A classification system was developed using the existing mass spectral data of cannabinoids to determine the presence of previously unknown cannabinoid-related substances. Principle Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLSDA) were used to develop the classification system using mass spectra obtained from freely available spectral databases such as the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) database. Genetic Algorithm (GA) was used to select the most discriminatory mass to charge (m/z) ratios of the mass spectral data. First, a binary classification model was developed to discriminate cannabinoids and cannabinoid-related compounds from other drug classes. Then, a classification model was developed to discriminate classical and synthetic cannabinoids. Finally, submodels were developed to discriminate the presence and absence of functional groups found on commonly encountered synthetic cannabinoids. Hierarchical Cluster Analysis (HCA) was used to determine the possible drug classes, and HCA along with chemical structure similarities resulted in the different unique groups seen with synthetic cannabinoids that were classified. These groups include indole, indazole, naphthalene, 4-Fluorobenzyl (FUB), and 1-amino-3,3-dimethyl-1-oxobutan-2-yl (BUT).[2] Classification models were developed for the determination of the presence or absence of aforementioned functional groups in an unknown cannabinoid. Current results show that these models are highly accurate (>95%) and applicable in determining the presence of cannabinoid-related substances.

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3. Richard G. Brereton, Pattern recognition in chemometrics, *Chemometrics and Intelligent Laboratory Systems*, Volume 149, Part B, 2015, Pages 90-96.

Drug Classification; Novel Synthetic Cannabinoids; Chemometric Modeling

B123   **A Qualitative and Quantitative Analysis of Particulate Spread During Simulated Illicit Drug Manufacturing: Implications for Collection of Evidence and Crime Scene Safety**

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Learning Overview: After attending this presentation, attendees will have learned about ongoing efforts to understand the spread of particulate that results from different illicit drug manufacturing activities and about considerations for maximizing the chances of collecting actionable evidence and minimizing potential safety concerns for those on scene.

Impact Statement: This presentation will impact the forensic science community by providing critical information on the spread of drug particulate throughout a home due to illicit drug manufacturing events through qualitative and quantitative data and visuals.

Illicit manufacturing of drugs of abuse—from small-scale personal use efforts to large-scale operations—continues to be a concern in the United States and abroad. These efforts can include synthesizing psychoactive compounds from precursors, packaging powdered material, and using pill presses or other means to prepare samples for distribution. When these operations are discovered, evidence must be collected that can provide a link to actual material(s) being synthesized or handled. If the house has been cleaned up in any way, this information may only be obtainable via collection of particulate residues. It is also important that personnel entering the home be aware of possible dangers, even if they are explicitly visible.

To help address these questions, we have begun a project where illicit drug manufacturing activities (i.e., weighing powders, grinding powders, packaging material, etc.) are completed using inert materials inside a home that has been equipped with numerous sensors and sample collectors to enable analysis of airborne and surface particulate movement and concentration. Particle counters are placed in rooms throughout the home to provide real-time information regarding the generation and movement of airborne particulate. These measurements are coupled with laser light sheet imaging to provide real-time visualization of the airborne particulate generation. This data is coupled with quantitative information on particulate concentrations from surfaces throughout the home, obtained using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). These surface collections are completed immediately after the activities take place, as well as one day and one week after to better capture the level of resuspension and resettling that occurs. Factors in the home (i.e., air temperature, whether the HVAC system is running) or how the activities are carried out (i.e., messy versus deliberate) are altered to see the effects these parameters have on particulate generation and movement. Finally, other targeted collections or activities, such as measuring the total amount of material on the person's hand afterward or understanding how much material is transferred to a doorknob, are completed to help develop informed, data-driven sampling practices.

Initial studies completed using an 80 % lactose, 20 % caffeine mixture have begun to provide insight into the level of contamination that can be expected throughout a home where these activities have occurred. Surface levels of caffeine, the minor component in the mixture, were found to exceed $1\mu g\ cm^{-2}$ in the area immediately around where activities took place. Particulate concentrations in excess of $0.01\mu g\ cm^{-2}$ were found in adjacent rooms. Surface concentrations changed over the one-week study, providing insight into redeposition throughout the house due to HVAC air circulation. Using laser light sheet imaging, the magnitude of airborne particulate that resulted from these different activities was easily visualized, and stark differences in particulate movement as the result of the HVAC being on or off could be understood. The persistence of the airborne particulate, and its implications for personnel entering a home where drug manufacturing is suspected, could also be captured. Through these studies, it was found that milligrams of material remain on the person's hand post-activity and can be transferred onto a range of different surfaces, increasing the probability of collection with targeted sampling.

Through this work, a combination of visual aids (videos and images) and quantitative data will be presented to convey ongoing efforts to inform best practices for collecting trace residues in homes where suspected manufacturing occurred. These tools can be used to determine what surfaces to sample, what contamination levels can be expected based on whether or not a room has been deliberately cleaned, and what considerations should be made to ensure the safety of those entering a contaminated scene.

Clandestine; Seized Drugs; Crime Scene Investigation

B124   Streamlining the Extraction of Challenging Samples Using the HID NIMBUS® Presto

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Learning Overview: After attending this presentation, attendees will understand how the HID NIMBUS® Presto System can be used to increase lab efficiency and produce similar results in DNA recovery and autosomal allele recovery to manual extraction methods for challenging forensic sample types, including burned, buried, and surface decomposed bones, hair, nails, and teeth from decomposed cadavers, "touch" samples, and fired cartridge casings.

Impact Statement: This presentation will impact the forensic science community by demonstrating the HID NIMBUS® Presto System's capability to extract forensically challenging samples and yield comparable or better results than via manual extraction.

Automated extraction enhances sample throughput, minimizes errors, and maintains consistency within a forensic laboratory. However, automation can lead to higher amounts of DNA loss compared to manual methods. Therefore, it is necessary to assess the efficacy of a new automated purification platform, the HID NIMBUS® Presto System, compared to manual extraction for challenging forensic samples. The HID NIMBUS® Presto system combines the THERMO FISHER™ KingFisher™ Presto Purification system and the Hamilton NIMBUS® automated liquid handling workstation, utilizing PrepFiler® and PrepFiler® BTA extraction scripts. In this project, various challenging sample types such as bones, hair, nails, teeth, "touch" samples, and fired cartridges were processed using both the HID NIMBUS® Presto System and manual PrepFiler® chemistry. Extracts were quantified on the ABI® 7500 system using QuantiFiler® Trio. Short Tandem Repeat (STR) analysis was conducted using the VeriFiler™ Plus kit, which targets 25 markers, including 23 autosomal STRs and two gender discrimination markers, with a maximum input of 0.5ng. This kit was specifically designed to maximize sensitivity for challenging casework samples by allowing up to 17.5mL of sample input for amplification.

This study found no significant difference in DNA recovery between manual extraction and the HID NIMBUS® Presto System for fired cartridge casings, teeth, surface decomposed, and burned bones ($p > 0.05$). However, the HID NIMBUS® Presto System yielded statistically significantly higher amounts of DNA for "touch," hair, nails, and buried bone samples ($p < 0.05$). In terms of autosomal allele recovery, there was no significant difference for "touch," teeth, surface decomposed samples, and fired cartridge casings ($p > 0.05$). Generally, the HID NIMBUS® Presto System demonstrated significantly higher autosomal allele recovery for nail and hair samples ($p < 0.05$). Conversely, manual extraction outperformed the HID NIMBUS® Presto System regarding DNA recovery for buried and burned samples ($p < 0.05$). Importantly, more samples met the minimum threshold of 1pg/µL to proceed to STR typing when processed with the HID NIMBUS® Presto System compared to manual extraction. Overall, this study shows that the automated HID NIMBUS® Presto System can provide a more streamlined workflow and faster turnaround time compared to manual extraction without negatively affecting DNA recovery and allele recovery from extremely challenging samples.

Automation; DNA Purification; Low-Template

B125   The Effects of Oily Contaminants on DNA Extraction Efficiency Using DNA IQ™

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Learning Overview: The goal of this presentation is to demonstrate the challenges of DNA extraction in the presence of common oily contaminants and their effects on DNA yield. Following the presentation, attendees will better understand the impact that the contaminants have on DNA yield, the problem these contaminants pose to casework, and potential remedies for this.

Impact Statement: This presentation will impact the forensic science community by demonstrating the issues posed by these contaminants and the mitigation practices explored. Forensic analysts will be informed of the issue and encouraged to investigate further approaches to lessen the impact the contaminants have on sample DNA yields.

In forensic investigations, unknown evidence DNA profiles are compared to reference samples to include or exclude individuals. The DNA yield and profile quality from evidentiary samples are impacted by the origin, number of contributors, environmental exposure, and contaminants present within the samples. Widely used solid phase extraction chemistries remove most of the contaminants that can cause inhibition of Polymerase Chain Reaction (PCR) reactions, such as indigo dye.[1] Additionally, newer Short Tandem Repeat (STR) typing kits have improved capabilities to suppress the effects of PCR inhibitors. Despite these improvements, certain contaminants still negatively impact the DNA extraction process and ultimately the success of STR profile development. Common contaminants that can severely impact the DNA extraction process using solid-phase extraction chemistries include motor oil, hand cream, and personal lubricants.[2,3] The DNA IQ™ resin was reported to clump in the presence of certain contaminants, which is problematic when used on an automation workstation because it results in statistically significant reduced DNA yields.[2] Products such as Nivea® hand lotion, Silky® personal lubricant, and Replens® Vaginal Moisturizer, all commonly contain glycerin and carbomers.

This study examined whether adding Sodium Dodecyl Sulfate (SDS) and alcohol to the lysis incubation increased DNA yields and the quality of samples contaminated with Nivea®, Silky®, and Replens® for samples containing blood or saliva. Samples were processed using standard operating procedures for manual and automated extractions from the Virginia Department of Forensic sciences (VDFS).[4]

For blood, manual extraction using 0.75% SDS significantly increased DNA yield for uncontaminated samples and increased DNA yield for Silky®-contaminated samples, but not significantly. Adding 0.75% SDS to Nivea®-contaminated samples decreased yield, but the reduction was not statistically significant. Adding 0.75% SDS to lysis incubation prior to automated DNA purification increased DNA yield for all contaminated samples and significantly for uncontaminated samples. When 20% alcohol and 0.75% SDS were combined during manual extraction, the DNA yield increased for all sample types and significantly for Nivea®-contaminated and Silky®-contaminated samples. When the samples were incubated lysis buffer with combined SDS and alcohol, followed by automated DNA purification, all samples had an increased DNA yield; a significant increase for uncontaminated and Nivea®-contaminated.

A similar impact on DNA yield was observed in saliva samples with the additives in the lysis incubation buffer. For saliva, manual extraction using 0.75% SDS generated unchanged average DNA yields for all samples. The addition of 0.75% SDS to lysis incubations followed by automated DNA purification resulted in significantly increased average DNA yields for the uncontaminated samples. The Nivea®-contaminated samples had an increase in DNA yield and Silky®-contaminated had a decrease, neither of which was significantly different from controls for automated samples. Following incubation in lysis buffer with 20% alcohol, average DNA yields of all sample types were increased for both manual and automated DNA purification. The mixture of 0.75% SDS and 20% alcohol during lysis incubations significantly increased average DNA yields for uncontaminated and Nivea®-contaminated samples, while DNA yields decreased for Silky®-contaminated samples, but not significantly.

DNA yields for samples contaminated with Nivea® and Silky® can be increased when purifying DNA with the DNA IQ™ System through the inclusion of additives such as SDS and reagent ethanol during the cells' lysis incubation step.

References:
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Forensic Science; DNA Extraction; Contaminants

B126   The Development of a Method for Complete Collagenase I Digestion of *Bos Taurus* Bone Samples

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Learning Overview: After attending this presentation, attendees will understand a unique approach for sampling DNA from aged forensic bone samples, ways of integrating it into the traditional workflow, and how it can improve the collection of DNA from challenging biological samples. Attendees will also gain an understanding of the applications for chemical excision of cells in forensic analysis.

Impact Statement: This presentation will positively impact the forensic DNA community by optimizing a unique approach to forensic DNA analysis of degraded skeletal remains, which will have broad applications across numerous fields of study. This research aims to improve the ability to isolate genomic material from compromised skeletal remains.

Traditional methods for processing forensic bone samples involve pulverization of bone samples to achieve adequate exposure of genetic material to the extraction buffer.[1,2] However, this technique can introduce numerous Polymerase Chain Reaction (PCR) inhibitors, including those found in the extracellular matrix found in mature bone and competition from excessive microbial DNA, in addition to the degradation from pulverization that results in further damage to already degraded genetic material.[3]

Previous research by Li has demonstrated that collagenase I, a matrix metalloproteinase that breaks down Type I collagen in bone, can be a potent method for digesting forensic bone samples prior to DNA extraction.[4] Li did not observe any detrimental effects on Short Tandem Repeat (STR) profiles when utilizing collagenase alone, indicating that the combination of total demineralization collagenase I digestion utilized in this study may be an effective method for obtaining DNA from forensic bone samples. Additionally, research by Yonenaga et al. showed that cell viability was maintained when collagenase was utilized to digest cartilage samples.[5]

The purpose of this project was to evaluate a combination of the total demineralization described by Loreille et al. and treatment with collagenase I in aged and weathered bovine samples.[1] Bovine was chosen as a model organism due to reduced ethical restrictions and microscopic and collagen matrix similarity to human bone. The goal was to utilize chemical excision methods to release osteocytes from the matrix with minimal degradation and reduced inhibition.

Slices of demineralized bovine bone samples were digested with various concentrations of collagenase I solutions for varying lengths of time. Samples were evaluated using several different nuclear dyes and fluorescence microscopy, and the optimal dye was chosen based on binding mechanism, preparation time, and nuclear visibility to be used for visualization of the digested samples. Propidium iodide showed an affinity for collagen, as there was increased fluorescence with less-digested samples. Acridine orange allowed for some visualization of nuclei, but also required significant preparation time. Diamond Nucleic Acid Dye was ultimately chosen due to its ease of use and increased visibility of matrix structures and nuclei. Images were captured and efficacy of collagenase I digestion under different reaction parameters was evaluated.

Based on microscopy and observation of samples after digestion, collagenase digestion was demonstrated to be an effective method for removing the bone matrix while maintaining the integrity of the nuclei. At lower concentrations and shorter time periods for digestion, the demineralized fragments were still observable in the sample tubes. Bone matrix was still visible, and trabeculae and osteons were visualized. Under these parameters, nuclei were visible, but very few were free, and the majority trapped within the matrix. In contrast, under high concentrations and longer digestion times, the samples were easily pipetted from the tube onto the slide, more nuclei were freely visible against the black background, and no matrix was distinguished.

In conclusion, digestion of bone samples with collagenase I was shown to be an effective mechanism for improving front-end sampling of weathered, aged forensic bone samples. By utilizing tissue slicing and a chemical excision method, the PCR inhibitor-containing matrix can be removed with little damage to the DNA. Future research will involve investigating the usage of partial digestion with collagenase combined with cell capture methods to effectively isolate bone cells prior to DNA extraction.

References:
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Bone; Forensic DNA; Human Remains

B127 A Simple Tooth Preparation Method for the Applied Biosystems® RapidHIT™ ID Instrument

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Learning Overview: After attending this presentation, attendees will have a better understanding of rapid DNA technology and its ability to produce Short Tandem Repeat (STR) profiles from dental remains as an approach to reducing the backlog of Disaster Victim Identification (DVI) samples.

Impact Statement: This presentation will impact the forensic science community by introducing data to demonstrate an alternative method to processing dental remain samples. The implementation of the RapidHIT™ ID instrument could potentially allow for a more expedited, less labor-intensive processing of DVI samples to obtain STR profiles in forensic DNA laboratories or on-scene at disaster events.

DVI refers to the process of forensic identification of unknown individuals following a mass disaster or fatality incident.[1] In events of mass disaster, human dental structures are often the only viable DNA source remaining, as soft tissues are more easily degraded, contaminated, or otherwise compromised. Due to the protective nature of dental enamel and dentin surrounding the inner pulpal tissue, a labor-intensive sample preparation performed by extensively trained personnel is necessary to expose the nuclear material preceding the traditional forensic DNA workflow.[2,3] In high-volume cases, such as those often submitted for DVI, the current forensic laboratory procedures for tooth sample DNA processing have shown to simply be inefficient. Thus, this study evaluates the ability of the Applied Biosystems® RapidHIT™ ID instrument, which incorporates rapid DNA technology to develop an STR profile in as little as 90 minutes, to produce viable STR profiles from tooth samples when coupled with a simplified sample preparation protocol.[4] Although the RapidHIT™ ID instrument has been validated for use with buccal swab (reference) samples, minimal research exists describing its ability to process dental or skeletal remains.

In this study, a fast, low-tech tooth cleaning/preparation protocol, incorporating a Dremel® rotary tool and household hammer for pulverization, was developed and tested on a sample set of ten deciduous teeth, which were subsequently processed on the RapidHIT™ ID instrument. Additionally, a parallel sample set of ten deciduous teeth of the same type and age were processed using the same cleaning protocol paired with a coffee grinder for fine-powder pulverization prior to being processed using a traditional forensic DNA laboratory-based workflow. All samples were stored at -20°C for approximately 1 to 21 years prior to processing.

The average percent of expected STR alleles detected from tooth samples processed on the RapidHIT™ ID was 99.0%, whereas those processed manually using traditional methods was 99.8%. Although the average STR allele peak height for the RapidHIT™ ID sample set is significantly lower (2,483 Relative Fluorescence Units [RFU] versus 4,004 RFU for the manual set), both produced peak heights that were well within the internally validated optimal peak height ranges and well above established analytical and stochastic thresholds. Additionally, the average intralocus heterozygote peak height ratio was 0.80 for samples analyzed using the RapidHIT™ ID, as compared to 0.86 for those processed manually, demonstrating acceptable intralocus balance. Not surprisingly, the RapidHIT™ ID sample profiles exhibited greater interlocus imbalance; thus, caution should be used for analysis of challenged casework-type samples if more than a single contributor is expected. Minimal artifacts were noted in profiles of tooth samples analyzed using the RapidHIT™ ID, regardless of how aged the sample was; however, 9 of 10 samples analyzed did require analyst review due to incomplete adenylation (-A peaks) and/or high baseline noise in the Y-indel target area for female samples.

These results suggest that, when coupled with a simple, quick sample preparation protocol, the RapidHIT™ ID instrument can successfully produce complete STR profiles from aged tooth samples with minimal allelic drop-out and high-profile quality. Future studies should explore the viability of this method using more challenged tooth samples of varying ages, tooth pathologies, and those that are exposed to harsher environmental conditions. Further simplification of the sample preparation process should also be explored in order to make this approach more amenable to on-scene application.

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Rapid DNA; STRs; Unidentified Persons

B128   The Application of Ancient DNA Methodologies to Badly Burned Forensic Samples

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Learning Overview: This presentation examines the incorporation of methodologies adopted from the analyses of ancient DNA applied to the isolation and analyses of degraded DNA from badly burned forensic samples. Attendees will learn about new techniques and guidelines for the successful DNA identification of severely thermally altered remains.

Impact Statement: This presentation will impact the forensic science community by serving as an optimal laboratory strategy guide for basic sampling strategies and locations, DNA extraction methods, and Short Tandem Repeat (STR) profiling when dealing with highly degraded, burned skeletal samples.

Identifying human remains using DNA analyses is a vital component of forensic investigation. These highly accurate analyses generally rely on the recovery of high-quality endogenous DNA that may not always be available. This is especially true when utilizing degraded source material.

The decomposition of DNA can alter not the amount of DNA retained in source tissue, the quality of the DNA, and alter its base composition, making downstream analysis problematic. As such, the field of ancient DNA (aDNA) analysis has invested heavily in the development of optimized protocols for the sampling, extraction, and analyses of DNA recovered from archaeological skeletal. In a forensic context, the use of these same techniques in modern, degraded skeletal samples may increase the likelihood of successful DNA identification[1].

The exposure of tissue to extreme temperatures affects DNA recovery and quality in a similar fashion to that observed in archaeological remains. Additionally, while soft tissue may still be present, many current guidelines recommend the removal and discarding of this charred material, as it is hypothesized that the extreme levels of morphological degradation render this substrate unusable for DNA identification.

Here we present a systematic investigation comparing forensic and aDNA laboratory protocols: the Dabney 2019 extraction protocol and the Lorielle 2007 protocol.[2,3] We examine DNA yields across a range of levels of thermal alteration on different skeletal locations and an assessment of DNA preservation in severely charred soft tissues using the QIAGEN® DNeasy® blood and tissue extraction kit.

Ten donor cadavers were systematically exposed to extreme temperatures (i.e., burned) at the University of Tennessee Anthropology Research Center. From each donor, approximately ten samples representing all regions of the body (i.e., thorax, long bones, etc.) were collected and sent to Arizona State University for processing. Each sample was then visually examined and assigned a burn scoring on a 1–5 scale, with 1 being the least thermally altered and 5 being the highest (i.e., cremains) based on observed morphological condition. Using both extraction protocols, DNA was isolated from each skeletal sample, as well as from corresponding tissue samples. The resulting DNA extracts were then assessed for total DNA recovery (Qubit® HS DNA assay and Agilent® TapeStation D5000 HS), endogenous DNA content (Quantifilier® Trio), and STR profile recovery (Promega® Powerplex® Fusion 6C).

Preliminary results indicate that our standard DNA quantification techniques (Qubit® fluorometry, TapeStation, and Quantifiler® Trio) are not a reliable predictor of successful STR profile recovery, likely due to high levels of co-extracted inhibitors. We additionally find that charred tissue samples consistently returned higher concentrations of both raw and endogenous human DNA as well as more robust STR profile recovery. This indicates that a re-evaluation of previously established sampling guidelines for severely thermally altered remains recommending the removal of this substrate may be necessary moving forward. In terms of our skeletal samples, the complete demineralization protocol developed by Lorielle et al. generally performed well at lower-medium levels of thermal alteration while the aDNA Dabney protocol was more suited for STR profile recovery at higher estimated levels of thermal alteration.

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DNA; Bone; Victim Identification

B129   A DNA Analysis of Degraded Skeletal Remains Obtained From the Petrous Bone, Tooth, Femur, Tibia, and Humerus: A Comparison Study

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Learning Overview: The goal of this presentation is to determine that petrous bone yields significantly more DNA than teeth, the femur, the tibia, or the humerus; yet the number of alleles obtained from petrous bone and teeth is approximately comparable. In the former, more reportable markers are obtained than in the latter, which results in obtaining more valuable genetic data. In conclusion, it is highly recommended to sample both petrous bones and teeth when performing DNA analysis on highly degraded human remains. Petrous bone or tooth sampling is recommended if DNA typing will be performed on skeletal remains that have undergone environmental degradation. We have been able to increase the success rate in the remains that are currently being examined thanks to this insight.

Impact Statement: This presentation will impact the forensic science community by informing attendees that since only bones and teeth remain after long periods of environmental exposure and degradation, DNA typing from skeletal remains is a useful tool in forensic, archaeological, and ancient DNA studies. Humidity, high temperature, salinity, and low pH are environmental elements that have a detrimental effect on DNA preservation, causing molecular damage and fragmentation that renders laboratory recovery of the DNA difficult. In this situation, the forensic DNA community may find it advantageous to perform ancient DNA techniques.

The only biological material that remains for a long period of time is skeletal remains. However, environmental factors like humidity, temperature, and pH have an effect on DNA preservation, making skeletal remains a difficult sample for DNA analysis.

The selection of samples is an important consideration, and conventionally, the most valuable genetic material substrates have been suggested to be the femur and the tooth. Due to its higher DNA yield, the cochlear region of the petrous bone has recently been recommended as an alternative better option. The objective of this study is to assess petrous bone's efficiency in comparison to other cranium samples (teeth) and post-cranial long bones (tibia, femur, and humerus). From 42 different individuals, a total of 92 samples were collected. The samples were extracted using a procedure for organic extraction, and the DNA was measured using a Quantifiler® Trio kit and amplified using a GlobalFiler® kit. In quantification data, petrous bone performs better than other bone fragments, yielding 20–30 times more DNA than the others, as demonstrated by the results. Regarding observed alleles, DNA profile data showed similarities between petrous bone and tooth samples; nevertheless, the volume of DNA extracted from petrous bones allowed us to obtain more informative DNA profiles of superior quality.[1-8]

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Forensic Genetics; Ancient DNA Analysis; Degraded Bone and Tooth Remains

B130   Comparing Likelihood Ratios of Degraded DNA Mixture Profiles Using DNA-View® Mixture Solution™

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Learning Overview: After attending this presentation, attendees will have learned about the performance of a non-Markov Chain Monte Carlo (MCMC) continuous-model probabilistic genotyping software that was used in the analysis of complex three-person mixtures.

Impact Statement: This presentation will impact the forensic science community by demonstrating that the Mixture Solution™ software is a viable alternative to MCMC methods for the analysis of complex DNA mixtures, particularly mixtures that consist of degraded DNA.

Interpreting DNA profiles manually can potentially call into question subjectivity between analysts who may interpret specific results differently. There are multiple features of a DNA profile that can complicate interpretation, which include allelic drop-out and drop-in, allele sharing, and Polymerase Chain Reaction (PCR) artifacts, as well as degradation of the DNA itself, which can be caused by various environmental factors. Developments in DNA profile interpretation using probabilistic genotyping software have been made to assist in the complicated task of deconvoluting and interpreting a challenging mixture. Among these programs is DNA-View® Mixture Solution™, a continuous-model probabilistic genotyping software.[1] Mixture Solution™ is unique in that it is not based on the MCMC approach used by other programs such as STRmix™ and TrueAllele®. Mixture Solution™ starts with a hypothesis (either H_p or H_d) and uses it to compute M (a representation of the mixture data), whereas MCMC methods use the mixture data to generate a probability computation for a list of multiple possible deconvolutions. This difference allows Mixture Solution™ to calculate the hypotheses independently, eliminating the need for the analyst to make a guess about the number of contributors to the mixture.

In this research, Mixture Solution™ was used to provide statistical analyses for three-person DNA mixtures where one or more of the contributors had been degraded by exposure to heat, through the assignment of a likelihood ratio between two given hypotheses based on the mixture data. The calculated likelihood ratio would either support the hypothesis that the person of interest contributed to the mixture, or support the alternate hypothesis, that the person of interest was not one of the contributors. The three-person mixtures were prepared at four different contributor ratios with varying combinations of three levels of degradation: no degradation, partial degradation, and full degradation, using controlled heating to systematically degrade the DNA template prior to amplification. Using two hypothesis tests, Mixture Solution™ was used to compute likelihood ratios for each of the mixtures with a variety of defined people of interest.

All computed likelihood ratios that favored the ground truth hypothesis provided "moderate support" or higher. However, when the DNA from a person of interest was degraded, decreases in the likelihood ratio values were observed when compared to the values computed for undegraded DNA from the same person of interest. These decreases occurred primarily as a result of allelic dropout caused by degradation. While allele sharing was also determined to be another major factor in the differences between likelihood ratio values across DNA mixtures with varying levels of degradation, stutter had little impact on changes in likelihood ratios. Results showed that Mixture Solution™ successfully generated appropriate likelihood ratios for 97% of the computations performed for each of the 20 mixtures. Significant levels of dropout resulting from the degradation of the DNA of the person of interest in the remaining 3% of computations pushed the likelihood ratio values into the "uninformative" range.

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Forensic DNA; Probabilistic Genotyping; Mixture Analysis

B131   A Picture Is Worth 10,000 LRs: The Evaluation and Implementation of Tools to Improve Interpretation and Reporting of Mixtures Using Probabilistic Genotyping

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Learning Overview: After attending this presentation, attendees will have been introduced to tools that may help improve concordance between experts and laboratories for the final step of the DNA interpretation process for samples with low Likelihood Ratios (LRs) and whether to report the LR as inclusive or inconclusive with regard to the Person Of Interest (POI).

Impact Statement: This presentation will impact the forensic science community by introducing practitioners, investigators, and attorneys to a method that comprises a reporting utility threshold that is determined by the characteristics of the specific case sample itself rather than from some series of validation samples that have nothing to do with the evidence in question.

In the United States, there are two predominant options for Probabilistic Genotyping (PG); STRmix™ and TrueAllele®. These two software programs are based on different models yet reach similar results in most mixture examples. However, because of the differences between models, differences in the resulting LRs are both expected and observed in both published studies and in case samples.

This study recruited STRmix™ laboratories to investigate differences in the reporting of LRs for the POI from a set of common DNA samples. Two sets of samples from the publicly available PROVEDIt data set were provided, along with the DNA reference of a trace level true donor, to see if there are differences between laboratories that use the same PG software. The laboratories were told that the samples would range from single source to four-person mixtures, as the goal of this study was to focus on the reporting of inclusionary versus inconclusive conclusions for the POI.

There have been several concordance studies for both inter- and intra-laboratory experiments reported in the literature, but most of these focus on the entire process. Each laboratory uses their complete protocol, including the determination of Number of Contributors (NoC), Analytical Thresholds (AT), STRmix™ parameters, and, finally, reporting statements that mention the inclusionary, exclusionary, inconclusive, or uninformative status of the LR with respect to the POI. This study provided some basic information about NoC, and all laboratories used the same AT and STRmix™ settings in an effort to isolate differences to the final, and arguably most impactful step, the determination of the potential donor status of the POI.

Each laboratory agreed to committing at least two volunteers to interpret either Set A or Set B (n=8 in each set) mixtures according to the validated protocols of each laboratory. The LRs and the report wording used for each sample was submitted for comparison. The initial interpretations varied between exclusion, inconclusive, and inclusionary conclusions for the same samples and references.

Many of the inconclusive statements were related to complexity thresholds determined during validation using internal samples that had nothing to do with the samples in this study. Such statements were based on "inconclusive zones" (e.g., LRs between 2 and 1,000) or other forms of mixture complexity or minimum data requirements.

Two companion tools were then introduced to the laboratories; Advent*LR* and DBLR™ that allow for non-donor testing (H_d True) first proposed by Gill and others.[1-4] These tools allow for the exploration of 10,000 or more non-donor profiles and their resulting LRs and comparison to the LR of the POI in the case. Schuerman proposed a utility threshold of the 99.9 percentile LR as a decision point for reporting an LR as inclusionary or inconclusive due to a risk of an adventitious inclusion.[1] Both tools present a visual output that can show how the LR of the POI compares to the population at large.

Laboratories were trained on the use of these two tools and then asked to swab mixture set and repeat the experiment. The only difference is that the reporting statements in the second round of interpretation were to be guided by the 99.9 percentile utility threshold.

The reporting results using these tools will be presented along with the change in concordance for the most important step in DNA analysis: the inclusion or not of the POI as a possible contributor.

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Probabilistic Genotyping; Low DNA; Interpretation

B132   A Comparison of the Two Most Widely Used Probabilistic Genotyping Systems Supports Accuracy and Concordance

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Learning Overview: This presentation will inform the forensic community on how STRmix™ and TrueAllele® Casework Probabilistic Genotyping (PG) systems generated concordant results for the majority of samples tested and provide possible explanations for those instances where the two PG systems disagreed.

Impact Statement: This presentation will impact the forensic science community and the DNA examiner's understanding of how PG systems function and will increase their expertise in this area of DNA analysis.

The development of PG systems to quantitatively analyze DNA mixture samples has been transformative in forensic science. TrueAllele® Casework (TA) and STRmix™ are the two most widely used PG systems. These systems model electropherogram data, utilizing both similar and dissimilar mathematical approaches.[1,2] The systems were interrogated with 48 challenging two-, three-, and four-person mock casework samples, for a total of 152 Likelihood Ratio (LR) comparisons. Of the 48 different mock casework mixtures, 35 contained at least one degraded DNA sample, and 46 out of the 48 contained at least one low-template DNA contributor (<100 pg). TA and STRmix™ converged on the same result ~91% of the time for mock casework (both systems included, were inconclusive, or excluded the reference profile). The 9% of the LR comparisons where results from the two PG systems were not in agreement corresponded to 14 LR comparisons. Seven of these comparisons resulted in one system including a low-level contributor and the other providing an inconclusive log(LR), and five of these resulted in one system excluding a low-level contributor while the other provided an inconclusive log(LR). The remaining two were contradictory (inclusion versus exclusion). The instances of differing results were likely due to dissimilarities in how the systems model allelic drop-out and stutter. The PG systems exhibited high correlations for both estimated contributor-specific template quantities (>92%) and log(LR)s produced (>87%) for mock casework samples. When log(LR) comparisons were parsed to those for contributors <100pg and those >100pg, the correlation dropped significantly for those log(LR)s < 100pg (~64%) and increased for those >100pg (~89%). Despite the differences between the systems in log(LR)s observed for low-template contributors, both were able to detect contributors at ~1% if sufficient allele information was present given their respective modeling systems, demonstrating very high sensitivity for both. The findings in this study comparing the results from the TrueAllele® Casework and STRmix™ systems will be presented as well as possible explanations to account for the limited instances of disagreement.

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Probabilistic Genotyping; Criminalistics; DNA

B133   Beyond STRs: A Snippet Into the Challenges of Interpreting iiSNPs

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Learning Overview: The aim of this presentation is to summarize some of the considerations in the interpretation of Next Generation Sequencing (NGS) Single Nucleotide Polymorphism (SNP) mixtures and show some deconvolution results of NGS SNP mixtures using published biological models implemented for the interpretation of NGS autosomal Short Tandem Repeat (aSTR) mixtures using a probabilistic genotyping solution.

Impact Statement: This presentation will impact the forensic science community by providing the community with some background on SNPs and will demonstrate a possible probabilistic genotyping solution for the interpretation of mixtures generated using this technology.

Some forensic laboratories have shown a growing interest in adopting NGS or Massive Parallel Sequencing (MPS) technologies for a wide range of applications, including genetic genealogy, missing-persons identification, phenotyping, and paternity testing. A key advantage of these technologies is their ability to amplify a diverse set of markers in a single reaction, encompassing aSTRs, Y-chromosomal Short Tandem Repeats (Y-STRs), X-chromosomal Short Tandem Repeats (X-STRs), and SNPs.

Unlike contemporary Capillary Electrophoresis (CE) methodologies for the analysis of aSTRs, NGS can detect sequence variations in a stretch of STRs. Because of this potential benefit of detecting variation at the sequence level, numerous studies in recent years have investigated the behavior of aSTRs in NGS DNA profiles. This led to the development of quantitative models for describing expected NGS DNA profiles.[1-4] As a result, probabilistic genotyping solutions have been developed, enabling the interpretation of aSTR mixtures using sequencing technology. Several of these solutions have undergone rigorous developmental validation, gaining recognition through publication and presentations at conferences.[5,6]

Despite significant progress in the interpretation of aSTR markers in NGS DNA profiles and the added benefit of detecting sequence variation, current methodologies are still only harnessing a fraction of the information within a DNA profile amplified using sequencing multiplexes.[7-9] Notably, data regarding the identity of informative SNP (iiSNP) markers are often disregarded during the interpretation process.

This presentation examines the behavior of iiSNPs in forensic DNA profiles. We will highlight the distinctive characteristics of iiSNPs compared with aSTRs, including stutter, allelic balance, and the biallelic nature of SNPs. Additionally, we will present the Likelihood Ratio (LR) results obtained after the interpretation of 51 profiles each with 94 iiSNP markers using a probabilistic genotyping solution.

These profiles include 26 single-source profiles with varying target template amounts, and 25 two-person mixtures experimentally designed to exhibit varying mixture proportions and template amounts. To add depth to our findings, we compare the LR results from the interpretation of the iiSNPs with those derived from an alternative probabilistic method. Additionally, these results were compared with the interpretation of aSTR markers found within the same profile. The integration of iiSNPs alongside aSTRs in forensic DNA analysis aims to utilize more information from the DNA profile, potentially enhancing the discrimination power of forensic investigations.

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Next Generation Sequencing; Probabilistic Genotyping; Mixture Analysis

B134   An Evaluation of the FORCE Panel on Alternative Reference Materials

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Learning Overview: After attending this presentation, attendees will understand how low-level DNA samples, such as hair and fingernails, can be used as alternative reference samples utilizing the FORensic Capture Enrichment (FORCE) panel and Massively Parallel Sequencing (MPS).

Impact Statement: This presentation will impact the forensic science community by teaching about emerging technologies such as the targeted Single Nucleotide Polymorphism (SNP) panel that enables low-level DNA samples to be used as alternative reference materials in cases where buccal swabs are not easily accessible or when high-quality DNA are unable to be collected. Hence, this technology, when implemented, will support challenging cases such as identifying human remains of missing persons.

A frequent problem encountered in crime scenes is poor quality samples that may be environmentally degraded or contain low-level DNA. Short Tandem Repeat (STR) analysis for human identification requires samples of standard forensic quality, which is one reason that SNPs are becoming more accepted and validated in the forensic community.[1] Buccal swabs are considered the gold standard of reference samples; however, in cases where buccal swabs are unable to be collected (such as from elderly individuals), alternative reference materials can be used. Some examples of low-level DNA samples include naturally shed hair without the root, deteriorated bones samples, and fingernails. To implement a more efficient technique to analyze all qualities of DNA samples, the FORCE panel made of forensically relevant SNP markers was developed.[2] This panel targets SNP markers for identity, ancestry, phenotype, and X- and Y-chromosomal SNPs.[2] Furthermore, the FORCE panel is compatible with MPS. As a result, this panel is an effective assay for degraded DNA samples, which can ultimately reinforce criminal investigative clues of suspect genealogy that cannot be performed with STR testing.

This project evaluated the FORCE panel on alternative reference samples such as fingernails and hair in comparison to buccal swabs. Preliminary experiments were conducted to optimize the DNA extraction from three sample types: (1) hair with root, (2) hair without root, and (3) fingernails. In tier one of this study, an extraction method for hair and fingernails was developed. Male and female samples were collected for each alternative reference sample type and extracted in triplicate. To prevent exogenous DNA contamination, samples were washed with a 5% Tergazyme™ cleaning solution and rinsed with ethanol and deionized water. The QIAGEN® EZ1® DNA Investigator® Kit was used following the trace protocol to extract DNA on the QIAGEN® EZ1® XL and samples were quantified with the Quantifiler™ Trio kit on the QuantStudio™5. The results showed that the rooted hair produced low degradation index values (0.98) and high DNA concentration with an average of 3.3ng/µL. The fingernail samples produced relatively low degradation (3.7) and average DNA concentration of 0.6ng/µL. However, the rootless hair had very high degradation index (6.8) and low DNA concentration averaging 0.02ng/µL. After the initial extraction optimization phase, for tier two of this study, five donors were recruited for each sample type (n=15), in addition to buccal swabs (n=5), and each sample was extracted based on the optimized protocol. Following the extraction, quantification and normalization of the extracts were performed. Libraries were prepared using the QIAseq® Targeted DNA HC Panel following the manufacturer's guidelines. The libraries were quantified with the Qubit™ fluorometer and the Agilent® 2100 bioanalyzer was used as a quality control check. The purified, pooled libraries were sequenced on the MiSeq® FGx®. Analysis was performed on the QIAGEN® CLC Genomics Workbench system using a custom pipeline. The hair and fingernail profiles were compared to buccal swab results to determine whether low level DNA samples possess adequate quality and quantity of DNA for analysis.

The results of this study demonstrate that hair and fingernails may be suitable as alternative reference materials for the FORCE panel in cases where buccal swabs are not readily available. These findings will provide the forensic community with more options when it comes to reference materials for human identification.

The opinions or assertions presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the Defense Health Agency, or the Armed Forces Medical Examiner System.

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Forensic DNA; SNP; Next Generation Sequencing

B135 Variant Allele Characterization in STR Markers Using Next Generation Sequencing

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Learning Overview: The goal of this presentation is to inform attendees about the development of a protocol for use in monoplex amplification and simultaneous sequencing of up to 24 autosomal Short Tandem Repeat (STR) loci used in forensic DNA Capillary Electrophoresis (CE) testing kits when unexpected CE results are obtained.

Impact Statement: This presentation will impact the forensic science community by demonstrating that a uniform monoplex Next Generation Sequencing (NGS) protocol for 24 autosomal STR markers makes the Variant Allele Sequencing Program a more efficient service that the National Institute of Standards and Technology (NIST) provides to forensic laboratories.

The Variant Allele Sequencing Program at NIST has traditionally used Sanger sequencing to identify genomic variations within Polymerase chain Reaction (PCR) amplicons containing STRs, particularly variants that result in CE null alleles and alleles that fall outside of the CE allele sizing bins provided by kit manufacturers.[1] However, this process has a low throughput capacity, making it time- and labor-intensive. To address this problem, a protocol was developed at NIST to provide the forensic community with a quicker and more straightforward sequencing method with NGS. NGS technology is a powerful tool for diagnostics and is already widely used in clinical testing to identify mutations across the genome.[2] By applying NGS technology to forensic samples with unexpected CE-STR results, additional information can be gathered about the molecular basis for variant alleles, and this information can be published and shared across the forensic community. The development of this protocol also increases awareness and encourages the integration of NGS technology in forensic laboratories to further improve forensic DNA typing for human identification.

The goal of this project was to identify uniform conditions (target input amount of DNA, PCR cycle number, and primer annealing temperature) for the initial monoplex amplification step that can be used for 24 autosomal markers found in commercial forensic CE typing kits. Primers for this protocol were originally designed to amplify regions differing from commercial kit amplicons.[1] For this work, amplicon size and annealing temperatures were also taken into consideration, and primers for 13 of the 24 loci were redesigned from the original primers to yield amplicon sizes below 500 base pairs (accommodating 2 x 250 read length sequencing chemistry). By designing primers with similar annealing temperatures, decreased amplification efficiency due to the thermal instability of these markers can be minimized.[3] Through empirical testing, the ideal amplification parameters were found to be 1ng/µL input DNA with a 60ºC primer annealing temperature, and 35 PCR amplification cycles. Following amplification, quantitation, and library preparation, the samples were sequenced using a Verogen® MiSeq® FGx® and resulting sequence data were aligned to the GRCh38 Human Genome Reference sequence. These results provide an understanding of the genomic basis (single nucleotide polymorphism, insertion, or deletion) causing the CE STR variant allele, provided the variant is included within the large NGS amplicon. A uniform monoplex amplification protocol that can be used for sequencing 24 autosomal STR markers either together or separately makes the Variant Allele Sequencing Program a more efficient service that NIST provides to forensic laboratories.

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Next Generation Sequencing; Sanger Sequencing; Short Tandem Repeats

B136 **Streamlining Degraded Sample Processing in Forensic Laboratories: Developing a Decision Tree for Capillary Electrophoresis and Next Generation Sequencing Platforms**

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Learning Overview: The goal of this presentation is to compare Capillary Electrophoresis (CE) and Next Generation Sequencing (NGS) chemistries in forensic laboratories, focusing on the ForenSeq™ MainstAY Kit and Investigator® 24plex QS Kit. This presentation aims to develop a decision tree for streamlined selection between CE and NGS platforms for effective processing of degraded samples.

Impact Statement: This study's findings on the comparative evaluation of CE and NGS chemistries in forensic DNA analysis have significant implications for laboratories facing the decision of choosing the most suitable analytical method. The development of a decision tree to select between CE and NGS platforms will streamline the processing of degraded samples, improving efficiency and accuracy in forensic investigations. By providing valuable insight into the strengths and limitations of each technique, this research will impact the forensic science community by contributing to advancing forensic DNA analysis and enhancing the capabilities of forensic laboratories in handling challenging casework.

Forensic laboratories face a critical decision when choosing between CE and NGS chemistries for their analytical needs, especially with the recent approval of the ForenSeq™ MainstAY Kit by the National DNA Index System (NDIS). CE offers rapid and cost-effective DNA analysis, particularly for Short Tandem Repeat (STR) genotyping. On the other hand, NGS provides a comprehensive analysis of DNA, enabling the identification of sequence variants and complex mixtures, offering enhanced sensitivity, and generating large amounts of data. However, implementing NGS technologies requires significant investments in equipment, training, and data analysis expertise, which may be excessive for routine forensic casework.

To determine the suitability of NGS over CE chemistries, quantification and degradation indices can serve as decision points for forensic laboratories, given the reported sensitivity of NGS and smaller amplicon sizes in most NGS chemistries. The ForenSeq™ MainstAY Kit contains 21 autosomal STR loci less than 250bp, whereas the Investigator® 24plex QS Kit has only 10 autosomal STR loci completely less than 250bp.

This study evaluates the ForenSeq™ MainstAY Kit and the Investigator® 24plex QS Kit, both approved by NDIS. The MainstAY kit includes 27 autosomal STRs and 25 Y-chromosomal Short Tandem Repeat (Y-STRs), while the 24plex QS kit contains 21 autosomal STRs. The evaluation involved enzymatically degraded samples and real-world challenging samples. Controlled degradation was carried out using the Turbo DNA-free Kit with increasing amounts of DNase enzyme (0–2 units) to artificially degrade DNA extracts to different degrees. Preliminary results showed that 0.05 units of DNase severely affected the Investigator® 24plex, with only 22% of alleles recovered, while Signature Prep showed autosomal STR recovery at 66%. This trend is expected to be found with MainstAY as well. Furthermore, various challenging samples with degradation indices ranging from 1 to 300 were tested, with genotypes of shared loci consistent between the NGS and CE methods. The MainstAY kit demonstrated similar or improved percent recovery compared to CE for these samples, and even in cases with reduced percent recovery, it recovered more loci, providing more information than the CE method.

Additional studies will include controlled degradation experiments with the MainstAY kit and evaluate comparative recovery in a wider range of skeletal remains, leading to the development of a decision tree for laboratories to process degraded samples.

Next Generation Sequencing; Forensic DNA; Degraded DNA

B137   Maximum Recovery of DNA From Degraded Remains

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Learning Overview: After attending this presentation, attendees will understand how technical advances used in isolating and sequencing genomic DNA from ancient remains opens new doors for the analysis of modern degraded tissue. This presentation will discuss the efficiency of isolating genomic DNA using ancient DNA and modern forensic extraction methods and their success rates for subsequent Short Tandem Repeat (STR) and mitochondrial genome analyses. Attendees will also learn about DNA damage patterns as assessed using bioinformatic analyses of genomic data revealing how hot desert environments impact DNA preservation in skeletal remains in the American Southwest.

Impact Statement: This presentation will impact the forensic identification community by presenting the results of the use of ancient DNA techniques applied to challenging cases. When standard DNA techniques fail due to tissue degradation, DNA extraction and Next Generation Sequencing (NGS) methods targeting small DNA fragments are viable options.

According to the National Missing and Unidentified Persons System (NamUs), as of February 2023, there are over 600,000 unidentified human remains in the United States. An average of 4,400 are added each year, of which roughly 1,000 remain unidentified. Degradation of these remains presents technical challenges for their identification by researchers and government agencies alike. Techniques used to isolate ancient DNA (aDNA) from archaeological samples could be efficient in cases of highly degraded forensic remains. For example, a method for DNA extraction (using guanidine hydrochloride) was developed by Dabney et al. to recover DNA fragments as small as 30–50bp in size.[1] This method has been used to successfully recover analyzable mitochondrial DNA (mtDNA) data from paleoanthropological samples as old as ~400,000 years.[2]

For this study, the Maricopa County Office of the Medical Examiner (MCOME) provided 75 skeletal samples representing 42 individuals who have remained unidentified by standard forensic procedures, such as STRs. DNA from bone and teeth samples was extracted using the Dabney protocol as well as a forensic protocol developed by Loreille and colleagues for degraded samples[3]. The DNA extracted was used to create double- and single-stranded libraries. These libraries were then used for targeted enrichment of the mitochondrial DNA genome performed using biotinylated mitochondrial RNA baits synthesized from the *H. sapiens* Representative Global Diversity Panel (197 mtDNA sequences) (Daicel Arbor Biosciences, Ann Arbor, MI). **Single Nucleotide Polymorphism (**SNP) capture was completed using a custom SNP panel targeting ~4,200 SNPs (Daicel Arbor Biosciences, Ann Arbor, MI). These enriched libraries were then subjected to Illumina® sequencing.

We found that the Dabney extraction method resulted in an average 4.4-fold improvement in DNA yield when compared to the Loreille extraction method. From the double-stranded DNA libraries, we generated mitochondrial genomes ranging from 0.3-246.8x depth of coverage with average fragment sizes of 89bp from 62 samples. Sequencing reads were not recovered from 13 samples, likely due to lack of sufficient DNA. Analyses of the mtDNA sequence data from the single-stranded libraries and the sequencing of the genome-wide SNP enriched libraries are currently underway. Using these data as well as additional analyses of DNA damage patterns and preservation across skeletal elements and environmental contexts, we aim to identify the optimal means of DNA recovery from degraded skeletal tissues.

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Mitochondrial DNA; Next Generation Sequencing; Degraded DNA

B138   An Assessment and Validation of the IDseek® mYSTR™ Reverse Complement PCR Technology for Y-Chromosomal STR Profiling Utilizing the MiSeq® FGx System

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Learning Overview: After attending this presentation, attendees will learn about a novel technology using a Reverse-Complement Polymerase Chain Reaction (RC-PCR) -based Massively Parallel Sequencing (MPS) library preparation solution for Y-chromosomal Short Tandem Repeat (Y-STR) profiling. Attendees will learn its kinetics, performance, and how it is beneficial compared to the conventional Capillary Electrophoresis (CE) -based methodology. Attendees will also be introduced to a user-friendly custom pipeline to analyze the sequencing data using MixtureAce™.

Impact Statement: This presentation will impact the forensic science community by providing an alternative Y-STR amplification technology for Y-STR analysis utilizing IDseek® mYSTR™ Y-Chromosomal STR Profiling kit (v2.0) and MPS technology. The chemistry incorporates RC-PCR technology, which simplifies the library target enrichment step required for MPS.

RC-PCR is an efficient target enrichment method that helps to simplify the laborious and time-consuming processes of MPS. RC-PCR allows for simultaneous amplification and tagging of a targeted sequence in a single tube.[1,2] Due to the single tube reaction mechanics, there is a reduction for possible sample switches and a decrease in potential contamination. In addition to the manual advantages, RC-PCR is also highly sensitive and specific because the specific primers are synthesized during the reaction. This allows for the concentrations of primers and amplicons to be more congruent.

The IDseek® mYSTR™ Y-Chromosomal STR Profiling kit employs the RC-PCR technology for the amplification of 30 Y-chromosomal STR markers plus Amelogenin in a single multiplex, closed tube reaction. Previous work was completed to assess concordance of the IDseek® mYSTR™ Y-Chromosomal STR Profiling kit with a Lebanese population (N=83) on the MiSeq® FGx. This was completed by comparing the mYSTR™ haplotypes with data previously generated with the PowerPlex® Y23 and AB Yfiler® Plus PCR amplification systems and CE methods at the 24 overlapping loci. The assessment demonstrated that the IDseek® mYSTR™ Y-Chromosomal STR Profiling kit can produce high-quality results while simplifying the MPS library preparation method. Since the original study, the kit has been updated to include three rapidly mutating Y-STRs (DYS449, DYS518, and DYS612). This study aims to assess the new version (v2.0) of the IDseek® mYSTR™ Y-Chromosomal STR Profiling kit and validate the kit in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines.

The IDseek® mYSTR™ Y-Chromosomal STR Profiling kit (v2.0) was assessed by comparing haplotype results to the AB Yfiler™ Plus PCR amplification system and CE method for a small subset of previously tested Lebanese samples. This assessment shows if the IDseek® mYSTR™ Y-Chromosomal STR Profiling kit (v2.0) performs reliably and as expected. Precision and accuracy studies, sensitivity and stochastic studies, mixture studies, mock evidence sample studies, and a contamination assessment were performed during the validation. Raw FASTQ data files were processed by MixtureAce™ via a custom pipeline. Male and female two-person mixtures with a range of concentrations were evaluated to determine the sensitivity of the IDseek® mYSTR™ Y-Chromosomal STR Profiling kit (v2.0). Additionally, inter-locus balance and stutter filters for each locus were determined and novel sequence-based alleles identified. RC-PCR has been shown to be beneficial for the MPS workflow and produces reliable results while reducing hands-on laboratory time as compared to other library enrichment methods. This technology would be useful for challenging forensic exhibits such as sexual assault or kinship cases due to its high sensitivity and incorporation of MPS technology allowing for increased recovery of genetic information.

References:
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Next Generation Sequencing; Y-STR; Reverse Complement PCR

B139   Visualizing Sequencing Artifacts in ForenSeq™ NGS Data

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Learning Overview: The aim of this presentation is to reveal the presence of a previously uncharacterized artifact in sequencing data and showcase the methods that led to their discovery.

Impact Statement: This presentation will impact the forensic science community by raising awareness of high-read sequencing artifacts in Next Generation Sequencing (NGS) data that can potentially be misidentified as alleles.

NGS has captured the interest of the forensic community leading to the emergence of novel sequencing kits and the development of analysis and interpretation methods.[1-6] Like other Polymerase Chain Reaction (PCR) -based assays for forensic DNA interpretation, one of the key objectives of sequencing technology is to amplify the allelic product or amplicon. However, two artifactual by-products of sequencing exist: stutter and noise sequences. An understanding of the behavior of these artifacts is important in order to generate models that can distinguish between allelic and artifactual sequences to aid in the deconvolution of a DNA profile.

Stutter sequences are fairly well characterized, and models have been built to aid in the classification of different types of stutter artifacts. However, noise artifacts or sequences have not yet been sufficiently modeled. Similar to the different types of stutters (for example, back, forward, and double back stutter), there are also different types of noise, including base substitutions, insertions, and deletions. While noise sequences often have a low number of read counts, there are instances where noise artifacts can confound the interpretation of a DNA profile, potentially leading to exclusions of a known contributor.

In our analyses of Verogen™ ForenSeq™ datasets with ground truth data, we encountered noise sequences of abnormally high reads that have the potential of being mistaken as minor alleles, such as those seen in mixed DNA profiles. Further investigation into these sequences revealed similar sequences from across several loci and samples within the multiplex. Analyzing at the base level, we found that a large portion of the error bases (insertions, deletions, substitutions) on the noise sequences from various loci and samples align to singular positions in the original read, forming a spike in the number of noise sequences. The positions of these noise spikes differ between plates and sequencing runs, and also correlate with a drop in base quality scores.

We hypothesize that these types of temporal, plate-wide noise spikes are produced during the sequencing process in the base-addition cycles, potentially from physical disturbances of the sequencing machine during its operation.

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Next Generation Sequencing; Sequencing Artifacts; ForenSeq™

B140   Expanding the STRSeq BioProject to Support Sequenced-Based STR Nomenclature

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Learning Overview: This presentation will inform attendees about the development and expansion of the STRSeq BioProject, a Short Tandem Repeat (STR) sequence catalog. Attendees will learn how this expanded catalog addresses the need for STR sequence nomenclature guidance and its ability to increase interlaboratory communication, facilitating the implementation of Next Generation Sequencing (NGS), also known as Massively Parallel Sequencing (MPS), in forensic laboratories.

Impact Statement: This presentation will impact the forensic science community by providing an up-to-date resource for users of NGS for STR sequence nomenclature guidance.

The rise in the use of NGS in forensic DNA testing has led to a need for STR sequence nomenclature resources. This was highlighted in a 2016 European Network of Forensic Science Institutes (ENFSI) survey, which identified a lack of resources available to generate standardized formatting and nomenclature as the main concern for the integration of sequenced-based analysis in forensic labs.[1] In the same year, an International Society of Forensic Genetics (ISFG) Commission published considerations on minimal nomenclature considerations, and a second ISFG Commission on STR nomenclature was convened in 2021, with recommendations expected in 2023.[2]

To address the needs discussed in the ENSFI survey and 2016 ISFG Commission report, the STRSeq BioProject was initiated in 2017.[3] In the years since, GenBank records were created for unique STR sequences identified in population studies by the National Institute of Standards and Technology (NIST) and partner laboratories. Additionally, during this time, many other research groups have published STR sequence population data developed from commercially available assays; therefore, a search was performed for sequence data published between 2016 and 2023 and not yet considered for the STRSeq BioProject. These published STR sequences were compared against over 2,500 existing STRSeq GenBank records to identify sequence-based alleles novel to STRSeq. This resulted in the compilation of STR sequence data from over 40 publications, which consisted of 28 autosomal STR, 7 X-chromosomal Short Tandem Repeat (X-STR), and 28 Y-chromosomal Short Tandem Repeat (Y-STR) markers. Novel sequences were cataloged into STRSeq following the forthcoming guidance of the ISFG DNA Commission on STR Sequence Nomenclature.

This updated catalog will help to unify reporting of data regardless of the forensic STR sequencing kit used for analysis. In addition, this expansion of known allelic diversity will aid the development of bioinformatic and mixture interpretation methods. When using STRSeq for a search, the output generated will provide the user a more comprehensive list of characteristics than were previously available, including updated manufacturer recommended reporting ranges, ISFG minimum reporting range, bracketed repeat region according to current ISFG guidelines, and additional variations of flanking regions from the current human genome reference assembly (GRCh38). Overall, the addition of novel allele sequences from population data and updating associated characteristics facilitates implementation of STR sequencing and improves sharing of data between laboratories.

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Forensic DNA; STRSeq BioProject; Next Generation Sequencing

B141 An Exploration of a Sequence-Specific Stutter Model for STR Analysis in Next Generation Sequencing

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Learning Overview: After attending this presentation, attendees will have learned about a proposed stutter model for Next Generation Sequencing (NGS) based on sequence characteristics rather than locus or allele number. Attendees will hear about the potential for more accurate stutter filters for human interpretation and improved stutter modeling in downstream Probabilistic Genotyping (PG) tools.

Impact Statement: This presentation will impact the forensic science community by introducing a novel stutter model based on the sequence of Short Tandem Repeat (STR) markers, which appears to predict the occurrence of stutter more accurately in complex loci than current models using simple locus thresholds or allele number. This model could be used to improve stutter filtering accuracy in Short Tandem Repeat (STR) analysis and probabilistic genotyping software and ease the adoption and validation of NGS technology in forensic laboratories.

Amplification of STRs using Polymerase Chain Reaction (PCR) is the foundation of current forensic DNA analysis methods throughout the world. While it remains the gold standard, an inevitable drawback of this process is the production of stutter artifacts due to strand slippage during PCR. Stutter appears as smaller peaks typically positioned one or two repeat units away from an allele peak on an electropherogram and can cause issues in interpretation or mixture deconvolution if not accounted for effectively. Current DNA analysis software attempts to recognize the expected occurrence of stutter and allow analysts to remove it from the allelic data. These stutter models have evolved over time from locus-specific thresholds to allele-specific filters based on a linear relationship between stutter peak height ratio and allele number.[1] While this allele-specific stutter modeling offers improvements over the locus-specific thresholds in more accurately predicting the occurrence of stutter in simple loci, loci with more complex repeat units tend to show major deviation from the linear model, suggesting that additional factors may still need to be considered.

The advancement and growing adoption of NGS technology offers exciting new possibilities for stutter modeling. NGS still allows laboratories to assess STR marker length but provides the additional ability to view their sequences, which can reveal differences in STR alleles that would have appeared identical on CE. These differences could potentially be used to develop a more accurate model for stutter filtering based not only on allele, but also on characteristics of the sequence.

This presentation details part of a study that sequenced batches of 96 and 32 single-source samples for autosomal and Y-chromosomal Short Tandem Repeats (Y-STRs) using the QIAGEN® ForenSeq™ MainstAY Kit. These sequence data were used to develop a model for sequence-specific stutter to explore its potential effectiveness in comparison to locus- or allele-based stutter filter model. While preliminary data suggests that additional sequence characteristics may still need to be considered, this presentation will demonstrate stutter ratio plots showing improved R2 measures of linearity at complex loci when considering the Longest Uninterrupted Stretch (LUS) in the repeat unit of the STR sequence rather than allele number.[2] Others have looked at various characteristics of NGS stutter before; however, this stutter model is the first to also incorporate the simplicity of the Sequence Identifier (SID) nomenclature first described by Young et al.[3-5]

These NGS stutter models will ultimately need to be incorporated into analysis and PG software to take full advantage of the potential interpretation and deconvolution improvements. While stutter data from many more samples will be needed, along with validation tools and downstream software, this presentation will serve as an introduction of sequence-specific stutter to the forensic community.

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Next Generation Sequencing; Modeling; STRs

B142 **Using the Chelation Filtration Method to Improve DNA Profile Recovery From Fired Cartridge Casings (FCCs) Prior to DNA Processing**

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Learning Overview: This presentation will allow a large and broad group of attendees to learn about a simple, cost-effective, and quick method of processing FCCs that can be used prior to DNA processing and will not interfere with the DNA processing steps or any other downstream analysis of the casings.

Impact Statement: This presentation will impact the forensic science community by addressing a topic that is a difficulty for the forensic community, which is the large amount of gun crimes that are increasingly common. The chelation filtration method is unique in that it is very simple to implement and can be done by crime scene personnel for a relatively low cost. The method does not require expensive instrumentation or any changes to the downstream DNA processing. It has been proven to succeed in 15–20% of real-world cases, helping to provide investigative leads where swabbing would not have helped.

Gun-related crimes are the cause of many homicides and injuries every year across the United States. According to the Federal Bureau of Investigation (FBI) statistics, firearms were used in 68% of murders, 41% of robberies, and 21% of aggravated assaults in 2011 alone.[1] Cartridges and casings left on the scene often link shootings to a firearm and many times are the only evidence. Traditional swabbing of FCCs has yielded usable profiles only approximately 1–2% of the time, causing many laboratories to refrain from processing FCCs.

A method for collection of the cellular material from the FCCs for downstream DNA analysis was developed and evaluated. Chelation filtration is a simple, easy-to-perform, two-step filtration method using Chelex® resin in the initial incubation step and only requires a vacuum device along with two filter devices. The process does not create any need for alterations to downstream DNA analysis. Comparisons of the Chelation filtration method (N=20) to traditional swabbing (N=20) were conducted and the quantity of DNA recovered (ng) as well as the percentage of expected alleles observed was evaluated using Analysis of Variance (ANOVA) analysis, resulting in 40% (P<0.001) more alleles detected with the Chelation filtration method. In addition, the process was further refined to enhance the DNA recovery by using different filter materials and incubation steps.

An anecdotal study of 122 cases was performed to evaluate the effectiveness of the Chelation filtration method on real casework evidence, where 21 interpretable DNA profiles were obtained yielding five Combined DNA Index System (CODIS) matches. With the assistance of STRmix™ analysis, over 50% of those profiles were eligible for statistical calculations, and STRmix™ was also used to deduce profiles for CODIS entry. The Chelation Filtration method can be an invaluable tool to help solve gun crimes when there is little to no other evidence besides FCCs. One case also showed that after the Chelation Filtration method was utilized, a fingerprint was also developed on the casing using the Recover Latent Fingerprint Technology (LFT) system.

Reference:
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Crime Scene Investigation; Cartridge Casings; DNA

B143   Evaluation of Single Cells Produces Highly Informative Forensic Comparisons Across Multifarious Admixtures

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Learning Overview: After attending this presentation, attendees will know how to determine sub-source weights of evidence from single-cell data by clustering, evaluating the Likelihood Ratio (LR) per cluster, then averaging the LRs across clusters. Attendees will be aware of automated procedures that support the efficient construction and validation of single-cell laboratory procedures and will also be aware of numerous laboratory approaches to single-cell analysis.

Impact Statement: This presentation will impact the forensic science community by demonstrating that, regardless of the number of contributors that donated to the mixture, single-cell analysis results in LRs approaching the inverse of the random match probability and does so while being able to associate what DNA originated from what cell and cell-type.

The consistency between DNA evidence and Person(s) of Interest (PoI) is summarized by the LR: the probability of the data given the PoI contributed divided by the probability given they did not. It is often the case that there are several PoI who may have individually or jointly contributed to the stain. If there is more than one PoI or the Number of Contributors (NoC) cannot easily be determined, then several sets of hypotheses are needed, requiring significant resources to complete the interpretation.

Recent technological developments in laboratory systems offer a way forward by enabling production of single-cell data. The scale of a single-cell experiment ranges from tens to millions of cells per sample and is dependent on the isolation and preparation method employed. Smaller-scale experiments typically use micromanipulation strategies coupled with tube-based amplification, while large-scale experiments exploit the use of barcodes, nanodroplets or picowells, and next generation sequencing (NGS) to parallelize the data generating process. Regardless of scale, there are two features common to all single-cell data generating processes: (1) that intact cell or nuclei are isolated before the DNA/RNA is extracted, and (2) that the extraction and amplification (or library preparation) occurs in the same vessel to which the cell was added. These two features explain the strength of single cell processing in that by isolating the cell before lysis, the two alleles of any donor are paired and fully resolved from DNA of other interference donors, and by extracting and amplifying the DNA in the same vessel, allele drop-out associated with fractionating the extract into two components—one that is stored and one that is amplified—is abated.

We use an *in silico* laboratory system, named ReSOLVIt, to test numerous laboratory scenarios and automatically determine what laboratory treatment offers a limit of detection of one copy. We show that laboratory outcomes are consistent with theory. We then experimentally generate 643 single-cell Electropherograms (scEPGs) and stochastically mix them to produce admixtures with up to 5 contributors with 17–75 cells and minor ratios as low as 3.5%.

We describe the development of a forensically cogent single-cell interpretation strategy that: clusters scEPGs into collections, each originating from one genetic source; determines an LR for each cluster of scEPGs for each PoI; then provides a whole-sample or *sub*-source evaluation. By using Model-Based Clustering (MBC) and a bespoke algorithm, named EESCIt for Evidentiary Evaluation of Single Cells, to compute *sub*-source single-cell LRs, we show that 99.2% of the comparisons gave logLR values >0, and of these all rendered log LR >5, regardless of the number of donors or whether the smallest contributor donated less than 20% of the cells, greatly expanding the collection of cases for which DNA forensics provides informative results.

Single-Cell Data; Forensic DNA; Mixture Analysis

B144 **Discovering the Mysteries About Degradation of Extracted DNA Stored in Different Conditions: Updates After 400 Days of Testings**

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Learning Overview: After attending this presentation, attendees will understand how long-term room temperature preservation of DNA extracts affects the survival and completeness of Short Tandem Repeat (STR) peaks, as well as the performance of phenotype panels for human identification.

Impact Statement: This presentation will impact the forensic science community by providing guidelines on the best approaches to conserve DNA extracts and informing attendees on the survival of DNA in less-ideal preservation scenarios, opening the path for potential future analyses on cold cases' material preserved in non-optimal conditions.

The appropriate storage of DNA extracts is crucial for the successful outcome of forensic genetic analyses, including human identification via STR analysis and the application of phenotype and ancestry panels to infer the morphological features and the geographical origin of the contributor of the trace. Despite the National Institute of Standards and Technology (NIST) guidelines recommending freezing DNA extracts for long-term preservation, it is possible that samples are kept in non-optimal conditions (such as in fridges or even at room temperature) for several reasons (e.g., faulty freezers, movement of the samples from the laboratory to crime bureaus at room temperature at the closure of the case).[1] In these circumstances, little is known regarding the survival of DNA and its suitability for future STR and **Single Nucleotide Polymorphism (**SNP) analyses.

Last year, an experiment was conducted to evaluate the preservation of DNA extracts originating from buccal swabs and their mixtures (ratio 1:15) when samples were stored at different temperatures (+20°C, +4°C, and -20°C) for up to 90 days.[2] Results showed a surprising survival of DNA at room temperature; quantification with Quantifiler® Trio showed a lack of degradation (degradation index 0.7) for all the three storage conditions and STR profiles obtained with GlobalFiler® PCR amplification kit run on both 3500 Series Genetic Analyzer and SeqStudio™ Genetic Analyzer revealed similar completeness for both -20°C and +20°C conditions, and less ideal results for samples kept at +4°C.

Due to the unexpected results obtained, the experiment was further prolonged for up to 400 days (expected completion in January 2024) to evaluate to which extent long-term storage of DNA extracts in non-ideal conditions affects samples quality. Specifically, after the initial 90 days of experiments, a subset of the samples was kept in the previously described conditions, and a second subset was moved to a cupboard located outdoors (uncontrolled temperature conditions). Under the controlled conditions, samples at +4°C were the most evaporated ones after 150 days, as showed by their increased concentration in comparison with the others kept at +20°C and at -4°C. Degradation indexes at T=150 days were comparable at all temperatures for both for the single profiles and for the mixture and generally showed no degradation (minimum degradation index = 0.83 and maximum degradation index = 1.17).

Regarding the subset relocated outdoors, after 150 days from the movement of the samples (overall age of the samples T=240 days), degradation indexes were still recorded to be between 0.8–1.0 for both single profiles and for the mixture. The STR profiles for all samples (e.g., both under controlled and uncontrolled temperatures) were generally complete even after 240 days, including the profile of the minor contributor to the mixture.

Degradation indexes and STR profiles will be evaluated again after 400 days from the start of the experiments and the latest results will be presented at the conference. Additionally, selected samples will be also analyzed with a newly developed in-house ancestry and phenotype panel, to evaluate the reliability of the NGS data on samples stored in non-optimal conditions.

Overall, results presented at the conference will clarify if single and mixture extracts preserved for long-time, non-optimal conditions can reveal information (STR, phenotype, and ancestry) useful to identify the contributor/s to the trace and may open new avenues for the analysis of samples originated from cold cases that were not always preserved by freezing.

References:
1. S.M. Ballou et al., The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers, 2013.
2. N.Procopio et al., Discovering the Mysteries about Degradation of Extracted DNA from Trace Evidence when Stored in Different Conditions: State of the Art and Future Perspectives. Proceedings of the American Academy of Forensic Sciences, 75th Annual Scientific Conference. Orlando, FL. 2023.

DNA Preservation; STR; SNP

B145 **Understanding the Stability of Environmental DNA (eDNA) From Four Biological Taxa and Their Association With Soil and Dust Evidence**

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Learning Overview: After attending this presentation, attendees will better understand: (1) how picking/scraping was found to be an appropriate method for eDNA analysis, (2) how mock geologic evidence items were collected for eDNA analysis, (3) the steps in the eDNA analysis workflow, (4) the analysis of sequencing data using bioinformatics, and (5) the stability of bacterial, fungal, plant, and arthropod eDNA from mock geologic evidence.

Impact Statement: If the aims of this research are met, this presentation will impact the forensic science community by informing attendees that DNA from bacteria, fungi, plants, and arthropods in soil and dust may be used to supplement forensic geology investigations with additional evidentiary information.

Geologic materials, such as soil and dust, are valuable types of trace evidence and are submitted to crime laboratories. Forensic geologists aim to analyze the inorganic components (e.g*.,* mineral content) and determine their physical properties (e.g*.*, color and pH) for sample-to-sample comparisons or to identify an evidentiary sample's origin. However, sample size is often a limiting factor in these analyses; supplemental methods requiring a small amount of geologic material as input could provide additional evidentiary information. DNA metabarcoding is a commonly used approach to identify the biological taxa that are present in environmental samples by amplifying and sequencing short, informative regions of the genome and is not restricted by sample amount. The goal of this research was to determine the utility and stability of environmental DNA from four biological taxa associated with soil and dust for sample-to-sample comparisons and sample origin. To accomplish this, four taxa, bacteria (16S), fungi (ITS1), arthropods (*COI*), and plants (ITS2, *trnL*) recovered from each sample were characterized (n, 1,026) via DNA metabarcoding.

An initial soil isolation study was performed to determine the most suitable approach (picking/scraping, swabbing, and sonication) to remove soil from mock evidence for environmental DNA analysis. Following soil removal, DNA was isolated using the PowerSoil® Pro Kit. DNA extracts were amplified using Polymerase Chain Reaction (PCR) primers specific to 16S, ITS1, ITS2, *COI*, and *trnL*. Libraries were then prepared and sequenced on an Illumina® MiniSeq™. Raw sequencing reads are processed through a bioinformatic pipeline that identifies Amplicon Sequence Variants (ASVs) via DADA2 and searches the ASVs against GenBank for taxonomic identification. Picking and scraping of soil produced the highest amount of DNA compared to swabbing ($p=0.0025$) and sonication ($p=0.0068$). While all three methods recovered similar taxonomic assignments, picked/scraped samples tended to cluster more consistently together with the soil reference in multidimensional space and thus was the method chosen for soil isolation in subsequent experiments.

Following the soil isolation study, five mock geologic evidence items were collected monthly from an agricultural and urban location in North Carolina over a one-year period. Mock items included: (1) soil scraped from t-shirts, boot soles, and trowels; (2) exposed dust collected from brick pavers using polyurethane swabs; and (3) dry dust from air filters (~1" X 1" area used). DNA was isolated from mock geologic evidence using the PowerSoil® Pro Kit, and libraries were prepared using custom-indexed primers and subsequently sequenced using the Illumina® MiSeq™. After sequencing, the bioinformatic pipeline was used to process sequencing reads to characterize the bacterial, fungal, plant, and arthropod communities. Important findings from this research include: (1) despite the low DNA concentrations of dust samples, it is still possible to characterize the biological communities in dust; (2) the wet lab workflow successfully recovered taxa associated with forensic mock evidence; and (3) it is apparent that there are changes in the biological communities over time and between locations. This presentation will also include a preliminary assessment of temporal and spatial variables on the recovery of bacteria, fungi, arthropods, and plants from mock geologic evidence.

Environmental DNA; Forensic Geology; Next Generation Sequencing

B146   A New Method for Determining Time-Since-Deposition of DNA Samples Containing Saliva and/or "Touch" Epidermal Cells

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Learning Overview: After attending this presentation, attendees will better understand the correlation between median autofluorescence of cell populations and the time elapsed since their deposition and its implications in determining the "time-since-deposition" for a biological evidence sample.

Impact Statement: This presentation will impact the forensic science community by informing attendees that determining the time-since-deposition of biological material found at the scene of a crime can provide invaluable information for investigators. It can help define the time frame in which the evidence was deposited and, consequently, the period during which the individual who deposited the evidence was present at the scene.

With the increase in sensitivity of DNA profiling, questions about how and when the DNA was deposited have become a driving issue in forensic cases. However, there are few scientific techniques that can be coupled to a DNA profiling workflow to resolve these questions. The objective of this study was to investigate the changes in the autofluorescence of cell populations recovered from saliva and/or "touch" epidermal samples over time and evaluate its potential for determining the time-since-deposition of an unknown DNA sample. To develop this signature, a series of biological samples consisting of either epidermal cells deposited by handling a substrate or saliva cells deposited directly onto a non-porous surface were created. Imaging Flow Cytometry (IFC) was then used to characterize the morphology and autofluorescence profiles of individual cells within each sample, followed by multivariate modeling and predictive classification. Results from saliva cell samples showed noticeable differences in the autofluorescence profiles at different time points, primarily in wavelengths between 535nm and 580nm. Median autofluorescence intensity exhibited a directly proportional relationship with time ranging between 0 to 180 days. Additionally, a clear increase in the heterogeneity of autofluorescence values was also observed with increasing time (i.e., interquartile range of the distribution of fluorescence intensity). The increase was observed to be gradual for the time points between zero and seven days, where the median autofluorescence increased from ~200 Relative Fluorescence Units (RFU) to ~1,000 RFU, but showed a non-linear/exponential increase from T7 to T90, increasing from 1,000 RFU to ~9,000 RFU.

Results from "touch" epidermal cell populations also exhibited distinct changes across the time series, with the most pronounced differences occurring in three time-since-deposition groups: <1 week, between 1 week and 2 months, and >2 months. Differences across age groups were largely driven by decreases in brightfield contrast and increases in the intensity of autofluorescence in wavelengths between 535nm and 580nm. To further test this approach for forensic casework, 47 individual donor cell populations spanning each time-since-deposition group were classified blindly against the reference donor data set. Samples containing at least 75 cells showed classification accuracies of ~95%. Classification accuracies for individual cells varied slightly across time groups: ~97% (<1 week), ~92% (1 week–2 months), and ~98% (>2 months). Overall, these results indicate that autofluorescence and morphological analyses may provide probative information regarding time-since-deposition for many types of trace DNA samples in forensic casework.

Time Since Deposition; Flow Cytometry; Autofluorescence

B147   A Biological Survey of Nucleated vs. Non-Nucleated Epithelial Cells Within Touch DNA Samples Using Autofluorescence Profiling

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Learning Overview: After attending this presentation, attendees will better understand the proportion of nucleated epithelial cells in touch evidence samples and how this information can provide probative value to the analysis of biological evidence in forensic casework.

Impact Statement: This presentation will impact the forensic science community by providing a new method to rapidly survey the types of cells in touch DNA evidence and potentially predict the quantity of amplifiable DNA and the quality of the Short Tandem Repeat (STR) profile.

Touch biological samples are commonly collected in forensic investigations and can originate from multiple sources. While touch DNA can connect an unknown individual to the crime scene, these samples may contain epidermal cells, saliva cells, and free DNA from the individual and/or the surrounding environment. The relative contribution of each of these DNA sources to a given sample can have a significant impact on the quantity and quality of DNA recovered from the sample. It can also have implications for various activity-level propositions for the circumstances in which it was deposited. However, there are few methods that allow investigators to assess these components before subjecting the sample to a DNA profiling workflow. To address this issue, we developed a new method to characterize the proportion of nucleated epithelial cells, non-nucleated epidermal cells, and cell-free DNA in touch samples using flow cytometry in a high throughput and non-destructive manner.

To identify signatures for differentiating nucleated versus non-nucleated epithelial cells, a total of 30 saliva reference and 30 touch reference samples were collected from different individuals and compared across ten time points, ranging from immediate collection to three months. Results showed distinct differences in the intensity of autofluorescence between these cell sources, particularly at wavelengths between 450nm–500nm. For example, epidermal cells showed median fluorescence intensity in the range of 300 to 450 Relative Fluorescence Units (RFU), whereas saliva cells exhibited lower median intensity in the range of 150 to 300 RFUs. Additionally, we observed some disparities in autofluorescence profiles across contributor cell populations from the same tissue type as well as differences in the abundance of cells deposited onto the surface. As the time-since-deposition increased, median autofluorescence intensity decreased in saliva cells but did not show any systemic changes in epidermal cell populations.

Next, we used these differences to characterize the proportion of nucleated epithelial cells and non-nucleated epidermal cells in 40 mock casework samples that were composed of either saliva and epidermal cells, or epidermal cells deposited from multiple contributors onto the same substrate. Results showed that saliva cells could be easily detected and quantified in cell populations where the ratio of saliva to epidermal cells ranged between 1:10, 1:1, and 10:1. Interestingly, cells consistent with nucleated epithelial cells were detected in some mixtures (~1%) that were deposited only through touch, suggesting a possible role for these cell types in touch DNA samples. DNA profiling showed a strong correlation (R2~0.94) between the abundance of saliva cells, the quantity of amplifiable DNA, and the quality of the STR profile quantity of amplifiable DNA Overall, these results indicate the potential to differentiate epidermal cells from other cell types and quickly predict the quantity of DNA in an evidence sample. This can enhance the analysis of trace biological samples and provide valuable insights into the possible mechanisms for presence, abundance, and quality of DNA in evidence samples.

Flow Cytometry; Cell Biology; DNA Profiling

B148   Proactive Crime Scene Response Improves Criminal Investigations

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Learning Overview: After attending this presentation, attendees will understand some principles of the proactive crime scene approach, utilizing targeted forensic results in real time to investigate crimes. Actual cases and business cases will further support the use of this technique to maximize the value of evidence in providing investigative leads.

Impact Statement: This presentation will impact the forensic community by providing a model to improve the positive impact on the objective evidence provided by forensic science. A model will assist forensic science service providers to implement the Proactive Crime Scene approach, supported by the cost savings of a range of business models, coupled with case examples.

The Proactive Crime Scene Response utilizes targeted forensic analytical results to direct criminal investigations in real time.[1] Forensic laboratories maximize the value of analytical evidence through recognition, documentation, collection, and preservation of evidentiary items located at the crime scene and subsequent identification of high value samples. A seamless flow of analysis is based on improved education, coordination, and communication between the crime scene investigators and forensic scientists. This approach enables greater focus on high-value evidence with decreased response time and greater impact on investigational direction. Real-time data from the resulting focused forensic analyses coupled with the use of databases provides primary investigative leads. These leads supply suspect identities, the whereabouts at the time of crime commission, links to other crimes, and other critical collaborative crime-solving information.

Forensic evidence provides actionable, objective information to investigators, including suspect identities and linking of similar crimes. Forensic evidence is perishable, as its value diminishes over time, with most investigative costs spent at earlier stages of the investigation. Timely objective forensic intelligence saves investigative resources, more accurately eliminating incorrect suspects from suspicion while directing investigations to the correct suspects. When a backlog exists at the forensic laboratory, a large component of the total turnaround time is due to the case waiting to start analysis, not the analysis itself. Providing targeted analysis on high evidentiary-value items is an interim step that can be provided immediately. Employing a two-step screening and confirmation process using Rapid DNA and the National Integrated Ballistic Information Network (NIBIN) provides quicker leads for suspect elimination and inclusion. These leads are then confirmed in accredited forensic laboratories for judicial proceedings as necessary.

The Proactive Crime Scene Response has several components, including communication, forensic scientist expert liaison, training, and education, streamlined analysis on high-value evidentiary items, and rapid reporting. Communication includes laboratory notification of crimes with a potential forensic component, active outreach to crime scene investigators, and real-time support with scientific expertise and dedicated forensic science expert liaisons. Ongoing education and training include in-person and remote learning, webinars, short videos, handbooks, and cheat sheets. Streamlined forensic analysis ensures analysis commences immediately on high evidentiary-value items with reduced impact on the standard evidence flow. Finally, streamlined reporting includes Laboratory Information Management System (LIMS) electronic reporting or other electronic means of providing timely results to investigators and justice system officials. LIMS also provides result compilation to provide bigger picture observations where Memorandum of Understandings (MOUs) between police agencies permit sharing of aggregate forensic laboratory-compiled information and data.

Resources providing timely forensic analysis are well spent as demonstrated by criminal and business case examples. Case examples are provided that highlight the successful application of key aspects of the Proactive Crime Scene Response model. Business cases covering a spectrum of forensic and database applications will illustrate a large cost saving, providing further demonstration of the effectiveness of this approach. Recommendations for implementation including Rapid DNA and NIBIN will be included.

Turnover of crime scene specialists and investigators and increasing forensic technology requires an ongoing forensic science education, to include interpretation of results impacting the investigation at hand. The presented investigator-forensic investigator partnership is a means to alleviate the risks of investigator turnover while realizing improvements in rapidly evolving forensic technology and optimizing the value of forensic intelligence. Quicker and more cost-effective crime solving, prevention, and elimination of the wrongfully suspected support implementation of this Proactive Crime Scene Response.

Reference:
1.   Ray A. Wickenheiser, Proactive crime scene response optimizes crime investigation, in press, *Forensic Science International: Synergy* 6, 2023, 100325, https://doi.org/10.1016/j.fsisyn.2023.100325.

DNA; Crime Scene Investigation; Rapid DNA

B149   An Interagency Study of Scene Investigation Using Virtual Reality (VR) Tools

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Learning Overview: After attending this presentation, attendees will understand how crime scene investigators across the nation compared processing virtual reality crime scenes as well as the capabilities of using VR as a crime scene training tool.

Impact Statement: This presentation will impact the forensic science community by providing empirical data related to how investigators across the nation process crime scenes with the goal of identifying areas in scene investigation that would benefit from standards development and exploring the capabilities of using VR as a crime scene training tool.

The actions taken during a crime scene investigation play a pivotal role in subsequent forensic analysis and ultimately case resolution. Because of this, incorporating quality assurance standards in the work being done at the scene is important to improving the overall quality of processing and collecting evidence being sent to the laboratories, thereby increasing the capabilities of laboratories. Currently, the two accrediting bodies for forensic testing, American National Standards Institute (ANSI) National Accreditation Board (ANAB) and the American Association for Laboratory Accreditation (A2LA), show less than 80 unique agencies across the United States are accredited under the International Organization for Standardization (ISO) 17025 or ISO 17020 standards in scene investigation. Additionally, when it comes to proficiency and competency testing, required quality assurance measurements for accreditation, there are very limited options for third-party testing due to the complex nature of scene investigation. While many agencies routinely perform scene examinations, the vast majority are not accredited, and the level of training, resources, and opportunities to practice actual scene investigation varies from jurisdiction to jurisdiction.[1]

To facilitate the development of standards for crime scene and improve the repeatability, reproducibility, and accuracy in the practice of forensic science, RTI International, in cooperation with the National Institute of Standards and Technology (NIST) as part of cooperative agreement 70NANB21H098, conducted a national interagency comparison study on crime scene processing using high-resolution VR scene recreations. VR has been found to be a successful training tool in many disciplines and enables the evaluation of problem-solving, decision-making, and technical skills in an immersive simulation of real-world experiences that can be easily repeated, amplified, and adapted as needed. After two initial phases of information gathering at the 2022 Organization of Scientific Area Committees (OSAC) for Forensic Science meeting in Orlando, FL, and the 2022 International Association for Identification (IAI) Conference in Omaha, NE, three virtual crime scene scenarios (sexual assault, assault, and overdose) and a training module were created along with a variety of crime scene processing tools. VR headsets were shipped out to 66 agencies across the country varying in jurisdiction, agency, and crime scene unit size. The participants (n=163) varied in years of experience, number of scenes processed in the past three years, and VR experience were asked to participate in the training module, then process two of the virtual scenes. Data was collected after each scene was processed and reviewed to compare participants' performance in crime scene processing, such as how long it took to process scenes, how many times gloves were changed, or number of photographs taken. Insight from this study would also be used to assess the effectiveness of VR as a training tool or potential means for agencies to assess members' proficiency and competency in scene investigation.

Reference:
1. National Forensic Science Technology Center (U.S.). (2013). *Crime scene investigation: a guide for law enforcement*. Bureau of Justice Assistance, U.S. Department of Justice, National Institute of Justice, NIST, NFSTC.

Crime Scene Investigation; Virtual Reality; Quality Assurance

B150   The Presence of Inorganic Gunshot Residue (iGSR) in Police Vehicles: A Study at the Zagreb County Police Administration in Croatia

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Learning Overview: This presentation will address the risk of contamination of police vehicles by iGSR, covering characteristic and indicative particles. After attending this presentation, attendees will understand which parts and to what extent police vehicles could be contaminated with GSR when the vehicle is used to transport people related to criminal events and other everyday police tasks and what could be contributing factors.

Impact Statement: This presentation will impact the forensic science community by demonstrating not only the prevalence of the contamination in the samples analyzed but also by showing a statistical relationship between the presence and the number of GSR particles at different parts of vehicles (driver's/back seats), as well as the connection between the indicative and characteristic GSR particles.

Although identifying iGSR particles has been a method of choice to prove that a person was involved in a firearm-related incident, it is crucial to remain aware of potential sources of contamination and secondary transfer of these particles. Studies have shown that iGSR can be present in the police environment, including police officers, facilities, and the vehicles used to transport potential suspects.[1-3] Therefore, the study aimed to analyze the vehicle contamination on the level of the police administration unit, examine the relationship between contamination of different parts of the vehicle and different types of particles, and identify factors that could contribute to the contamination.

A study was conducted at the Zagreb Country Police Administration, the largest police administration unit in Croatia, on the vehicles used for event participants' transportation and other police tasks. The sampling was conducted from December 8, 2021, to April 13, 2022, and comprised 65 vehicles (margin of error: ±6% at a 95% confidence level) from 17 police stations. The samples were collected from the driver's seat, back seat, and backrest with GSR stubs and examined using Scanning Electron Microscopy and Energy Dispersive X-ray (SEM/EDX) analysis by an accredited method within the Forensic Science Centre. Any vehicle with at least one GSR particle in at least one sample was considered a contaminated vehicle, whereby characteristic and indicative particles were considered separately. The proportion of contaminated vehicles and the number of particles were calculated depending on the location and type of particles. A logistic regression was used to examine the possible influence of variables collected by the questionnaire (conducted at the police station level) on vehicle contamination.

Characteristic particles were detected in 63.1% of vehicles, 33.8% on the driver's seat and 24.6% on the back seat/backrest. Indicative particles were found in 70.77% of vehicles, on the driver's seat in 33.8%, on the back seat in 30.77%, and on the backrest in 35.38% of vehicles. McNemar's chi-square test revealed no significant differences between the proportion of positive samples depending on the vehicle part nor the type of the GSR particles (P >0.05). Overall, 228 characteristics and 166 indicative GSR particles were found, but without significant correlation between them (P=0.346). The most common number for both types of GSR particles was one. Logistic regression showed that, among the considered variables, vehicle contamination could be affected by the transportation of persons who participated in firearm-related incidents (P=0.030), suggesting caution in such cases.

The high prevalence of contaminated vehicles may point to risk from secondary contamination, but to a limited extent, due to the low number of particles found. The study demonstrated that a similar degree of contamination could be expected in the driver's seat and back seat/backrest. The results indicate that contamination with indicative particles does not necessarily imply the presence of characteristic GSR particles, despite no difference in the proportion of vehicles positive for both types.

References:
1. Gerard RV, Lindsay E, McVicar MJ, Randall ED, Gapinska A. Observations of gunshot residue associated with police officers, their equipment, and their vehicles. *J Can Soc Forensic Sci*. 2012;45(2):57–63.
2. Berk RE, Rochowicz SA, Wong M, Kopina MA. Gunshot residue in Chicago Police vehicles and facilities: An empirical study. *J Forensic Sci*. 2007;52(4):838–41.
3. Anders DH, Miller SA, Graziano CR, Castellano J, Conte J. Technical note: Presence of gunshot residue in and around a police station. *Int J Legal Med*. 2020;134(6):2195–8.

Gun Shot Residue; Police; Scanning Electron Microscopy

B151   The Prevalence of GSR and the Mitigating Quality Procedures at Harris County Institute of Forensic Sciences

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Learning Overview: This presentation will summarize the results of an in-house Gunshot Residue (GSR) prevalence study and demonstrate the need for effective mitigating procedures. After attending this presentation, attendees will be familiar with the persistence of GSR particles, the need for appropriate quality controls, including universal and locally developed procedures, and why these procedures ensure robust analytical results.

Impact Statement: This presentation will impact the forensic science community by demonstrating what it means to ensure contaminant-free analytical results in the category of GSR testing and the applicability of GSR in a criminal investigation, given the prevalence of GSR particles in the operational forensic laboratory.

The persistence of GSR in a forensic laboratory is a concern for trace evidence analysts and the criminal justice community. To help mitigate this concern, a localized study of GSR within the Harris County Institute of Forensic Sciences was conducted to determine the presence and prevalence of background GSR. An additional objective was to determine the potential for transfer of GSR within the building and the effectiveness of mitigating quality assurance procedures.

In this study, a variety of surfaces were sampled throughout the five active floors of the building, including the hands of various employees, small surfaces frequently touched by hands (e.g., water dispensers, door handles, elevator buttons), and common areas, such as breakrooms and conference rooms. Each sample was collected using a commercially available GSR collection kit utilizing a carbon adhesive stub. Analysis was performed using Scanning Electron Microscopy/Energy Dispersive X-Ray spectroscopy (SEM/EDX) with an automated software to detect particles characteristic of GSR. The same procedures employed for evidence analysis were followed in this study. Quality control samples used to monitor GSR contamination in the analytical environment were also included as part of the study.

GSR was detected on surfaces and items frequented by firearms examiners and firearms evidence, such as evidence technician carts utilized in transporting evidence to and from the firearms laboratory. However, GSR was also detected on surfaces that, without targeted mitigating procedures, could lead to potential transfer to a trace evidence analyst and inadvertently affect GSR analysis. Surfaces testing positive for the presence of GSR particles included elevator buttons, hands of evidence intake personnel, and other surfaces within the evidence receiving area. No GSR was found to be in the vicinity of the trace evidence laboratory, including sampled door handles, office spaces, and breakrooms. The results of this study highlight the effectiveness of existing procedures and suggests the need for continuous monitoring and vigilance on the part of trace evidence personnel.

In conclusion, this presentation will demonstrate the prevalence of GSR in an operational forensic laboratory, the need to monitor background levels of GSR as an important consideration to ensure the quality of the results, and provide a set of procedures to mitigate concerns of GSR persistence.

Gunshot Residue; GSR Prevalence; Quality Procedures

B152 **Utilizing Gas Chromatography/Mass Spectrometry (GC/MS) to Determine the Effect of Solvent Extraction Parameters on Forensic Smokeless Powder Analysis**

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Learning Overview: This presentation will utilize GC/MS data to demonstrate how certain instrument parameters and an optimized solvent extraction method can increase the accuracy of forensic smokeless powder analysis. After attending this presentation, attendees will understand how instrumental parameters influence smokeless powder analytes during analysis and why current extraction methods are insufficient or impractical for forensic science applications.

Impact Statement: This presentation will advance the forensic science community's understanding of smokeless powder analysis and offer an optimized solvent extraction method that could strengthen the interpretation of data collected for cases involving such samples.

Since its invention in the 1800s, smokeless powder has served as an energetic material in ammunition for civilians and military applications. Due to its commercial availability and use in pipe bombs, it has also been an important component of forensic explosives casework. During wartime, research originally focused on developing methods that could evaluate the stability of powders, but today the focus has shifted to developing instrumental techniques that enable forensic identification and comparison work.[1,2]

The analysis of smokeless powders is analytically straightforward; however, the complex chemistry of these powders and the proprietary nature of production makes determining a powder's exact formulation difficult. GC/MS has typically been utilized for analyzing intact and post-blast smokeless powder.[3] The effect of instrument parameters (inlet, transfer line, and source temperature) on analyte detection was evaluated. Despite the vast amount of research that has been conducted to develop instrumental methods capable of analyzing smokeless powders, to this author's knowledge, no research has been conducted to develop an optimized solvent extraction method for use prior to instrumental analysis.

In this study, a 13-minute GC/MS method that can detect 14 analytes was utilized to develop an optimized solvent extraction method for 28 powders of double-base tube morphology. Results indicated that Dichloromethane (DCM), the solvent traditionally used for smokeless powder extractions, was not sufficient because it had an average extraction efficiency of approximately 4–30% and required a large amount of sample (approximately 10mg) to detect minor and trace analytes. When a solvent mixture of 1:1 DCM and Acetonitrile (ACN) was used with a 15-minute extraction time, the ability to detect minor and trace analytes present in sample amounts as low as 0.50mg was possible. This extraction method was applied to other selected morphologies. As the mass of the individual particle decreased, the number of particles needed for successful extraction of the minor and trace analytes increased.

References:
1. Wiggam, D.R., Goodyear, E.S. (1932) Stability test of nitroglycerin-nitrocellulose smokeless powder. *Industrial & Engineering Chemistry Analytical, 4* (1), 73-76. DOI: 10.1021/ac50077a033.
2. Davis, Tenney L., Ashdown, Avery A. (1925) Transformations of Diphenylamine during the Aging of Smokeless Powder. *Industrial & Engineering Chemistry*, *17* (7), 674-675. DOI: 10.1021/ie50187a007.
3. Beveridge, A. *Forensic Investigation of Explosions*, 2nd edition. International Forensic Science and Investigation Series. Taylor & Francis Group, 2012.

Smokeless Powder; GC/MS; Sample Preparation

B153   Advancements in 3D Technology and Virtual Comparison Microscopy for Ballistic Identification

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Learning Overview: The goal of this presentation is to inform attendees that 3D imaging technologies are developing for use in firearms and toolmarks laboratories that can be used to supplement the current processes.

Impact Statement: This presentation will impact the forensic science community by reviewing how developing 3D imaging technologies can be implemented in laboratories to streamline the processing of evidence, provide easier access to imaging needed for examinations, and supplement the documentation and review process for casework, allowing the lab a more streamlined process to reduce the backlogs currently seen in many labs today.

In this session, we will explore the exciting advancements in 3D technology, database correlations, and Virtual Comparison Microscopy (VCM) as applied to ballistic identification, utilizing the state-of-the-art EVOFINDER® Ballistic Identification System. The system's capabilities enable the creation of a comprehensive database of 3D images of bullets and cartridge cases, revolutionizing the way forensic ballistic examinations are conducted.

Through the use of 3D imaging, historical sample images can be efficiently stored and organized in a database. This repository facilitates rapid and automated comparisons between samples, providing forensic examiners with unprecedented access to visualize and compare specimens virtually. This powerful combination of technologies helps expedite the identification process, leading to increased efficiency and better utilization of examiners' time while reducing backlog in ballistic investigations.

The utilization of 3D technology in ballistic identification represents a significant leap forward from traditional two-dimensional methods. By capturing intricate details and surface characteristics of bullets and cartridge cases, the EVOFINDER® system enhances the accuracy and reliability of forensic examinations. Furthermore, the implementation of database correlations enables the system to intelligently sort and match samples, presenting potential matches to examiners for further analysis.

The incorporation of VCM adds another layer of versatility to the EVOFINDER® system. With VCM, examiners can compare samples from different locations, facilitating collaboration and information-sharing among forensic teams regardless of geographical barriers. This capability not only enhances the accuracy of ballistic identification but also fosters a more interconnected and collaborative forensic community.

In conclusion, the EVOFINDER® Ballistic Identification System, powered by 3D technology, database correlations, and virtual comparison microscopy, has transformed the landscape of ballistic investigations. Its ability to efficiently manage vast image databases, enable virtual examinations, and accelerate the identification process has a profound impact on forensic science. By harnessing these advancements, forensic examiners can uncover critical evidence faster, leading to more effective criminal investigations and bolstering public safety.

3D Technology; Ballistics; VCM

B154   Feature Similarity (FS) and Congruent Matching Features (CMF) Methods for Comparison of Impressed Toolmarks in Firearm Evidence Identification

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Learning Overview: The goal of this presentation is to introduce the FS and CMF methods for objective comparison of impressed toolmarks in firearm evidence identification.

Impact Statement: This presentation will impact the forensic science community by comparing the performance of the FS and CMF methods with the existing Areal Cross Correlation Function (ACCF) and Congruent Matching Cells (CMC) methods, respectively, using three datasets of breech face and firing pin impressions with granular, parallel, and circular mark characteristics, respectively. The FS and CMF methods show promising improvements in the separation of Known Matching (KM) and Known Non-Matching (KNM) comparison scores.

The FS and CMF methods are introduced for objective comparison of impressed toolmarks in firearm evidence identification. Both methods seek to combine the advantages of areal and feature-based comparisons of toolmark images (i.e., the pixel-wise comparison of the surface topography of both samples and comparison of the type and distribution pattern of individual features on the topography images). Both methods apply optimized height thresholding combined with the Maximally Stable Extremal Regions (MSER) method to generate pixel blobs that represent distinct features on band-pass filtered topography images.

For the FS method, we use a trinary feature map to represent the detected peak and valley features. The similarity of two compared feature maps is expressed by the Feature Similarity Score (FSS), which is normalized as the FS value that represents the correlation coefficient of the compared feature maps. A pattern search algorithm is applied to find the image registration position and orientation that yields the maximum FS value. Compared with other areal comparison methods, such as those based on the normalized ACCF, the FS method is less affected by local regions with poor mark visibility or spurious marks with relatively large heights or depths that can decrease the accuracy of identification.

The CMF method is an extension of the CMC method. We extend the CMC method by replacing the regular grid of square correlation cells with irregular feature cells whose shape follows the feature blobs identified on the reference image, while covering the entire image region.

We compare the performance of the FS and CMF method with the ACCF and CMC method, respectively, using three datasets of breech face and firing pin impressions with granular, parallel, and circular mark characteristics, respectively. The FS and CMF method show promising improvements in the separation of KM and KNM comparison scores.[1-6]

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Firearms; Imaging; Identify

B155   The Impact Behavior of 9mm Jacketed Hollow Point Bullets on Laminated Glass

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Learning Overview: The goal of this presentation is to describe the significance and analysis of critical angle behavior of a 9mm jacketed hollow point bullet on laminated glass. The systematic nature of the study allows for further discussion of various trends in impact behavior relative to the angle of incidence.

Impact Statement: This presentation will describe a systematic approach for determining the critical angle of incidence between an ammunition-substrate pairing as well as elucidate impact behaviors that may be observed and analyzed in the process. This presentation will further bolster the knowledge base of shooting reconstruction with regard to the behavior of laminated glass (commonly encountered in automotive windshields and side glass) as a substrate.

The reconstruction of shooting incidents in forensic science investigation is critical to determine the sequence and circumstances that occurred during an incident. This is especially true in officer-involved shooting incidents, where event details may be in question or under scrutiny.[1] A specific understanding of the position, orientation, and shooting distance of an officer are often necessary to determine if the actions taken were justified.[2] To do so is often crucial to determine the trajectory of projectiles based on impact behavior.

Shooting reconstruction is a highly dynamic and complex process that in part may attempt to determine the origin and sequence of fired projectiles. These attempts are further complicated by the impact behavior of bullets at the critical angle of incidence. Critical angle refers to the transition from perforation or penetration behavior to ricochet behavior as bullet incidence angle is decreased. At and near the critical angle of incidence, it becomes more difficult to predict whether a bullet will perforate, ricochet, or a combination of both (upon fracturing). The ammunition tested in this study is a standard-issue cartridge used by agents of the United States Department of Homeland Security. By studying the impact behavior of 9mm Luger® +P Speer Gold Dot 124-grain jacketed hollow point ammunition on AS-1 laminated glass, we attempt to describe the phenomena at the critical angle for reference in shooting reconstruction. Using empirical testing methods, we tested a range of incident angles around the critical angle to create a distribution of impact behavior and developed a method to provide a more precise determination of the critical angle.

A number of studies have been conducted regarding bullet impacts in float glass.[3,4] However, studies of bullet behavior on laminated glass have generally small sample sizes and involved multiple impacts to each windshield.[5-7] Aside from affecting substrate integrity upon successive shots, this multi-impact approach also necessitates that impact sites be spaced apart on the windshield, adding a target-curvature variable in the process. This study was conducted with 100 flat panes of laminated glass, with a single shot fired through each. This sample size is large enough to draw statistically significant inferences, provided consistent substrate integrity, and removed curvature as a confounding variable.

Ammunition was fired from an H-S Precision® Universal Receiver with a 9mm SAAMI® test barrel at the test panes held in a steel mount designed for this project. An Oehler Model 83 chronograph was used to measure the pre-impact. Post-impact behavior was recorded on witness paper, as well as with a Phantom® TMX 7510 high-speed camera. Trends in the distribution of glass fragments, the fragmentation of the bullet, and the relative loss of kinetic energy of the bullet from impact were recorded.

Unlike the full metal jacketed bullets of previous studies, the jacketed hollow point bullets in this study perforated the glass (at least partially) at much more oblique angles of incidence. These bullets also appear far more prone to jacket separation. Consistent perforation by jacket material occurred at an angle of incidence of 15-degrees and full ricochet of intact ammunition was not observed until an incidence angle of 10-degrees. In addition to reducing the maximum ricochet angle, the jacket separation created a broader transition across the critical angle than expected. Methods for defining the critical angle will be discussed.

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Ricochet; Shooting Reconstruction; Critical Angle

B156   The Interoperability of Firearm Toolmark 3D Measurements on Objective Similarity Metrics

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Learning Overview: After attending this presentation, attendees will better understand how 3D topography measurements of firearm toolmarks generated by different instruments and laboratories affect similarity metrics Areal Cross Correlation Function (ACCF) and Congruent Matching Cells (CMC).

Impact Statement: This presentation will impact the forensic science community by informing attendees that the adoption of 3D topography measurement is a major paradigm shift in the science of firearm and toolmark analysis. As with any novel technology, validation is required at a foundational level to ensure efficacy and meet *Daubert* criteria. The round robin inter-comparison proposed in this study is a well-established method to characterize, diagnose, and reduce differences in measurement results obtained by different labs and instruments. Equivalency of measurement results and comparison results is essential for building confidence in evidentiary findings obtained by forensic labs through this new technology. Results of the study provide data for *Daubert* hearings and will contribute to standardization efforts of procedures for measurement and quality control, as well as the development of standard operating procedures by forensic labs. Results will also help instrument manufacturers and researchers develop novel objective similarity metrics and weight of evidence estimation procedures.

There has been a major paradigm shift in firearm and toolmark analysis toward the use of 3D topography measurements. The new approach bolsters objectivity through the International System of Units (SI) traceable measurements. Several manufacturers are now offering specialized 3D microscopes for toolmark measurement that rely on differing measurement principles that each has advantages and challenges. These instruments are currently being used in laboratories for Virtual Comparison Microscopy (VCM). The ultimate goal for these instruments is to generate measurements used to report on the statistical weight of evidence of a comparison in case work.

For impressed toolmarks, there is no comprehensive study that characterizes the resulting differences in 3D data obtained at different forensic labs and their effect on objective similarity scores. This is an important gap in the quest for objective comparison results and quantitative weight of evidence reporting. This gap needs to be addressed to ensure consistency in results among labs and provide associated foundational data for future *Daubert* hearings.

The research evaluated the effect of measurement source variations on similarity metrics. This was accomplished through a round-robin study where each lab/instrument measures the same set of 120 cartridge cases fired from four sets of consecutively manufactured firearms. A similar inter-lab study was conducted for striated toolmarks on bullets where significant deviations were observed between some labs and instruments.[1] These findings were then used to improve measurement protocols, data processing, and analysis methods.

To quantify the differences between labs and technologies, each lab's measurements was analyzed using two well-established similarity scores: the normalized Areal Cross Correlation Function ($ACCF_{MAX}$) and the number of Congruent Matching Cells (CMC).[2,3] The results were used to generate Known Matching (KM) and Known Non-Matching (KNM) score distributions that were be used to statistically analyze for differences between labs and systems. Results will facilitate improvements in consistency of measurement results while providing the foundational research data required to defend the future use and interoperability of 3D measurements in case work.

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3D Technology; Firearms; Microscopy

B157   The Effect of Gunshot Angle of Incidence on Distance Determination

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Learning Overview: After attending this presentation, attendees will understand how the gunshot angle toward a person or object will affect the quantity, pattern, and density of Gunshot Residue (GSR) and the applicability of angled shots in GSR distance determinations.

Impact Statement: This presentation will impact the forensic science community by informing attendees that there are dynamics within angled shots that produce different GSR patterns and particle distributions. The angle at which the gun was held in relation to the target is one of the key conditions that can affect the deposition of GSR. Without consideration of this important element in the shooting incident, a distance determination can be significantly inaccurate.

GSR is a primary element produced by all firearms. The presence, absence, distribution and/or density, and overall pattern shape created by the various forms of GSR are widely used as a means of determining the distance between a firearm and a target: human, animal, garment, or other inanimate object. All forensic ballistics laboratories have protocols for making distance determinations based upon the GSR in which a test is performed with the same firearm and the same ammunition as was used in the actual incident.

These forensic distance determinations have been performed with the firearm aimed in an orthogonal (perpendicular) orientation toward a cotton fabric target. To determine the distance, several shots are made at different distances into the fabric target until the distance is sufficiently great that no GSR reaches the target material. The resulting patterns and density of GSR particulate (burned, partially burned, and unburned gunpowder particles) on the target material are then compared to the pattern diameter and density of GSR on the clothing, skin, or other objects from the actual incident. When a pattern and/or density on the test target material is similar in diameter and density to the actual shooting case object, a distance determination can be made.

This research project involved the use of 9mm and .45 Automatic Colt® Pistol (ACP) handguns. Testing was performed using four angles of incidence: 0", 30, 45, and 60 degrees. A total of 36 test shots were made. The GSR particles were identified and quantified using scientific image analysis software (ImageJ). Quantity of particles, density, and overall patterns were identified. Data were visualized and statistically analyzed by using R and GraphPad® Prism® (v 9. 4. 1) software. Analysis of Variance (ANOVA) followed by Dunnett's T3 multiple comparisons tests were performed to find statistically significant differences with their associated p-values.

When properly performed, this method can be used to accurately determine the approximate distance between a gun muzzle and a target. But there is a significant flaw in the process that this paper will address. Many actual, real-life shootings are done with some degree of angularity between the gun and the target. The angle at which the gun was fired toward the target will affect the amount and distribution of GSR. The actual difference in the amount of GSR and the resultant pattern has never before been quantified and examined. The research represented in this presentation demonstrates that there is a significant difference in the GSR patterns produced with angled shots.[1-8]

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Firearms; Gun Shot Residue; Ballistics

B158   A Needle in a Haystack: Are Those "Rare" Minutiae in Palms Really as Uncommon as You Think?

Heidi Eldridge*, The George Washington University, Washington, DC

WITHDRAWN

B159   Interdigital Frequencies of Pattern Types and Their Arrangements as a Key to Palm Print Identification

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Learning Overview: After attending this presentation, attendees will be informed about important features to be taken into consideration when providing a human identification based on a palm print fragment collected from a crime scene. Our data on pattern frequencies, and their arrangements in the interdigital intervals and distal bases of palm prints, bring a useful complementary element for human identification in the comparative process and in research in automated systems.

Impact Statement: This presentation will impact the forensic science community by bringing a different approach to the identification of a palm print fragment. In addition to the elements commonly considered for identification, such as minutiae and deltas, when the pattern frequencies and their arrangements in the interdigital intervals and distal bases are also analyzed, the global value of the analyzed characteristics is increased, which helps the examiner's decision-making when determining an identification. This different approach is particularly important in the forensic field, where the fragments analyzed in most of the cases are of poor quality, since they are left at crime scenes in conditions that are nothing like the ideal palm print collection conditions. Also, along with its usefulness in the comparative process and research, the results of this study can be helpful for technology companies to make available search filters in automated systems that consider the pattern arrangement frequencies.

Palm prints are important evidence that contribute to identifying who was present at a crime scene. Knowing the line systems that form the palm print ridges, as well as the patterns that occur in the palm print regions, is fundamental for human identification purposes. Thus, the aim of this study was to provide the frequency of these patterns—independently and in combination—in the interdigital intervals and distal bases of palm prints, helping the examiner to proper orient the palm print, determine the hand (left or right), as well as increase their certainty in providing an identification. The data can also contribute to automated systems by providing frequency values to be used as search filters.

In this study, 4,000 palmprints, 2,000 from the right hand and 2,000 from the left hand, were analyzed regarding the pattern types at the interdigital intervals and distal bases: loop, whorl, composite and open field in the intervals, and delta, plain arch, and tented arch in the bases. Pattern frequencies of each interval and base and its arrangements were calculated though *R* software. Statistical comparisons were made between right and left hands in two steps. First, differences between right and left hands from different people (3,054 individuals) were calculated with chi-squared and Fisher's exact tests. Subsequently, the differences between both hands of 473 individuals were analyzed using the Mcnemar's test. The results obtained show that the number of deltas varies according to the pattern types that occur. Among all the pattern arrangements mathematically possible, about 13% were observed in the studied population. Few of the possible pattern arrangements are observed in both hands. Many of the possible pattern arrangements observed occur in less than ten palm prints regarding each hand. When left and right hands were compared, it was found that there is an association between side and pattern type at all the intervals and at the base 4 for the independent data. That is, the pattern observed at all the intervals and the base 4 is associated with whether the hand is the left or the right one. For paired data, where individuals had both hands analyzed, there is no association between the side and the incident pattern at interval 2 and the base 1. However, at base 4, there is an association between the side and the pattern.

In conclusion, when a palm print fragment has low quality and/or few minutiae, the study of the pattern occurrence at each interdigital interval and distal base along with their arrangements increases the value of the global characteristics observed. Taken together, all these aspects contribute to the examiner's decision-making when determining an identification.

Identify; Latent Prints; Forensic Analysis

B160   Toward Consistency in Latent Print Examiners' Naming Conventions and Minutiae Frequency Estimations

Adele Quigley-McBride*, Simon Fraser University, Burnaby, BC, Canada; Heidi Eldridge*, The George Washington University, Washington, DC; Brett Gardner*, University of Virginia, Charlottesville, VA

Learning Overview: After attending this presentation, attendees will be aware of new data suggesting that fingerprint analysts vary in how they refer to fingerprint features (minutiae) and how often they estimate the frequency with which those minutiae appear in fingerprints. Attendees will also gain a better understanding of the extent to which fingerprint analysts report using these subjective estimates in their analyses. Finally, attendees will be able to identify and discuss potential sources of inconsistency in fingerprint analyses and conclusions.

Impact Statement: This presentation will impact the forensic science community by providing fingerprint analysts with a consensus view of naming conventions, perceived frequency of minutia types, and the use of subjective judgments about minutiae frequency in "value/no value" judgments and comparisons. This knowledge will allow for immediate reductions in variability between analysts and will also form the basis for future work comparing real-world minutiae counts to fingerprint analysts' perceptions of how frequent those minutiae are.

Forensic science literature has increasingly emphasized the importance of consistency and reliability across forensic disciplines in recent years. Latent fingerprint comparison, in particular, has received a high level of scrutiny as research on some of the core aspects of these analyses remains lacking. Assessing the appearance and placement of fingerprint minutiae (the shapes and features within friction ridge patterns) is central to the task of a fingerprint analyst, as is an analyst's ability to communicate their analytic process and the reasons for their expert opinion. Although we know that some minutiae are rarer than others, there are no large-scale studies assessing analysts' perceptions of, or actual, minutiae frequency.[1] People generally struggle with the task of estimating how often events or features occur, and analysts' estimates of the frequency with which different friction ridge patterns occur (loops, whorls, and arches) are often inconsistent with objective data.[2,3] Thus, we can expect that analyst's estimates will vary and that analysts' beliefs about minutiae rareness or commonness will affect how much weight is given to each feature during analyses.[4] Compounding these inconsistencies is a lack of conformity in the labels used for minutiae (e.g., the same circular shape is sometimes called a lake, an enclosure, or an eye), which adds to the variation seen in fingerprint analyses and reporting.

To assess analysts' perceptions of minutiae frequency and their naming conventions, we collected data in two online studies from a diverse sample of fingerprint analysts (*n* = 132 in Study 1, and *n* = 94 in Study 2). Most participants performed casework weekly in the United States with an average of 13 years of experience. We surveyed examiners at two time points, one month apart, with 75% (*n* = 99) completing both time points in Study 1 and 60% (*n* = 56) completing both in Study 2.

Study 1 revealed three important types of variation in fingerprint analysts' responses. First, there was considerable variation in the labels used to describe the same friction ridge shapes. Second, analysts often agreed on the relative rarity of minutia types (rank order data), but their estimates of the percentage of fingerprints in which they could expect to see examples of different minutia types varied substantially. Finally, analysts' estimates varied across time, especially for rare minutiae when they had recently encountered an example in their casework.

Analysts also reported how they used minutiae frequency in their "value/no value" judgments and comparison decisions. Most analysts (84%) reported using their perceptions of minutiae frequency when making "value/no value" judgments and when evaluating the level of correspondence between two fingerprints during a comparison analysis (89%).

Participants in Study 2 were asked similar questions, but were also asked to indicate whether particular minutia types were more common in the center or periphery of fingerprints, or in particular friction ridge pattern types (i.e., loop, whorl, or arch). There was even less consensus observed in these judgments compared with Study 1, and most analysts changed at least some of their responses one month later in the second survey.

Taken together, these data provide new insights into sources of variation in fingerprint analysis and suggest a need for standardization in minutiae naming conventions. Analysts' frequency perceptions varied substantially, both between analysts and for the same analyst across time. Thus, the weight that analysts give to different minutia types is likely to vary between analysts and at different times for the same analyst. How these subjective estimates compare to estimates calculated from actual counts will be assessed in future research. These studies can also serve as a model to other forensic disciplines seeking to increase consistency and reliability.

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Accuracy; Reliability; Fingerprint

B161   Not Everything Is as It Seems: The Importance of Minutiae

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Learning Overview: This presentation will depict three very different cases of hanged subjects to demonstrate the importance of correctly interpreting the data collected on the crime scene and the subsequent postmortem examination. After attending this presentation, attendees will better understand the importance of an accurate analysis of the location and crime scene in order to not be deceived.

Impact Statement: This presentation will impact the forensic science community by demonstrating that things are not always what they seem. Indeed, the forensic pathologist should pay more attention to analyzing the scene and medical history of patients and promote collaboration with other agencies involved at the crime scene.

Hanging is one of the most common methods of suicide and represents the most important of mechanical asphyxia, in which the airway is obstructed by compression of the neck caused by a taut ligature or by the weight of the victim or part of the victim. Hanging may be complete or incomplete (depending on whether the body is fully suspended), typical or atypical (depending on the position of the knot), interrupted, simulated, etc. Consequently, ligature marks on the neck may have typical features that are usually helpful in distinguishing hanging from ligature strangulation. Other elements that can be used to support the hypothesis of the suicidal intent of the deceased are circumstantial data and findings at the death scene. However, in some cases, the forensic examination could reveal certain elements or confounding features that may suggest the involvement of a third party. This study presents three cases of hanged persons in different and unusual situations.

The first is the case of a 63-year-old man who was found in the backyard of his downstairs neighbor with a broken cord around his neck and a considerable wound on his head. Outside the house, on two different balconies, two pieces of rope were found, similar to the one found around the man's neck. Inside the house of the deceased, more disturbing elements were discovered, including copious bloodstains on the walls and floor.

The second case is the one of a 50-year-old man found hanging from a tree. The strange thing in this case is that the man was found almost decapitated, with only a fine flap of skin connecting his head to the rest of his body. All other structures, such as vessels, respiratory tract, and even vertebrae, appeared to have been crushed, and a blood pool was found under his feet.

The last one is the case of a 41-year-old man who was also found hanging from a tree near his house, several days after his disappearance. Curiously, a knife was recovered in his hand as if he was defending himself from a possible attacker.

In all cases, only a meticulous evaluation of the scene, the remote medical history, and the results of the ensuing investigations allowed them to be definitively classified as suicidal events, unequivocally excluding the hypothesis of third-party intervention. Therefore, the aim of this work is to highlight the importance of the correct analysis of the case, a combination of precise interpretation of circumstantial and autoptic data in the presence of ambiguous circumstances, to make a differential diagnosis between suicide by hanging and suspension after a homicide.

Hanging; Crime Scene Investigation; Differential Diagnosis

B162   Dog Bites or Knife Wounds? A Case Report of Atypical Neck Injuries

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Learning Overview: The objective of this presentation is to demonstrate the value of existing literature in determining a production process and understanding the pathophysiology of lesion formation. Through this case of cervical wounds, we aim to illustrate that certain types of lesions can be easily confused. However, by considering the full range of injuries (cutaneous, subcutaneous, vascular, and bone), the mechanism becomes more apparent.

Impact Statement: This presentation will impact the forensic science community by serving as a reminder of the importance of considering both cutaneous and deep lesions as a whole in order to avoid confusion between different mechanisms of injuries.

A 24-year-old woman was brought to the emergency department following dog bites according to a witness at the scene. She quickly presented a cardiorespiratory arrest and, despite adapted treatment, passed away 12 hours later. Autopsy revealed 21 deep cervical wounds, with unclear bilateral carotid lesions, dilaceration of neck muscle masses, a laryngeal wound, and avulsion of the right lobe of the thyroid. Some of these wounds were buttonhole-shaped, with sharp edges and a rat's tail appearance, suggesting the use of a bladed weapon. In addition to these wounds, there were multiple circumferential superficial wounds in the cervical region, blunt facial trauma with no associated fracture, and ecchymosis on the internal face of the left arm. It was concluded that the cause of death was asphyxia and hemorrhagic syndrome. Considering all the lesions, the medical examiner suggested the intervention of a third person using a sharp object.

The investigation took a drastic turn following the coroner's findings, leading to the arrest and detention of the witness on charges of culpable homicide. A study of the medical records confirmed the presence of dissection of both carotid arteries, without vascular sectioning. Dissection of the left vertebral artery was also identified. Fractures of the cervical transverse processes had also been identified on a Computed Tomography (CT) scan performed in the emergency department.

This second forensic expert supported the conclusion that the cervical lesions were consistent with dog bites, particularly due to the dilacerated appearance of the muscle masses and the absence of clear tissue continuity. Furthermore, the association of vascular dissections and fractures of the cervical transverse processes can be attributed to hyper-extension and/or compression of the cervical spine[1-6].

All these types of lesions have already been described in the scientific literature in cases of dog attacks. Moreover, several cases have reported the presence of wounds resembling knife wounds following dog bites[7,8]. Death was described as secondary to cerebral anoxia resulting from multiple dissections of the neck vessels and asphyxia related to the laryngeal wound, aggravated by blood spoliation. All the observed lesions were determined to be consistent with dog bites, leading to the release of the defendant.

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Vital Wounds; Dog Bites; Homicide

B163   Odor Contamination in Explosive Canine Training Aids and Steps Toward Contaminant Mitigation Through Off-Gassing

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Learning Overview: After attending this presentation, attendees will understand how easily training aid contamination can occur during regular training and how to mitigate contamination through the use of off-gassing.

Impact Statement: The findings of this research will impact the forensic science community by directly aiding in improving explosives detection by allowing canine teams to be aware of how contaminants enter the headspace of their training aids as well as provide recommendations as to how to mitigate suspected contamination. The cleaner training material will allow for high proficiency in canines for lower false or missed alerts, heightening national security and improving current methods used by defense agencies. This research will also provide insight for standards boards to create standards for training aid handling, maintenance, and validation.

This presentation will inform attendees on the necessity of maintaining the integrity of canine training aids as they are prone to contamination, provide one technique for mitigating contamination, and understand how the headspace of training material can change in as fast as six months. This impacts the forensic community as it emphasizes the need for standards to ensure that training material for the sensitive biosensors are of high quality to yield high-quality detectors.

Explosives have become a major threat to those serving abroad or even to the civilian public. To ensure national security, it is essential to detect explosive threats before a detonation event. Canines have transformed explosive detection, becoming some of the most sensitive and reliable sensors available. To maintain the desired detection proficiency levels required by the American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 092 of a false alert rate of less than 10% and discrimination of non-target odors, it is important to train canines on materials with odors representative of true, clean explosives. Studies have found that originally certified canines have shown up to 40% false detection rates in non-testing scenarios, suggesting inconsistencies in training maintenance due to unsatisfactory training material.[1] With contaminants altering the odor profiles of explosive training aids, it is necessary to understand how unwanted odorants adsorb to and desorb from explosive training materials to mitigate contamination.

This study provides insight into how contaminants alter the headspace of training aids over time and how to minimize the detrimental effects of contaminated material. This was done in three experiments escalating in complexity. The first was understanding contamination in a laboratory environment and tracking contamination over a 2-week period to understand when contamination reaches its maximum. This was followed by allowing off-gassing of contaminated materials up to 24 hours to determine how long it takes to remove contaminants from the headspace of training material before storage. The next experiment used training aid surrogates to be handled and stored by canine teams in the same manner as their actual training aids. Surrogates were analyzed bimonthly to understand which environmental contaminants are easily adsorbed onto materials as well as which training materials are most prone to contamination and therefore must be monitored more frequently for presence of non-target odors.

The training aids in question were sampled by use of Headspace/Solid Phase Microextraction (HS/SPME) and their headspace profiles were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). The use of chemometrics during data analysis allowed for further conclusions regarding types of explosive materials and training aid substances that are most prone to contamination and would benefit most from contamination mitigation techniques. It was found that contamination of the explosives was less handler-dependent and systematic over time, while contamination of substrates was both handler-dependent and the dependent of the sorbent property of the material. It was also determined that some sorbent explosive materials will desorb quickly while others that may not initially adsorb large amounts of odor struggle with desorption of contaminants, affecting the contamination mitigation process.

All these experiments allowed for conclusions that can be directly presented to canine handlers and standards boards to form recommendations regarding training aid maintenance and validation. Results will also apply to those at defense agencies to modify current methods of training aid handling to uphold the integrity of the training material and canine detection proficiency to maintain the current expectations of national security.

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Canines; Mitigation; Explosion

B164   An Improved Analysis of Nylon Microplastic Trace Evidence Through Enhanced Signal-to-Noise Ratio in Room-Temperature Fluorescence Spectroscopy

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Learning Overview: The goal of this presentation is to teach attendees the advantages room-temperature fluorescence spectroscopy provides in the analysis of nylon trace evidence, as well as the how to maximize the potential of the technique for small (<500μm) samples.

Impact Statement: This presentation will impact the forensic science community by demonstrating how room-temperature fluorescence spectroscopy can provide more information about nylon evidence samples than other non-destructive techniques alone. This additional information is important because nylon is well-suited for 3D printing of weapons and keys, and the increasing availability of 3D printing technology is also increasing the risk of 3D printing for illegal activities. The ability to link trace evidence to its source is always impactful, and fluorescence spectroscopy can be used to make that link even stronger.

As 3D-printing technology continues to advance and become more accessible, forensic scientists will need more reliable and effective techniques for the analysis of its associated trace evidence. The analysis of microplastic trace evidence is often done non-destructively through spectroscopic techniques, especially Fourier-Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy. Destructive techniques, including Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) and Direct Analysis Mass Spectrometry (DART®), are occasionally used for analyzing microplastics; however, destructive techniques are commonly discouraged in forensic settings, and especially in trace evidence analysis, due to the desire to preserve limited evidence.

Room-temperature fluorescence spectroscopy, a non-destructive technique, has recently been shown to successfully analyze nylon microplastics through the use of a fiber-optic probe connected to a commercial spectrofluorometer.[1] Nylons, or polyamides, are a class of durable polymers that can be used to 3D print objects like weapons and keys. Though the chemical structure of nylon is not expected to be fluorescent, the synthesis and processing of nylons imparts fluorescent impurities in the backbone of the polymer that create unique fluorescence spectra, and these spectra can allow for discrimination beyond general polymer class (e.g., Nylon) or monomer subclass (e.g., Nylon 6/6).[2,3] However, because the fluorescence arises from impurities and not the bulk polymer itself, the recorded signal is relatively low, particularly when analyzing small trace evidence samples (less than 500μm).

Herein, we present improvements to the room-temperature fluorescence analysis of nylon microplastics that have increased the signal-to-noise ratio by an order of magnitude. Utilizing a neutral-density filter as a sample substrate and orienting the fiber-optic probe at an angle to the benchtop, microplastics from Nylon 12 and Nylon 6/6 as small as 125μm were routinely and successfully analyzed. The experimental procedure proposed here provides forensic scientists with the necessary tools to confidently analyze nylon trace evidence, be it from 3D-printed materials or otherwise, and make compelling arguments as to whether that evidence came from a specific source.

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Trace Analysis; Fluorescence; Polymers

B165   An Evaluation of a Comprehensive Raman Spectral Library for the Identification of Soil Minerals

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Learning Overview: After attending this presentation, attendees will understand the value of a comprehensive digital Raman spectral library for the identification of soil minerals.

Impact Statement: This presentation will impact the forensic science community by detailing the challenges and steps required for developing, testing, and utilizing a digital Raman spectral library for the identification of minerals.

Raman spectroscopy is a valuable tool for elucidating the chemical structure and more of an unknown sample. A comprehensive collection of data, or library, is required for the proper identification of any material. Searchable spectral libraries have demonstrated value for the identification of a plethora of different forensic samples, such as drugs, organic pigments, and polymers. Mineral analysis presents another opportunity where a comprehensive searchable Raman spectral library could aid in the identification of samples for both geological and forensic purposes. However, while there have been several collections of mineral spectra created, there remains to be a comprehensive searchable Raman spectral library. Software programs like KnowItAll currently only have a few hundred mineral spectra, while other online databases like RRUFF[2] allow one to view thousands of varying mineral spectra, but do not have a capability to compare an unknown sample to the library.[1] This demonstrates the need for a comprehensive searchable mineral database to provide for their automated identification.

The purpose of this research is to develop and evaluate a searchable mineral library of Raman spectra to assist in identification of the minerals found in unknown soil samples through our ongoing research with Particle-Correlated Raman Spectroscopy (PCRS). PCRS is an analytical technique that combines automated image analysis with Raman spectroscopy to provide morphological measurements and chemical identification of discrete particles within a complex mixture. Reliable and automated Raman spectral identification is critical for successful PCRS; however, it must also be rapid (no more than a few seconds for Raman analysis) because of the large number of particles to be targeted for analysis. Another challenge for mineral identification by Raman spectroscopy is the natural variations within a mineral variety that can cause spectral differences. Factors such as polymorphism and twinning can cause minerals of the same composition to have different spectral peaks from Raman spectroscopy. Additionally, the fluorescent properties of some minerals can cause effects on the spectra that overtake the weaker Raman scattering effect, making it more challenging to identify some minerals. Thus, it is important to evaluate the capabilities of a purpose-built, searchable, and comprehensive Raman spectral library of minerals.

The capabilities for the automated spectral library identification of mineral grains in soil were evaluated using a known set of 60 comminuted soil minerals. The RRUFF[2] database was imported into KnowItAll to create the comprehensive mineral library, and the ability to correctly identify the 60 comminuted minerals was evaluated using two laser excitation wavelengths (785nm and 532nm). When comparing the automated mineral library correct identification rate before and after the addition of the RRUFF[2] database, as expected, there was a noted increase in correct identifications of different minerals and a greater hit quality index regarding the strength of identification. With the addition of the RRUFF[2] mineral database to KnowItAll's searchable library, correct identifications went from 15 of 60 (25%) to 47 of 60 (78.3%) minerals. The ability of this Raman searchable library to identify more mineral spectra demonstrates the importance of having such a comprehensive library available for analysis of unknown samples. The results of this research have the potential to be applied to an overall mineral identification procedure that would be useful for both the forensic science and geology fields.

Reference:
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Raman Spectroscopy; Spectral Library; Mineral Identification

B166   Pigment Identification in Solution-Dyed Fibers

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Learning Overview: This presentation provides a primer on the microscopical recognition, classification, and identification of solution-dyed (i.e., pigment-colored) fibers. The goals of this presentation are intended to serve two main purposes: (1) to provide fundamental information about the pigment packages used to color solution-dyed fibers (e.g., the number and types of pigments used to color various polymers and brands of fiber); and (2) to provide a practical analytical approach to the study of solution-dyed fibers that can be integrated into existing trace evidence fiber comparison protocols.

Impact Statement: This presentation will impact the forensic science community by filling a knowledge gap regarding: (1) the recognition of solution-dyed fibers, (2) the characterization and identification of pigments used to color this class of fiber, and (3) the trends and significance related to solution-dyed fiber evidence.

Solution-dyed fibers are synthetic fibers colored during the manufacturing process by microscopic and submicroscopic solid pigment particles prior to extrusion. The use of pigments to color fibers provides improved weatherability and lightfastness over traditionally dyed fibers and represents an eco-friendly alternative method for producing colored fibers. As such, solution-dyed fibers have steadily gained market share and are being used in an increasing variety of polymers for an expanding range of applications (including trunk liners of vehicles as well as commercial and consumer carpeting). Despite their minute size, the visualization and identification of these fine pigment particles is accessible using robust methods that are already commonly employed (e.g., polarized light, fluorescence microscopy, and scanning electron microscopy/energy dispersive x-ray spectroscopy) or are becoming more common (e.g., Raman microspectroscopy) in trace evidence laboratories.

The act of recognizing that a fiber was colored by pigmentation represents a significant point of comparison in a fiber examination that is not presently included in many fiber examination protocols. This research aims to fill this knowledge gap by providing the results from an analytical approach that was developed and optimized to recognize and differentiate solution-dyed fibers from traditionally dyed fibers and has been expanded, in the present work, to characterize and identify the population of pigments in such fibers.

This research is based on the study of a population of 225 fibers selected from our internally developed, curated fiber reference collection consisting of thousands of solution-dyed fibers. The selected fibers were chosen to represent a range of manufacturers that span a variety of commercial applications, colors, and fiber types (e.g., polyolefin, nylon, polyester, rayon) selected to reflect market trends as well as the sources of fibers that are anticipated to be encountered as trace evidence. Building from our initial results, which provided a method to recognize solution-dyed fibers, this presentation is focused on expanding the approach to the process of identifying the range of pigments used in these fibers through a combination of chemical and elemental analyses.[1]

The identification process presented here explores several sample preparation methods and draws from prior microscopical examinations to direct analyses by Raman microspectroscopy (532 and 785nm lasers). Both "bulk" fiber analyses and particle-focused, confocal Raman measurements on cross sections have been explored to determine the optimal approach for identifying the suite of different pigments that are found in a given fiber. The identification is also supported by elemental analyses conducted by energy dispersive X-ray spectroscopy analyses of the fibers (longitudinal mounts) conducted in the scanning electron microscope. Ultimately, the approach as well as the tabulated identification data (i.e., the name of a pigment by its Color Index designation or chemical structure) provides new insight into the trends, and commonality (or rarity) of the pigments used to color fibers. The combination of this research is intended to provide examiners with a means by which these previously unexplored properties can be exploited in casework samples.

Reference:
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Raman Microscopy; Fibers; Microscopy

B167   Polarized Light Microscopy of Vegetable Fibers

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Learning Overview: After attending this presentation, attendees will understand the role of polarized light observations in the microscopical examination of a variety of vegetable fibers that may be encountered in forensic science casework.

Impact Statement: This presentation will impact the forensic science community by providing data to aid in the microscopic identification of several vegetable fibers that are now part of the modern environment.

Vegetable fibers have been used since antiquity for textiles and cordage. These fibers derive from a variety of sources: there are seed fibers, bast fibers from the stems of plants, leaf fibers, fruit fibers and stalk fibers. Vegetable fibers may occur as trace evidence in criminal cases. In forensic science laboratories, these fibers are identified microscopically using brightfield and polarized light microscopy. Petraco and Kubik present an analytical procedure for the identification of vegetable fibers.[1] The fibers are viewed with transmitted light (brightfield illumination), between crossed polars with the fibers oriented for maximum birefringence, between crossed polars with the fibers oriented at extinction, and, finally, between crossed polars at extinction with a first order red accessory plate inserted. The last observation is the Herzog or red plate test used to distinguish bast fibers based on the S-twist or Z-twist orientation of the cellulose molecules in the fibers. These observations permit the microscopical identification of the most common vegetable fibers. Petraco and Kubic present data for a selection of common plant fibers: abaca (Manila hemp), sisal, cotton, flax, hemp, jute, ramie and kapok.[1] In recent years, many other plant fibers have come into use and may be encountered in forensic casework. Agave fibers are used in exfoliating wash clothes; coconut (coir) fibers are used for pet and plant bedding, upholstery stuffing, and floor mats; corn fibers are used in brooms; raffia fibers are used in floral arrangements; and wheat straw and barley straw are used for floral arrangements and water clarification.

Samples of agave, barley straw, coir, corn, raffia, and wheat straw were obtained from retail sources. Many of the fibers comprising the samples are composed of bundles of cells (called fiber ultimates). To free fiber ultimates, fibers were treated with a mixture of equal volumes of 6% hydrogen peroxide and glacial acetic acid at 100°C for eight hours.[2] Fiber cross-sections may be useful for identification.[3] Fiber cross-sections were prepared by embedding fibers in drops of hot glue (One World Technologies, Inc., Anderson, SC), then cutting sections free hand from the hardened glue using single-edge razor blades. The fibers, fiber ultimates, and the fiber cross-sections were mounted on microscope slides with Permount® mounting medium. Two microscopes were used to examine the mounted samples: a stereomicroscope for reflected light and darkfield observations (AmScope, Irvine, CA) and a transmitted light polarizing microscope (AmScope, Irvine, CA). Photomicrographs were captured with an OMAC® 18-megapixel digital eyepiece camera (AmScope, Irvine, CA). The following photomicrographs were obtained for the fibers and fiber ultimates: (1) reflected light; (2) darkfield; (3) brightfield; (4) crossed polarizing filters (fibers oriented for maximum birefringence); (5) crossed polarizing filters (fibers oriented at extinction); and (6) crossed polarizing filters (fibers oriented at extinction with first order red plate inserted).

Photomicrographs were captured of the fiber cross-sections using the stereomicroscope using reflected light. The following results were observed for the fiber ultimates:

Fiber	Elongation	Estimated Retardation (nm)	Extinction	Herzog Test
Agave	+ve	200-800	Incomplete, lumen dark	Orange
Barley Straw	-ve	200	Incomplete	Blue
Coir	+ve	200	Undulose	Orange
Corn	+ve	200	Complete	Orange
Raffia	+ve	200-400	Complete, except at nodes	Orange
Wheat Straw	-ve	200	Incomplete	Blue

The most distinguishing features of the fibers were the colors and the fiber cross-sections. The fibers showed a number of colors: white (agave), tan (barley and wheat straw), light brown (corn and raffia), and dark brown (coir), reflecting the lignin retained in the fibers after processing. The cross-sections ranged from flattened ribbon (barley straw, raffia, and wheat straw) to flattened ovals (agave, coir, and corn).

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Fibers; Polarized Light Microscopy; Trace Analysis

B168   The Application of Particle-Correlated Raman Spectroscopic Analysis of Soils to Mock Casework Scenarios

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Learning Overview: After attending this presentation, attendees will understand the comparative capabilities of Particle-Correlated Raman Spectroscopy (PCRS) for the analysis of soil minerals collected from mock evidence samples, including footwear and shovels.

Impact Statement: This presentation will impact the forensic community by providing attendees with an understanding of how PCRS can be applied to forensic soil analysis, through the lens of simulated casework of soil traces on footwear and shovels. This will draw a connection between instrumentation that is being explored academically and the potential for its integration for use in crime laboratories.

Soil is a continuous but complex mixture, reaching across geological bodies. It is primarily composed of minerals, organic matter, organisms, gases, and water. Although it is continuous, soil is distinctly variable and differentiable, based on aspects such as geographic location, seasonal factors, and human interference. In combination with its highly transferable nature, the complexity of soil composition makes it a valuable material for forensic trace evidence and object-to-scene association. The development of forensic soil analysis is currently focused on mineralogy, with the traditional tool for mineral identification being the Polarized Light Microscopy (PLM). One development in forensic mineralogy focuses on employing elemental analysis, most often using the Scanning Electron Microscopy equipped with Energy Dispersive X-ray spectroscopy (SEM/EDX), to simultaneously visualize and analyze mineral grains. Other methods, including several spectroscopies as well as X-Ray Diffraction (XRD), have also been explored for application to forensic soil analysis. One method with excellent potential for the interrogation of a range of forensically relevant samples is Raman spectroscopy.[1] In particular, Raman spectroscopy has a demonstrated history for use in the field of geology for mineral identification; thus, its application to the forensic analysis of soils is a logical extension.

PCRS is a novel analytical method that combines automated image analysis with Raman spectroscopy, thus providing both microscopic morphological and chemical information about a single sample non-destructively. PCRS provides both qualitative and quantitative information about a sample. When applied to soil minerals, this information includes their identification, microscopic morphological characteristics (e.g., circularity, elongation, brightness), and particle size distributions. This information is valuable for forensic soil comparisons, but more research is needed to understand the significance of an association of these properties given the complexities of transfer and persistence.

In this project, PCRS is used to analyze soil particles collected from simulated evidence samples. Shoes and shovels were used to collect mock evidence from three different geographical locations: an urban park, a suburban residential area, and a rural woodland area. Known soil samples were also collected from these locations to serve as reference samples for comparison under PCRS analysis. The mock-evidence items were prepared in a method detailed by Stoney et al. for the analysis of very small particles.[2] The collected adhering soil was then cleaned to isolate the mineral grains per the method described by Palenk.[3] The particles in the size range of 90nm–180nm in diameter were then dispersed onto a Raman-inactive microscope slide and analyzed using PCRS. The results were compared to the reference samples that were treated and analyzed with the same method. Source consistency could then be determined using a set of match criteria that includes mineral identity, particle morphology, and composition percentage.

Analysis of the soil samples showed that it was possible to determine the source of soil collected from mock evidence by using PCRS, when reference samples from suspected sources are available for appropriate comparison.

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3. Palenik, S. (2007). Heavy minerals in forensic science. *Developments in Sedimentology*, 58, 937-961. https://doi.org/10.1016/S0070-4571(07)58037-4.

Particle-Correlated Raman Spectroscopy; Soil Analysis; Mock Evidence

B169 **The Capabilities and Limitations of Particle-Correlated Raman Spectroscopy (PCRS) for the Analysis of Forensic Soil Minerals**

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Learning Overview: After attending this presentation, attendees will understand the advantages and limitations of the application of PCRS to the forensic analysis of soil minerals.

Impact Statement: This presentation will impact the forensic science community by critically evaluating the capabilities of PCRS for the analysis of soil minerals through a direct comparison of results obtained from traditional methods.

This study evaluated the capabilities and limitations of PCRS for the analysis of soil samples. PCRS is an automated, integrated technique that combines image analysis with Raman spectroscopy to provide morphological properties and chemical information from a mixture of discrete particles, such as the population of mineral grains in a soil sample. Considering how valuable soil traces can be in providing linkages in a case, along with the long-stated criticisms of forensic soil analysis (e.g., subjective, time consuming, and labor intensive), there is a need for an automated system that provides an efficient, objective, and statistically comparable approach to the interrogation of soil samples. PCRS has the ability to provide information regarding the particle size distribution, grain morphology, and mineral identification, which permit samples to be compared. To develop PCRS for inclusion in a forensic soil workflow, the limitations and advantages of the method (e.g., dispersion, sample imaging and analysis, Raman spectroscopic analysis and interpretation) need to be evaluated, which has been the goal of the research that will be presented.

For the evaluation of PCRS as a tool to analyze soil minerals, single-blind PCRS was completed on four unknown, four-component mixtures of comminuted minerals and an additional ten disparate soil samples collected from the northeast United States. The 90–180mm fraction of minerals were obtained from each soil sample after washing and sieving using traditional methods.[1] Next, a computer-controlled vacuum-type powder dispersion system (operating at -20Pa and with a high-sample volume) was used to create a uniform strew distribution of microscopic mineral particles with good spatial separation. Following the dispersion of particles on a glass slide, the sample was imaged, and an image analysis routine was applied to isolate individual particles and obtain a range of morphological properties (e.g., circularity, elongation, area, translucency). The individual particles comprising a sample were each probed by Raman microspectroscopy using two different lasers excitations (532nm and 785nm). The resulting Raman spectra were identified via spectral library searching of the RRUFF[2] mineral database that contains close to 10,000 different Raman mineral spectra.[2] The morphological data associated with the particles in each mineral class was then used to generate size and shape distributions, delivering mineral-specific morphological information. Mineral size and morphological distributions for each soil sample were compared using multivariate statistical methods to evaluate their discriminating potential. The results of the PCRS method were then compared to those obtained using traditional methods for mineral identification, including polarized light microscopy and scanning electron microscopy equipped with energy dispersive X-ray spectroscopy. Similarities and distinctions of the results between these approaches have been evaluated to explore the utility of the present PCRS method for use in forensic soil casework.

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2. Lafuente B, Downs R T, Yang H, Stone N (2015) The power of databases: the RRUFF project. In: *Highlights in Mineralogical Crystallography*, T Armbruster and R M Danisi, eds. Berlin, Germany, W. De Gruyter, pp 1-30.

Forensic Soil Analysis; Raman Spectroscopy; PCRS

B170   The Differentiation of Architectural Paint by Sheen Using Reflectance Spectroscopy

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Learning Overview: After attending this presentation, attendees will better understand a new method of differentiating architectural paint in trace evidence examination based on a determination of paint sheen using primarily reflectance spectroscopy.

Impact Statement: This presentation will impact the forensic science community by helping to increase the evidentiary value of architectural paint in an objective manner.

Architectural paint is a form of transfer evidence that can be found in cases involving contact between a coated surface and another surface. Common cases in which this evidence type is found are breaking and entering, assault, homicides, and vandalism. When paint evidence is found in these situations, physical fit, chemical analysis, and physical observations are often made. Parameters for comparative analysis include color, layer structure, presence of contaminants, surface features, type of pigment, resinous vehicle, and other additives. These observations are performed using light and polarized microscopy as well as other common instrumental techniques such as infrared microspectroscopy, Raman microspectroscopy, and pyrolysis-gas chromatography/mass spectrometry.

Architectural paint is often categorized by a type of sheen such as flat, semi-gloss, gloss, eggshell, and satin. In this study, sheen of architectural paint chips was determined by measuring the reflectance of light from the paint surface using reflectance spectroscopy. This could further help differentiate paint evidence and may be helpful in distinguishing paint samples that are analytically similar using chemical, microscopic, or spectroscopic methods.

Using an Ocean Optics® reflectance probe and spectrophotometer, percent reflectance measurements were recorded from 350–800nm on dried samples of paint obtained commercially at an incident angel of 45-degrees covering an area approximately 36.79mm^2. Principal component to linear discriminant analysis was performed on this spectral data using RStudio.

A total of 50 white paint samples of one product type from a single manufacturer (ten samples for each of the five sheens tested) were examined by this method. Resulting linear discriminant plots showed that the five tested sheens (gloss, semigloss, satin, eggshell, and flat) were clearly separated when using between three to five principal components, indicating that paint within a product type of a manufacturer can be differentiated by sheen in this manner. Another 50 white samples from the same manufacturer, but from different products, were observed in this manner, and the result showed that sheens could still be differentiated from each other. Finally, 40 different pigmented samples from a second manufacturer were tested, and it was determined that color changes the resulting reflectance spectra necessitating that comparisons need to be made with similar colors. In total, 140 samples were analyzed, and from these results it can be concluded that when the manufacturer and the product type are identified through chemical and spectroscopic methods, the sheen can also be accurately determined when samples have similar pigmentation.

As was shown in this study, sheen was often the only distinguishing characteristic between paints within a product line and between manufacturers when Raman and infrared microspectroscopy yielded similar spectra. As a result, architectural and maintenance paints can be further differentiated by sheen, giving more evidentiary power to this type of physical evidence.

Paint; Spectroscopy; Statistics

B171   The NIST Footwear Impression Comparison System

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Learning Overview: After attending this presentation, attendees will better understand how the National Institute of Standards and Technology (NIST) Footwear Impressions Comparison System (FICS) evaluates evidential strength scores for wear patterns and acquired characteristics. Attendees will also learn FICS performed similarly well to human examiners when distinguishing between mated and close-non-match comparisons from the recent black box study for footwear examiners.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the NIST FICS has been developed to provide algorithmic support to examiner assessments of evidential strength related to wear patterns and acquired characteristics (often called RACS). Its performance on comparisons from the recent black box study for footwear examiners demonstrates that FICS achieved a critical milestone in supporting examiner conclusions with quantitative algorithms.

In particular, FICS was built to discriminate between mated comparisons (i.e., two impressions from the same shoe) and close-non-match comparisons (i.e., two impressions from different shoes of the same make, model, size, and side). This presentation will overview the end-to-end FICS comparison process, including manual markup, alignment, and evaluation of similarity metrics for comparing wear characteristics and acquired characteristics (often referred to as RACs). We will summarize results from applying FICs to comparisons in the recent black box study for footwear examiners .[1] Two key aspects of this presentation are that FICS's performance is assessed using comparisons resembling casework, and the results can be compared directly with the performance of (anonymous) examiners who evaluated those same comparisons while participating in the black box study.

To apply FICs, a user annotates which image subregions contain information regarding the outsole pattern of interest (called the region of interest) and indicates the resolution for each impression image. Additionally, RACs are marked in the test impression. Test impressions are initially aligned to questioned impressions using manually selected anchor points, and the alignment is refined using numerical optimization. Following alignment, similarities used for assessing correspondence of wear characteristics are computed across a grid of overlapping tiles.

FICS then uses a blind lineup approach to convert wear similarity metrics into a score reflecting evidential strength. That is, each questioned impression is compared not only to a test impression from the Shoe Of Interest (SOI) but also to test impressions from other shoes of the same make, model, and size as the SOI. For each tile location, the maximum similarity score between the questioned impression and any of the test impressions in the lineup serves as an automated quality measure for the corresponding region of the questioned impression. Each tile also receives a score for evidential strength based on the extent to which the similarity for the SOI stands above the similarities obtained for the other shoes in the lineup (i.e., how strongly pattern information in the questioned impression tile picks the SOI out from a crowd of similar shoes). The evidential strength scores across tiles are combined using a weighted average that considers the tile quality score and how much of the tile falls within the region of interest.

For acquired characteristics, FICS performs local alignment and similarity evaluation for each RAC marked in the test impression. A RAC marked in a given test impression is compared with the corresponding region in the questioned impression as well as the corresponding regions in the other test impressions in the lineup. A score for the RAC's evidential strength is assigned based on the extent to which the RAC's similarity with the questioned impression stands out among similarities obtained when comparing the RAC to test impressions from other shoes in the lineup (i.e., how strongly a RAC pattern in the test impression from the SOI points to the questioned impression among a crowd of shoes similar to the SOI). Evidential strength scores for RACs and wear patterns are combined to produce a final evidential strength score.

Using this approach, the FICs system discriminated between mated and close-non-match comparisons in the Federal Bureau of Investigation (FBI) black box study with similar efficacy as participating footwear examiners. To our knowledge, this represents the first direct comparison between examiner and algorithmic assessments of evidential strength for footwear impression evidence. In total, the FICS results illustrate a promising pathway to supporting examiner conclusions with quantitative algorithms.

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Footwear Evidence; Quantitative Evidence; Statistics

B172   An Investigation of Extraction Protocols for the Analysis and Quantitation of Cannabinoids in Gummy Matrices Using Liquid Chromatography and Photodiode Array/Ultraviolet Detection

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Learning Overview: After attending this presentation, attendees will understand current methodologies that utilize Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEchERS), other enzymatic techniques, and non-enzymatic methods for the extraction of cannabinoids within seized and purchased cannabis edibles. In addition, this presentation will discuss the use of Liquid Chromatography-Photodiode Array/Ultraviolet (LC-PDA/UV) detection for the quantitation of cannabinoids in gummy matrices.

Impact Statement: This presentation will impact the forensic science community by providing cannabinoid extraction method comparisons in addition to quantitative results as they relate to product labeling. This information can be used to improve upon the extraction methods currently used within the field and provide important data as to the true cannabinoid content of edibles.

The passing of the Farm Bill (2018) resulted in a major change to the legal status of cannabis, reclassifying hemp and removing it from the Controlled Substances Act (CSA).[1] This bill differentiates hemp and marijuana from one another based on the amount of Tetrahydrocannabinol (THC) present, stating that to be classified as hemp, there must be no more than 0.3% THC by dry weight present. Because of this new distinction, labs are now faced with the new issue of having to determine whether a seized sample is marijuana or hemp. In this vein, low THC and relatively high Cannabidiol (CBD) are indicative of hemp. This project seeks to compare and contrast different extraction methods that could be used for the quantification of cannabinoids to help overcome this differentiation problem in complex matrices.

In this study, numerous extraction methods were evaluated on various gummy samples to examine cannabinoid recovery. A modified LC-PDA method previously developed was used to screen each sample for 11 cannabinoids.[2] The modification (an extended purge time) was necessary to reduce carryover of other matrix components between samples. Extraction methods were compared to one another across different cannabis-containing gummies and across a suite of non-cannabis gummy samples spiked with known concentrations of cannabinoids to assess recovery, repeatability, and to look for potential interferences.

Sample dissociation methods investigated included enzyme digestion (alpha-amylase), cryo-grinding, and agitation/shaking with and without heating. Dissolution was generally done in an aqueous solution, with the optional addition of base (ammonium hydroxide), followed by the addition of acetonitrile, methanol, or isopropanol to extract cannabinoids. Liquid-Liquid Extraction (LLE) via the QuEChERS method was investigated for acetonitrile extractions. At the time of writing, an amylase digestion followed by addition of acetonitrile and a QuEChERS extraction performed the best across all sample types and studies for cannabinoids analyzed. This extraction method had percent Relative Standard Deviations (RSDs) that typically ranged from 2–10%. Preliminary results showed good recoveries from spiked samples and consistency with label claims on a small number of commercial samples. In comparison, other extraction methods yielded lower recoveries or exhibited issues during the processing of some samples (e.g., problems filtering). Additional studies will be conducted with gummies made from scratch and spiked with known cannabinoid concentrations to quantitatively assess recovery efficiencies in realistic samples. Method comparisons and the results of these studies will be presented.

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Cannabis; Liquid Chromatography; Edibles

B173   The Development of a Δ9-THC Colorimetric Breathalyzer Using 3D Printing Manufacture

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Learning Overview: This presentation will demonstrate the development of a **Δ9-Tetrahydrocannabinol (**Δ9-THC) breathalyzer for the detection of Δ9-THC recent use in site. After attending this presentation, attendees will better understand the mechanism of the colorimetric reaction applied to detect Δ9-THC, the application of this reaction to a support base that will work as a prototype for a colorimetric-based Δ9-THC breathalyzer.

Impact Statement: This presentation will impact the forensic science community by showing the development of the basic chemical foundations needed for the development of a portable colorimetric device for the in site detection of Δ9-THC in air exhaled for the early detection of driving under the influence of marijuana.

Today, the recreational use of marijuana is legal in 31 states and the District of Columbia. Several studies show that after the legalization, a 6.0% increase in injury crash rates and a 4.0% increase in fatal crash rates were observed.[1] Several factors must be considered to reduce driving under marijuana influence, such as preventive, educational, and punitive activities. In site detection of marijuana recent use is one of the possible measures to be adopted. Alcohol breathalyzers are a reality, and they help law enforcement to perform site evaluations quickly and easily. Thus, a Δ9-THC breathalyzer could bring similar advantages. Unfortunately, this is not yet a reality for Δ9-THC. The current devices are collection devices where the exhaled air is stored and must be further analyzed in a laboratory to allow Δ9-THC identification.[2]

In this project, the development of the Δ9-THC breathalyzer is based on the application of an additive manufacturing solid device made by 3D printing.[3] To create this support, a commercial Anycubic® 3D polymerizable resin was mixed with different Fast Blue dyes and were evaluated in the presence of several cannabinoids. The Fast Blue dye family is known to produce a colorimetric response in the presence of THC.[5-7] Several dyes from the Fast Blue family were studied (B, BB, and RR), in concentrations varying from 1.0 to 6%w/v. The results showed that the Fast Blue B in a concentration of 4% w/v in the solid resin is capable of reacting with 0.01mg with a linear response ranging from 0.01–0.50mg of Δ9-THC. Achieving lower concentrations are extremely important as the concentration of Δ9-THC in exhaled air are described to be in the range of 1ng/30 L of exhaled air.[2] In recent years, due to the increasing demand for both product complexity and multi-functionality, many new materials have been tested for 3D printability, including a forensic science application.[4] The results obtained in this work are the initial fundamental chemical foundation needed for the construction of a reliable semi-quantitative breathalyzer device to be applied in Driving Under the Influence of Drugs (DUID) monitoring in the United States.

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THC; Impaired Driving; 3D Technology

B174   Then vs. Now vs. the Future: An Overview of Extraction Protocols for Cannabis-Derived and Cannabinoid-Infused Products and the Development of a Universal Extraction Method

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Learning Overview: After attending this presentation, attendees will better understand how cannabinoids can be extracted from a broad range of cannabis-derived or cannabinoid-infused complex matrix products with a rapid and universal extraction protocol for detection and quantification purposes.

Impact Statement: This presentation will impact practices in the forensic science and criminal justice fields by providing a universal optimized procedure for the extraction of cannabinoids infused within complex edible and non-edible matrices.

In 2019, the National Institute of Justice's report to Congress stated that there is a critical need for the development of standardized protocols for the analysis of cannabis and cannabis-derived products. This is because well-established protocols for the analysis of cannabis plant material are generally suboptimal for the plethora of cannabinoid-containing goods that have become exceptionally popular since the decriminalization and legalization of marijuana at the state level in the United States. These processes, which are highly nuanced and complex-matrix dependent, are generally resource-intensive, time-consuming, and require extensive sample preparation. For example, products with high sugar and carbohydrate content, such as candies and honey, are subjected to dissolution in water, organic solvent extraction, sonication, and filtration, followed by evaporation of the solvent and derivatization (to protect the carboxylic groups if analyzed by Gas Chromatography/Mass Spectrometry [GC/MS]).[1] Lipophilic/oily products are extracted with solvents of varying polarity, washed, dried, and reconstituted or subjected to Solid-Phase Extraction (SPE). Solid foods such as brownies and cookies are often ground/homogenized prior to extraction.[2,3] Aqueous products (if carbonated) are degassed by sonication, extracted with acetic acid, acetonitrile, and "extraction salts" by applying the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique.[4] Usually, the QuEChERS extraction method involves two steps: (1) liquid-liquid extraction; and (2) dispersive solid-phase extraction clean-up. Recently, slight modifications of this technique have been employed for the extraction of cannabinoids from food products.[5,6] Although many of these protocols have been studied for decades and are routine, these approaches to sample analysis require a perpetual need for highly specialized and nuanced method development in order to accommodate the ever-changing complex matrices that are encountered by crime labs, often at great expense in terms of time and material resources.

To address the challenge of the need for nuanced methods development, this study focused on the creation of a more universal extraction protocol that is applicable to multiple matrix types, featuring the QuEChERS DisQue CEN salts.[7] The approach involves suspension of the cannabinoid-containing sample in water, vortexing of the suspension, sonication (only required for chocolates and gelatin candies), addition of acetonitrile followed by DisQue CEN salts (1g trisodium citrate dihydrate, 0.5g disodium hydrogen citrate sesquihydrate, 1g NaCl, and 4gMgSO$_4$) and vortexing again before allowing the layers (top acetonitrile layer, which contains cannabinoids, and the bottom aqueous layer, which contains the salts and other matrix constituents) to separate. Rapid analysis of the layers by Direct Analysis in Real-Time High Resolution Mass Spectrometry (DART®-HRMS) revealed that the cannabinoids were reliably extracted into the acetonitrile layer in a single step with recoveries of up to 99%, with no cannabinoids detected in the water-salts-matrix constituent layer. This protocol was then successfully applied to a wide range of samples prepared or spiked in-house with CBD and THC standards, including: (1) gelatin candies; (2) chocolates; (3) marshmallows; (4) beverages such as coffee, sodas, and liqueurs; (5) butters and oils; and (6) personal-care products such as balms and lotions (commercially available).[8]

Overall, the development of this universal, simple, rapid, robust, and cost-effective analytical method for the extraction of cannabinoids can assist forensic science practitioners by: (1) streamlining sample analysis using a sample preparation protocol that can be applied to a vast range of matrix types; (2) saving time through fast analysis; and (3) contributing to a reduction in sample testing backlogs.

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Extraction; Cannabis; Mass Spectrometry

B175 Trace Metals in Cannabis Seized by Law Enforcement in Ghana and Multivariate Analysis to Distinguish Among Different Cannabis Farms

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Learning Overview: After attending this presentation, attendees will be able to understand how trace metals are used as tools to predict the origin of the cannabis plant from different parts of Ghana.

Impact Statement: Attendees will be made aware of the impact of Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) instrumentation for reducing the trafficking of narcotics across international borders using metal contents.

For hundreds of years, cannabis has been one of the most known cultivated plants due to its variety of uses, including as a psychoactive drug, as well as for medicinal activity. Although prohibiting cannabis products, the countries of the African continent are the largest producers of cannabis in the world; a fact that makes the trafficking of cannabis-based illicit drugs a high priority for local law enforcement authorities. The latter are exceedingly interested in the use of chemical analyses for facilitating quantification, identification, and tracing of the origin of seized cannabis samples.

A number of different analytical techniques, as well as the interpretation of their data such as chemometrics, have been used for probing the geographical sources of plants and soils.[1-3] Among them, ICP/MS has been the method of choice for the determination of toxic metals and metalloids of plants and soils, with low detection limits down to 0.1ppb, simple sample preparation, high throughput, and the ability to measure many elements simultaneously.[2,4,5] Targeting these goals, and focusing on the country of Ghana, the present study used ICP/MS for the determination of 12 elements (Pb, Cu, Ca, Mg, Mn, Zn, Cd, As, Hg, Fe, Na, and K) in cannabis seized by Ghana's law enforcement authorities and soils of cannabis farms. Furthermore, multivariate analysis was applied to distinguish among different cannabis farms and match them with the samples. As a result, 22 seized cannabis samples and 12 other cannabis samples with their respective soils were analyzed to reveal considerable As and Pb concentrations. As and Pb levels in cannabis were found to be up to 242ppb for As and 854ppb for Pb. Multivariate analysis was applied for separating different cannabis farms and seized samples based on elemental analysis, linking the seized samples with two Ghana regions.

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Toxic Metals; Cannabis; ICP/MS

B176   Are Smokable Hemp Products Correctly Labeled?

Walter B. Wilson*, National Institute of Standard and Technology, Gaithersbrug, MD; Aaron Urbas, National Institute of Standards and Technology, Gaithersburg, MD

Learning Overview: After attending this presentation, attendees will be informed about the sample preparation procedures, the Liquid Chromatography-Photodiode Array (LC-PDA) method at the National Institute of Standards and Technology (NIST), and inaccuracies of product labels for commercial smokable hemp plant samples for Δ9-Tetrahydrocannabinol (Δ9-THC), Δ8-Tetrahydrocannabinol (Δ8-THC), Tetrahydrocannabinolic Acid (THCA), and total Δ9-THC.

Impact Statement: This presentation will impact the forensic science community by providing a summary of analytical methods that NIST would like to help transfer to forensic laboratories in distinguishing seized plant samples as either legal hemp or illegal marijuana. Additionally, the forensic science community will obtain an example of how product labels are inaccurate and the typical levels of Δ9-THC, Δ8-THC, THCA, and total Δ9-THC in smokable hemp products.

The passage of the 2018 Farm Bill has led to a large increase in hemp production in the United States in 2021 with approximately 76% of it focused on floral hemp that is used to produce hemp-derived finished products such as smokable hemp (e.g., manicured, roll your own, or cigarettes). As a result, forensic laboratories have seen a significant increase in confiscated cannabis samples, but few reliable analytical methods exist in the literature for differentiation between hemp and marijuana. Prior to the passage of the 2018 Farm Bill, forensic laboratories were required to perform non-quantitative confirmatory tests for the presence of Δ^9-THC, but new legislation defined hemp as cannabis containing mass fractions of decarboxylated-Δ^9-THC less than or equal to 0.3%. In response to the need for reliable quantitative methods, NIST has developed and evaluated analytical methods to provide forensic scientists the tools necessary.

In this presentation, 53 smokable hemp plant products were analyzed for Δ^8-THC, Δ^9-THC, THCA, and total Δ^9-THC by a published LC-PDA method using a methanol extraction procedure.[1] LC is the most widely employed separation technique by the cannabis industry because it permits the determination of total Δ^9-THC, which is calculated as the sum of Δ^9-THC and its acidic precursor, THCA. Over 90% of the samples analyzed by NIST were determined to have a total Δ^9-THC mass fraction above this limit, even though samples were being marketed as hemp with Δ^9-THC mass fractions below 0.3 %. Surprisingly, often the associated online documentation reported total Δ^9-THC mass fractions of ≥ 0.3%. Mass fractions determined by NIST were compared with manufacturer's online documentation for 22 samples. Measurements differed by ≈ 55 % for total Δ^9-THC, ≈ 68 % for THCA, and ≈ 18 % for Δ^9-THC. Poor agreement may result from method difference, sample inhomogeneity, batch-to-batch variability, changes due to storage conditions, and/or product labels or online documentation that are not representative of actual products.

Reference:
[1.] Abdur-Rahman, M., Wilson, W.B. Determination of 11 cannabinoids in hemp plant and oils by liquid chromatography and photodiode array detection. *Chromatographia*, 2022, 85, 115-125.

Cannabis; Delta-9-Tetrahydrocannabinol; Liquid Chromatography

B177   The Application of the Expert Algorithm for Substance Identification (EASI) to Resolve THC and CBD ESI-Mass Spectra

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Learning Overview: After attending this presentation, attendees will have learned how the correlations that exist within the abundances of fragment ions in replicate tandem mass spectra can be used to effectively discriminate between compounds of notoriously high spectral and structural similarity, such as delta-9-Tetrahydrocannabinol (THC) and delta-9-Cannabidiol (CBD).

Impact Statement: This presentation will impact the forensic community by providing a flexible algorithm capable of resolving and extrapolating between compounds collected under different conditions or on different instruments. EASI aims to minimize false identifications and improve forensic analysts' confidence in their identifications and testimonies.

Our central hypothesis is that the relative abundances of mass spectra fragments are not independent but are correlated. This hypothesis is supported by unimolecular fragmentation theories like Rice–Ramsperger–Kassel–Marcus (RRKM) and confirmed by Pearson correlation matrices for each compound.

In most existing library search algorithms, a query spectrum is compared to a library of discrete spectra of standards or known spectra. In our discussions, the average spectrum of many replicate spectra of a substance can be considered a consensus or exemplar spectrum. One problem with library spectra is that inter-laboratory and inter-day measurements can easily introduce abundance variations of up to $\pm20\%$. This variance is even more pronounced when collecting tandem mass spectra at different collision energies on different instruments. Such variance increases the difficulty in differentiating between compounds of similar mass spectra. EASI takes an informed approach that accounts for the different sources of variance by modeling the correlations within replicate spectra.

For this presentation, 60 replicate spectra were collected for THC and CBD at three different collision energies on an ESI-source Agilent® 6538 UHD Q-TOF. The total number of spectra is 360. The replicate spectra were randomly and evenly distributed to create training and test sets for General Linear Modeling (GLM). Since the branching ratios vary at different collision energies, the 29 most abundant mass fragments across all collision energies were selected as variables for the linear models and entered in a stepwise additional method. Additionally, one-way Analysis of Variance (ANOVA) was performed using the collision energy as the fixed factor to determine which fragments significantly differed between THC and CBD to better inform the variable selection. The models from each training set were used to predict each compound's fragment abundance. The measured and EASI-predicted abundances were evaluated using similarity and dissimilarity metrics, such as the dot-product and mean absolute residual. Each metric was then used as a binary classifier to generate a Receiver Operating Characteristic (ROC) curve to determine the true positives and false positive rates over a range of threshold values. The Area Under the Curve (AUC) was then calculated to assess the performance of each metric and its supporting model.

The residuals from the EASI models were generally smaller over the consensus-based approach at each collision energy.

Using all the modeled fragment abundances as input variables for classification, the consensus-based approach discriminated between THC and CBD with an average of 80% accuracy, but EASI distinguished between the conformers with an average of 91% accuracy. The residuals from the EASI and consensus-based models were used to build a binary logistic regression model at each collision energy using half the spectra (n=30 known positives and n=30 known negatives) as a training set and the other half as a test set. Using as few as two variables for discrimination (e.g., residuals for GLM models for *m/z* 91 and 165), EASI was typically able to resolve THC and CBD with greater than 99% accuracy, which exceeds the accuracy of conventional algorithms.

Algorithms; Correlations; Tandem Mass Spectrometry

C1   A Forensic Analysis of Veo Micromobility Mobile Application on iOS® Devices

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Learning Overview: Attendees of this presentation will learn about the forensic analysis of electric scooter and electric bike applications, specifically focusing on the Veo micromobility app on iOS®. This study aims to determine what forensically relevant data can be recovered from these apps to aid in investigations related to e-scooter and e-bike usage.

This presentation will cover the reasons behind the increasing popularity of micromobility vehicles such as e-scooters and e-bikes, including their ease of use, cost effectiveness, and environmentally friendly nature. The app-based nature of these vehicles, which allows users to start their ride by scanning the vehicle's identification and making payment, will also be discussed. Additionally, this presentation will provide a methodology for acquiring evidence from the Veo app and explore the artifacts that can be recovered from two types of forensic acquisitions. The focus will be on the specific data that can be obtained from the Veo app and other micromobility apps, considering their growing usage. Overall, this presentation will highlight the importance of forensic analysis in investigating e-scooter- and e-bike-related incidents and demonstrate how the data recovered from micromobility apps can be utilized in forensic investigations.

Impact Statement: This presentation will have a significant impact on the forensic science community by addressing the emerging field of forensic analysis related to micromobility vehicles and their associated applications in the following ways:

Knowledge Expansion: This presentation will provide valuable insights into the forensically relevant data that can be extracted from e-scooter and e-bike applications, focusing specifically on the Veo micromobility app on iOS®. This will expand the knowledge base of forensic scientists and investigators, enabling them to understand the potential digital evidence available in these types of cases.

Methodology Development: This presentation offers a methodology for acquiring evidence from the Veo app, which can serve as a foundation for further research and development in the field. By establishing a systematic approach, it helps standardize the process of extracting and analyzing data from micromobility applications, ensuring consistency and reliability in future investigations.

Enhanced Investigation Techniques: With the increasing prevalence of micromobility vehicles and their associated apps, forensic professionals need to stay updated on the latest investigation techniques. This presentation will equip attendees with knowledge on the artifacts that can be recovered from two types of forensic acquisitions, enabling them to leverage this information in their investigations. This knowledge can potentially assist in linking individuals to specific e-scooter or e-bike usage, identifying patterns of behavior, and reconstructing events relevant to a case.

Adaptation to Technological Advances: By focusing on the forensic analysis of micromobility apps, this presentation acknowledges the evolving landscape of digital technology and its impact on forensic science. It highlights the need for forensic professionals to keep pace with emerging trends and technologies, ensuring they can effectively analyze and interpret evidence from novel sources.

Collaboration and Networking: This presentation serves as a platform for forensic scientists, investigators, and researchers to connect and exchange ideas. It fosters collaboration within the forensic science community, promoting the sharing of knowledge, methodologies, and best practices. This collaboration can lead to further advancements in the field and facilitate interdisciplinary approaches to forensic investigations.

Overall, this presentation will contribute to the growth and development of the forensic science community, empowering professionals with the knowledge and tools necessary to navigate the challenges presented by the increasing use of micromobility vehicles and their associated applications.

The escalating use of electric scooters (e-scooters) and electric bikes (e-bikes) necessitates the identification of forensically relevant data recoverable from associated applications to support investigations. These micromobility vehicles have gained widespread popularity due to their user-friendly features, cost-effective and efficient transportation capabilities, as well as their environmentally friendly nature. Leveraging app-based functionality, users can seamlessly initiate their rides by scanning vehicle IDs and making payments. This study focuses on the forensic analysis of such applications, with a specific emphasis on the Veo micromobility app on iOS®. We present a systematic methodology for evidence acquisition from the Veo app, enabling forensic professionals to standardize the acquisition of evidence from the Veo app and similar micromobility applications. Additionally, the recoverable artifacts from two types of forensic acquisitions are discussed. The recovered artifacts bear significant practical implications for forensic investigations related to e-scooter and e-bike incidents, including accidents, thefts, or other criminal activities involving these vehicles. The extracted data can provide invaluable information about rider identities, travel patterns, usage frequency, geolocation data, payment history, and potential communication records. Such evidence assists investigators in establishing timelines, reconstructing events, identifying potential witnesses, linking individuals to specific vehicle usage, and ultimately facilitating case resolution.

Additionally, this study encourages collaboration and knowledge sharing within the forensic science community. Educators and law enforcement officials will have the opportunity to engage in discussions, exchange ideas, and explore interdisciplinary approaches to enhance their investigative capabilities in the growing domain of micromobility app forensics. Adapting forensic expertise to encompass the evolving digital landscape, including the analysis of emerging technologies like micromobility apps, is essential for professionals to stay abreast of technological advancements and effectively serve justice in the digital age. With Veo and other micromobility app usage on the rise, this study emphasizes the acquisition of forensically relevant data and its application in forensic investigations.

Cyber Forensics; Digital Evidence; Apple® iOS®

C2 A Forensic Analysis on Major Android™ Cloud SDKs

Chen Shi*, Iowa State University, Ames, IA; Yong Guan, Iowa State University, Ames, IA

Learning Overview: After attending this presentation, attendees will understand how to use the comprehensive forensic analysis results of Android™ Cloud Software Development Kits (SDKs) in their casework investigation. This presentation will introduce the basics, challenges and limitations of the current mobile device forensics and demonstrate how to take advantage of the Android™ Cloud SDKs database and search/recover the possible evidence from the corresponding locations outside the mobile devices being investigated.

Impact Statement: This presentation will impact the forensic science community by demonstrating how this new forensic analysis results on the Android™ Cloud SDKs can simplify and speed up investigation procedures.

Forensic analysis on Android™ Cloud SDKs faces several challenges. Android Cloud SDKs often employ strong encryption techniques to protect user data. Forensic analysts may face difficulties in accessing and decrypting this data, especially without proper authentication credentials or encryption keys. In traditional digital forensics, physical access to a device is essential. However, with cloud-based data storage, the data is hosted remotely and obtaining physical access to the device may not be possible. This makes it challenging to conduct a standard forensic examination. On the other hand, cloud-based data can be distributed across various servers and data centers, leading to data fragmentation. Analyzing fragmented data and ensuring data integrity can be challenging, particularly when investigating time-sensitive cases.

Cloud data is dynamic, and real-time updates occur frequently. This makes it difficult for forensic analysts to capture a specific moment in time accurately, as the data may have changed by the time it is accessed. Cloud services can store vast amounts of data from numerous users. Analyzing such large volumes of data requires powerful and scalable forensic tools and techniques. Cloud service providers frequently update their Application Programming Interfaces (APIs) and SDKs to improve functionality and security. These changes can impact forensic tools that rely on specific APIs or access methods, requiring constant updates and adjustments to stay effective. Data transmitted between the mobile device and the Cloud is vulnerable to interception. Ensuring the security and integrity of network traffic during data collection is essential to maintain the integrity of the evidence. Many cloud services offer multi-device synchronization, where data is automatically shared and updated across different devices. This can complicate the forensic process, as the data may exist on multiple devices, each with its own unique challenges. Cloud service providers may implement anti-forensic measures to protect user data. These measures can include file obfuscation, data compression, or other techniques designed to thwart forensic analysis.

To address these challenges, we conducted a large-scale forensic analysis toward major Android™ Cloud SDKs where we manually analyzed the framework and APIs to determine how user data is processed by different Android™ Cloud SDKs. A comprehensive approach was proposed, and it's evaluated in terms of precision and time efficiency. Both static and dynamic program analysis approaches were applied on analyzing/resolving evidentiary data, such as the URL path and its corresponding evidence types. To preserve the advantages (e.g., better time efficiency) provided by our tools, a set of refined/improved automatic program analysis algorithms to analyze Android™ apps were implemented to create the forensic analysis results. The advantages of leveraging the results are: (1) mitigating errors caused by manual investigation; (2) improving the likelihood of discovering evidentiary data stored in specific format on the Cloud; and (3) fast-tracking the evidentiary date of real-world apps.

Forensic Investigation; Data; Software

C3 **Geodata Sleuthing: Geo-Contextualized Digital Footprints Analysis to Empower Cyber Forensic Investigations and Life Pattern Analysis**

Mohammad Meraj Mirza*, Taif University, Taif, Makkah, Saudi Arabia; Umit Karabiyik, Purdue University, West Lafayette, IN

Learning Overview: After attending this presentation, attendees will leave with the knowledge and understanding on unlocking valuable insights from geodata found in cyber investigations and the tools necessary for such a process. Moreover, this presentation will focus on geospatial techniques that can be harnessed to enrich various aspects of cyber forensics investigations by applying it to an iOS® case study. This empowerment will enable forensic experts to handle complex mobile forensics and incident response investigations confidently. This will help enhance their effectiveness and efficiency in solving cases. Finally, attendees will leave equipped with the knowledge, techniques, and tools necessary such as Geographic Information Systems (GIS) to embrace the power of geospatial analysis in their efforts to combat crimes and contribute to advancing forensic sciences.

Impact Statement: The presentation will have a significant impact on the forensic science community by demonstrating strategic integration of geodata and spatial analysis, showcasing its potential impact for mobile forensics investigations. Moreover, this presentation will provide cyber forensic professionals with techniques to reconstruct digital events, identify life patterns, and map locations crucial to investigations. By showcasing the potential of geospatial analysis in cyber forensics, this presentation will encourage researchers to explore further applications and advancements in the field. This newfound interest in research and development can lead to novel solutions and technologies that further strengthen the capabilities of the digital forensic science community.

As the digital landscape continues to evolve, cyber forensic professionals face an increasing need for up-to-date methodologies and tools to navigate the complexities of smart digital devices (e.g., mobile devices, the Internet of Things [IoT], and drones) investigations and deal with unprecedented challenges that are the results of complex digital incidents.[1] A National Institute of Justice (NIJ) -sponsored report emphasizes the growing significance of digital evidence in the investigation and prosecution of various crimes, as these devices often contain critical trails and information related to criminal activities, as well as actions and movements of the individuals involved (e.g., suspects, victims, and related persons).[2]

One of many critical personal data that can be found in these digital devices is geodata. Explicit geodata (e.g., Global Positioning System) and implicit (e.g., Wi-Fi Mac Address) evidence can play a significant role in criminal investigations where they can provide critical information about a user's whereabouts, their association with specific locations, and even help establish geo-timelines of events related to a crime. Therefore, the research provided a thorough process of leveraging geodata and spatial analysis to construct a representational geo-map of the digital trails involved in the case. By connecting what is known with potential insights from other intelligence domains, the research demonstrated how to enhance the digital forensic practitioner's ability to draw meaningful conclusions and make informed decisions by using an interdisciplinary approach. Throughout a case study, we highlighted the advantages of Pattern of Life (PoL) analysis, which unveils behavioral patterns and routine activities, shedding light on the user's actions and movements. Furthermore, the integration of activity-based intelligence, open-source intelligence, and location intelligence provides a geospatial context to the investigation, enabling cyber forensic analysts to visualize the subject's movements and interactions.

In addition, the research explored the confluence of cyber forensics, GIS, and intelligence domains to improve cyber forensic investigations in evidence collection, examination, analysis, coloration, and visualization. Moreover, we discussed interdisciplinary analytical techniques, including geospatial analysis to build knowledge with the help of geospatial intelligence, open-source intelligence, and activity-based intelligence. These geospatial investigation methods generate an exceptional understanding of the users' spatial-temporal behavioral patterns that can be mapped to identify spatiotemporal patterns to provide more accurate reasoning and explanation. Furthermore, the research highlighted the challenges that may arise in employing this interdisciplinary approach, such as data integration complexities. Finally, it emphasized the need for collaborative efforts between law enforcement agencies, legal professionals, and industry stakeholders to address the challenges and improve the use of geodata evidence in legal proceedings.

By the end of this presentation, attendees will gain valuable insights into overcoming challenges related to geodata and how to optimize the investigative process effectively. Moreover, they will have a comprehensive understanding of how geodata and spatial analysis, coupled with intelligence-related knowledge, can augment digital forensic practitioners' decision-making processes. Finally, the case study demonstrated serves as a tangible example of how to accomplish creating a transdisciplinary approach that can revolutionize digital investigations, providing a more comprehensive and geo-contextually meaningful analysis of incidents using GIS software.

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Cyber Forensics; GIS; Interdisciplinary

C4   The Development, Testing, and Comparison of Decontamination Methods for Drugs and Ignitable Liquids From Mobile Devices

Christopher Michael Haser*, Marshall University, Huntington, WV; Kimberly S. Kunkler, Marshall University, Huntington, WV; Lauren L. Richards-Waugh, Marshall University, Huntington, WV

Learning Overview: This presentation will discuss potential methods of decontamination for mobile devices to protect the health and safety of the analyst while preserving forensic evidence. After attending this presentation, attendees will understand previous methods of decontamination and how the process can be improved.[1]

Impact Statement: This presentation will impact the forensic community by demonstrating how the safety of Crime Scene Investigators (CSIs) and analysts can be maintained with an effective decontamination protocol. The quality of analysis may also be improved through safety and timeliness in the field and laboratory, promoting decreased backlogs and improved safety measures within the forensic community.

Mobile devices collected from clandestine labs can be contaminated with hazardous materials, including illicit drugs, drug precursors, and ignitable liquids used during the synthesis process. If seized mobile devices are not adequately decontaminated, laboratory analysts can be exposed to health hazards while performing their examinations. VTO Labs in Broomfield, CO, has developed a decontamination method comprised of three solutions—Easy DECON® Parts 1–3—that they claim cleans devices of fentanyl, carfentanil, and methamphetamine residue, thereby protecting CSIs and analysts from these potential hazards.

However, there are many other dangerous substances in clandestine labs that may also contaminate these devices. Previous research demonstrated the VTO Labs decontamination kit was effective at removing heroin and cocaine. Although the liquid decontamination kit from VTO Labs has been shown to work with drugs, it is also important to consider that mobile devices from clandestine labs could also be contaminated with ignitable liquids. Having a vessel that contains a decontamination solution able to remove both drugs and ignitable liquids would be beneficial. Activated carbon has been used for ignitable liquid extraction, most commonly in the form of activated Carbon strips (C-strips) or loose activated Carbon powder made into an adsorption package (C-bag).

This project aimed to use loose activated carbon (FLUEPAC® MC) as a method for decontamination of mobile devices. This activated carbon powder could be added to any signal-blocking transportation container to serve the purpose of potentially decontaminating the device from drugs and ignitable liquids. During the study, devices were contaminated separately; one set of devices was contaminated with a street sample of heroin (obtained from the Huntington Police Department and previously analyzed by the West Virginia State Police Forensic Laboratory), and the other set of devices was contaminated with a 1:1 mixture of gasoline and diesel fuel (IL mix).

The devices contaminated with drugs were swabbed pre- and post-decontamination with a methanol-soaked Kimwipe®, extracted with methanol, vortexed to separate any drug from the Kimwipe®, and analyzed using a Liquid Chromatograph coupled to a Tandem Mass Spectrometer (LC/MS/MS). The devices contaminated with the IL mix were wiped with a Kimwipe® spotted with approximately 1 milliliter of the mix, rinsed with distilled water, decontaminated using three different methods, (Dawn® dish soap and water, Elma Ultrasonics® Elma Tec Clean A1 and water, and activated carbon powder and water), then processed in accordance with ASTM International standard E1412-19. The samples were analyzed using Gas Chromatography coupled with Mass Spectrometry (GC/MS) and compared to determine the effectiveness of each method. Finally, a preliminary test was performed on a decontamination method developed during this project. A final cleaning step was added to the activated carbon decontamination method. Clean glassware was contaminated with the IL mix, decontaminated using activated carbon, then placed in either the Dawn® dish soap and water mix or Clean A1 and water mix to remove any activated carbon and IL mix that may still be present on the surface of the glassware. These samples were also tested using GC/MS.

Reference:
1. ASTM International. ASTM E1412-19: Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Activated Charcoal. ASTM International, West Conshohocken, PA, 2019. DOI: 10.1520/E1412-19, www.astm.org.

Drug Analysis; Fire Debris Analysis; Method Development

C5   Using File Structure to Authenticate Video Files Uploaded to Cloud Storage Via iOS® Mobile Web Browser

Brandon Epstein*, Medex Forensics, Freehold, NJ; Bertram Lyons*, Medex Forensics, Madison, WI

Learning Overview: After attending this presentation, attendees will understand how video file structure can be used to authenticate video files that have passed through an iOS® mobile web browser despite changes to the file's hash and/or semantic metadata.

Impact Statement: This presentation will impact the forensic science community by informing attendees that cloud platforms provide a reliable and efficient way to share and store digital video files. In addition to commercial storage and sharing platforms, law enforcement agencies are deploying purpose-built public evidence submission portals that allow citizens to submit video files as evidence directly to the Cloud, often through a cell phone's mobile web browser. While a reliable and efficient way to share and store digital video files, this submission process often changes a file's hash and metadata values, particularly in iOS® devices, making it difficult to authenticate a submission or determine if a complete, unedited file was received. Additionally, open video file submission portals may be prone to a deluge of non-original, non-pertinent video files from multiple sources, challenging law enforcement agencies to identify pertinent evidentiary video when investigating large-scale incidents.

In this project, camera-original exemplar files were created on an Apple® iPhone® and the file hashes, stream hashes, metadata attributes and file structure for each file was recorded. The camera's original files were then edited on the iPhone® and uploaded from the iOS® photos app to commercial cloud storage as well as a purpose-built public evidence submission platform. Those files were then downloaded from the Cloud and the same data evaluated. A second set of videos were edited within the iOS® photos app on the iPhone® and then evaluated. The upload and download were repeated, and the resultant files evaluated as well.

The generated file hashes, stream hashes, metadata attributes, and file structure for each file were then examined to determine what, if any, data has changed, and the ability to use that data to determine authenticity or provenance of a video file.[1] It was found that file hashes of video files on iPhone® did not match hashes of resultant uploaded/downloaded video from cloud storage, regardless of editing or submission of the camera original video.[2] Although file hashes did not match, stream hashes for all uploaded and corresponding downloaded videos were consistent. Despite the matching stream hashes, certain metadata attributes did change or were removed from all files when uploaded/downloaded from cloud storage. The consistent visual display of the videos as demonstrated through stream hashing, coupled with "normalized" metadata values serve to complicate authentication examinations.

When comparing file structure of camera original vs. edited files on Apple® devices, structural changes were noted between original and edited files. Additional structural changes were found after they were uploaded/downloaded from cloud storage. Even though the file structures were different, they changed consistently for the camera original and edited file, such that a specific structure can be linked to the provenance of the file.

This research found that file structure is a valid method to evaluate provenance and authenticity of video files uploaded to cloud storage via iOS® mobile web browser, even though other file attributes have changed. Using file structure can allow an examiner to authenticate citizen-submitted evidence video or triage through multiple submissions of potential video evidence to identify camera-original video at scale.

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1.   Wales, G., Smith, J, Lacey, D and Grigoras, C. Multimedia stream hashing: A forensic method for content verification. *Journal of Forensic Sciences*, vol. 68, pp. 289-300, 2023.
2.   Valenzuela, J. *Validation Testing of the Multimedia Created and Transmitted by the Axon Capture Mobile Application for Apple iOS®*. University of Colorado, Denver, 2022.

Video Analysis; Digital Evidence; Admissibility

C6   An Overview of Deepfake Technologies: From Creation to Detection in Forensics

Luca Guarnera*, University of Catania, Catania, Sicilia, Italy; Sebastiano Battiato, University of Catania, Catania, Sicilia, Italy

Learning Overview: After attending this presentation, attendees will be familiar with various deepfake creation and detection techniques. This presentation will attempt to present all existing solutions to date with a simple but schematic view. Attendees will gain that useful information in order to avoid being a victim of cyber attacks created through this powerful technology

Impact Statement: This presentation will have a major impact on the forensic science community by demonstrating the potential of deepfake technology and how it can pose a danger to every individual, industry, nation, and the entire world. An overview of the main methods of deepfake creation and detection will be given with the goal of giving users those tools and information useful to perform a critical analysis on any multimedia content in order to prevent cyber attacks carried out through this powerful technology.

Advancements in Artificial Intelligence (AI) techniques have given rise to significant challenges in the field of Multimedia Forensics, particularly with the emergence of the deepfake phenomenon. Deepfakes are images, video and audio generated or altered by powerful generative models such as Generative Adversarial Networks (GANs) and Diffusion Models (DMs).[1,2] While GANs have long been recognized for their ability to generate high-quality images, DMs offer distinct advantages, providing better control over the generative process and the ability to create images with a wide range of styles and content.[3] In fact, DMs have shown the potential to produce even more realistic images than GANs.

The AI-generated contents span diverse domains, including films, photography, video games, and virtual reality productions. A major concern of the deepfake phenomenon is the application on important people such as politicians and celebrities to spread misinformation. However, the most alarming aspect is the misuse of GANs and DMs to create pornographic deepfakes, posing a serious security threat. Notably, a staggering 96% of deepfakes available on the internet fall into this pornographic category. The malicious use of deepfakes extends to issues such as misinformation, cyberbullying, and privacy violation. In addition, deepfakes have been applied in the fields of art and entertainment, sparking ethical discussions about the limits of creativity and authenticity.

To counteract the illicit use of this powerful technology, novel forensic detection techniques are required to identify whether multimedia data has been manipulated or altered using GANs and DMs.

Regarding image deepfake detection methods in the state of the art, the primary focus lies in binary detection, distinguishing between real and AI-generated images.[4,5] Notably, some methods in the state-of-the-art have already demonstrated the ability to effectively differentiate between various GAN architectures and several DM engines.[6-12] Research showed that generative models leave unique fingerprints in the generated multimedia data, which can be used not only to identify deepfakes but also to recognize the specific architecture used during the creation process.[13] This can be extremely important in forensics in order to reconstruct the history of the multimedia data under analysis (forensic ballistics).[14]

In order to create increasingly sophisticated deepfake detection solutions, several challenges have been proposed by the scientific community such as the Deepfake Detection Challenge (DFDC) and the Face Deepfake Detection Challenge.[15,16] The latter has also launched a new challenge among researchers in the field: reconstructing the original image from deepfakes, a task that can be extremely important in forensics.

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Artificial Intelligence; Deepfake; Multimedia

C7 **The Application of Artificial Intelligence in Forensic Science AI4Forensics From Deepfakes to Risks and Challenges**

Zeno J. Geradts*, Netherlands Forensic Institute, Den Haag, Zuid-Holland, Netherlands

Learning Overview: After attending this presentation, attendees will have learned what the risks of Artificial Intelligence (AI) in forensic science are, how to cope with these, and how to work with AI in a proper way.

Impact Statement: This presentation will impact the forensic science community by showing examples of how AI is used in forensic science, what the challenges are, and why validation is important.

AI for forensics is a rapidly growing field, and the AI4Forensics Lab at the University of Amsterdam and the Netherlands Forensic Institute is leading the way. The lab is home to four PhD students and a postdoc who are working on a variety of projects, including speaker recognition, facial comparisons, chemical analysis of illicit drugs, and tracing criminal money flows.

One of the most exciting projects at the lab is the development of computer models for recognizing hidden messages in photos or videos. These models could be used to identify steganographic images, which are images that have been altered to contain hidden data. Steganographic images are often used by criminals to communicate with each other or to store sensitive information.

Another project at the lab is the development of methods for recognizing deepfakes. Deepfakes are videos or audio recordings that have been manipulated to make it appear as if someone is saying or doing something they never said or did. Deepfakes can be used to spread misinformation or to damage someone's reputation.

The AI4Forensics Lab is also working on projects that use data from mobile phones and smart watches to track people's movements. This data could be used to identify crime scenes or to track the movements of suspects. The lab is still in its early stages, but it has the potential to revolutionize the field of forensics. By applying AI to forensic evidence, the lab can help law enforcement solve crimes more quickly and efficiently.

Here are some of the key benefits of the AI4Forensics Lab:

- The lab brings together experts from the University of Amsterdam and the Netherlands Forensic Institute who have a wealth of experience in AI and forensics.

- The lab has a strong focus on research, which means that it is constantly developing new and innovative methods for applying AI to forensic evidence.

- The lab is open to collaboration with other organizations, which means that it can share its findings with a wider audience.

The AI4Forensics Lab is a significant step forward in the field of forensics. The lab has the potential to revolutionize the way that forensic science and AI are used. Validation and also the proper use of artificial intelligence in relation to the AI Act from the European Union will be implemented.

Artificial Intelligence; Forensic Analysis; Validation

C8   Searching for Hidden Data in Multimedia Files With UNCOVER

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Learning Overview: After attending this presentation, attendees will be aware of which methods exist for hiding data in many types of files. Attention will also be given regarding making new stego applications with language models such as ChatGPT. Detection of the hidden data as well as recovering the data is one of the aspects.

Impact Statement: This presentation will impact the forensic science community by presenting new methods for stego analysis and a real-world database.

Criminals and terrorists are increasingly using steganography to conceal incriminating information in digital files. Law Enforcement Agencies (LEAs) currently use commercial tools to detect hidden information in these files, but these tools are limited in their capabilities.

The UNCOVER project aims to fill the gaps in LEAs' ability to detect steganography.[1] With a consortium of 22 partners, including LEAs, forensic institutes, researchers, and industrial companies, UNCOVER will develop new steganalysis tools that are more powerful, user-friendly, and adaptable to the needs of LEAs.

The developed tools will be integrated into a flexible and user-friendly platform. End-users will play a key role in the project from proposal writing to final evaluation. Regular feedback loops with LEAs, forensic institutes, and external stakeholders will ensure that the developed solutions can be integrated into law enforcement's daily criminal investigation process. A set of clearly defined key performance indicators will allow for an objective assessment of progress and final results against defined objectives.

Below are some of the key benefits of the UNCOVER project:
- More powerful steganalysis tools that can detect a wider range of concealment methods.
- More user-friendly tools that are easier to use and interpret.
- Tools that are more adaptable to the needs of LEAs.
- A flexible and user-friendly platform for integrating the developed tools.
- Regular feedback loops with end-users to ensure that the developed solutions are fit for purpose.

The UNCOVER project is a significant step forward in the fight against steganography. The developed tools will allow LEAs to detect hidden information more effectively, which will help prevent and investigate criminal activity. A large database of 2 terabytes of images, videos, audio files, and text is developed to test the different tools. In this presentation, new challenges with language models such as ChatGPT are discussed on making new forms of data hiding based on python scripts, which may be harder to detect.

Reference:
[1.] https://cordis.europa.eu/project/id/101021687.

Artificial Intelligence; Steganography; Cyber Forensics

C9   The Accuracy of Time Stamps on Surveillance Recording Devices

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Learning Overview: After attending this presentation, attendees will understand how to assess the timing between video surveillance frames based on the time stamp digitally inserted into the pixel information of the video. This research explores the accuracy of those time stamps and methods for establishing timing in cases where there is incomplete metadata, held frames, or a video recording of the surveillance playback monitor.

Impact Statement: This presentation will impact the forensic science community by informing attendees that in today's world, video is becoming more common in forensic analyses. Unfortunately, video evidence can be inconsistent in the way it is recorded. In many cases, the forensic analyst is given a non-native video file or even a video recording of the playback monitor. In these cases, there is very little or no information relating to the timing between recorded frames. This can affect our ability to properly analyze the video. However, many times these video files contain digital timestamps inserted into the pixel information of each frame. Based upon my research, these timestamps can be used to establish frame-to-frame timing, if done carefully and correctly. We will also discuss the limitations found in the research by employing the timestamp.

The presence of video surveillance data has been increasing in recent years. With this increase, video analysis plays a major role in many forensic fields. Many articles, publications, and presentations have covered the importance and difficulty in properly establishing timing between frames captured by any given recording device. This timing is crucial to the forensic analysis to ensure accurate results. In many cases, the analyst is given video files that either do not contain all native metadata or are missing important data required to establish the timing accurately. In some cases, the files contain video recordings of the surveillance playback monitor, which eliminates all original metadata from the video recording. However, within many of these video files, timestamp information is imprinted into the pixel information of each frame. This study examines the accuracy of these timestamps to establish if they can be reliably used to determine the timing between frames.

To accomplish this research, four common recording devices and six different camera devices were utilized. Of these four recording devices, three were digital video recorders and one was a network video recorder. For the three digital video recorders, tests were run with the same four cameras all plugged in, then with only one camera. For the one network video recorder, tests were run with two cameras plugged in, then with only one camera. All videos recorded the Axon® (formerly Input-Ace) Lightboard as the control measurement for timing between each captured frame. The Lightboard was set to 1, 2, 3, and 4 millisecond settings, and the requirements for the number of frames captured by Axon® were met. Videos were recorded with each device and each camera configuration at 30, 15, and 4 frames per second to determine if the capture rate affected the accuracy of the embedded time stamp data. Once recorded, each video was processed using Axon Investigate's™ native "VFR Lightboard Calculator" to establish the actual timing between each frame of test video.

Subsequently, ten timestamp seconds within each video were selected and compared to the actual elapsed time reported by the Lightboard. In total, 54 videos were recorded across all configurations. This study demonstrates the timestamp is not always accurate from second to second. However, a pattern was observed, and a methodology was developed to accurately establish approximate timing between timestamp seconds. The average error between the theoretical time (based on the timestamp) and the actual time reported by the Lightboard across all analyzed timestamp seconds was 2.86 milliseconds (approximately 0.29%). The maximum error between theoretical time (based on the timestamp) and actual time for any timestamp second across all tests was 22 milliseconds (approximately 2.2%). These errors can be considered reasonable for the purposes of analyzing videos in forensic analyses. Inherent in this methodology is the limitation of using the average frame rate of the video, which does not account for timing variability between any two consecutive frames or dropped frames of video.

Reconstruction; Surveillance; Video Analysis

C10 Forensic Video Analysis of an Underwater Volcanic Explosion That Injured Multiple Tour Boat Passengers

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Learning Overview: After attending this presentation, attendees will be aware of how forensic analysis of video recordings taken by passengers aboard a tour boat were used to determine the vessel's location and proximity to a volcanic underwater explosion.

Impact Statement: This presentation will impact the forensic science community by showing how important data about an incident can be derived from video evidence, beyond just watching it on the screen, if analyzed fully.

The prolonged 2018 volcanic eruption of the Kilauea volcano in Hawaii provided once-in-a-lifetime opportunities for visitors to the island to witness spectacular lava flows in person. Tour boats ferried tourists to where the flows entered the ocean along the East Rift Zone. On an early morning in July, a major underwater explosion, called a lava bomb, exploded near one tour boat, injuring 23 people. Most passengers sustained minor burns and soft tissue injuries; however, one woman's femur was shattered, and she was airlifted to a hospital. Questions arose as to whether the vessel had entered the Coast Guard Safety Zone perimeter and how far the explosion was from the boat.

Five passengers were recording video at the time of the explosion; however, the images were very dark, and no landmarks were visible due to smoke and poor lighting conditions. The videos were brightened and synchronized using forensic software. One recording made on a cell phone contained Global Positioning System (GPS) information. An identical model cell phone was purchased and tested to determine the accuracy of its GPS readings. During weeks of continuous eruption, the coastline of Hawaii was constantly changing. Determining whether the vessel was too close to shore required comparing the GPS location data to United States Geological Survey mapping performed at the time of the incident.

Determining the explosion's proximity to the boat was problematic as there was no scale to the video images. The only object of known scale was the boat itself. The tour boat was scanned using a laser scanner. The position and orientation of one camera near the bow of the ship and one further aft were determined using photogrammetry software. Calibrations for optical distortion were performed using exemplar cameras. The laser scan point cloud data, calculated camera locations, and image planes were imported into a 3D modeling program. The origin of the explosion was identified in synchronized images from both cameras. Vectors were plotted from each camera location through their associated image planes to that point. The vectors from both cameras converged at a distance from the boat model. This method of triangulation provided an approximate distance to the explosion. To verify the accuracy of this technique, measurements were made to objects known distance while the vessel was laser scanned in port. Details on the techniques and results will be presented.

Forensic Imaging; 3D Technology; Explosion

C11   An MDCT Analysis of Copy-Paste Fragments Without Query in Digital Forensic Audio

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Learning Overview: The goal of this presentation is to disseminate the application of the Modified Discrete Cosine Transform (MDCT) coefficients to determine the presence of copy-paste fragments in digital audio recordings. Attendees will be presented with the proposed methodology, collected materials, analysis results and interpretation, limitations, and future research as well.

Impact Statement: This presentation will impact the forensic science community by introducing the application of MDCT analysis for copy-paste detection and digital audio authentication of evidence recordings.

The Scientific Working Group on Digital Evidence (SWGDE) best practice guidelines for forensic authentication of digital audio recordings and the European Network of Forensic Science Institutes (ENFSI) best practice manual for digital audio authenticity analysis present frameworks to analyze and authenticate digital audio recordings.[1,2] Audio samples copy-paste, insert, or clone are editing techniques that can chance the meaning of a recorded conversation and alter the authenticity of the initial recording. Previous work reports on applications of Mel-Frequency Cepstral Coefficients (MFCC), Local Binary Pattern (PBP), Discrete Fourier Transform (DFT), Short Time Fourier Transform (STFT), Content-Based Copy Detection (CBCD), and partial matching without query.[3-5]

This study is part of a larger research project regarding the MDCT coefficients and Map analyses for forensic purposes.[6] This presentation will provide the results of a preliminary study on the discrimination between original audio recordings and different other copy-paste tampered signals.

This study is CBCD independent, partial matching without query samples based, and reports on: (1) applications of MDCT to analyze the artifacts introduced by copy-pasting audio fragments; (2) the robustness of the proposed method against editing techniques like inverting, amplifying, and attenuating the paste fragments; (3) the robustness of the proposed method against added noise; (4) the robustness of the proposed method against lossy audio re-compression including social media; (5) an unbiased method to report the comparison results; and (6) the actual limits of the method and future research.

Future presentations will present extended results and applications of the proposed method. With these findings, the use of the proposed framework in forensic audio analyses, together with other scientific validated methods, is recommended.

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Forensic Audio; MDCT; Clone

C12   Navigating the SILK Road: An Examination of WeChat SILK Audio Files

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Learning Overview: The goal of this presentation is to examine the file structure and forensic implications of SILK v3 encoding and the WeChat audio file structure in mobile device analysis.

Impact Statement: WeChat is a global mobile communication application that allows users to send and receive audio messages. WeChat saves the audio using a SILK v3 codec but with a one-byte offset in the header. This unique header has caused issues with standard forensic tools parsing and playing the data and causes additional complications when the audio files require review by attorneys in civil litigation. This presentation will not only discuss WeChat audio data structure but also recommended methodologies to examine and review the WeChat audio files outside of commercial tools. This presentation will impact both law enforcement investigations and civil eDiscovery matters.

WeChat is a mobile device communication application developed by Tencent, a multinational technology company based in Shenzhen, China. The application is used world-wide but is especially popular in Asia-Pacific countries for both personal and business use. The WeChat application family has three different versions: WeChat International, Weixin, and WeChat Commercial. The international version is available to all mobile numbers outside of mainland China and stores its data in Europe and Singapore. Weixin is similar to WeChat International but used for mobile numbers within China and maintains all data within China. WeChat Commercial is used by businesses and employees within mainland China. The WeChat application has the capability to record and send voice messages, similar to other mobile device communication applications. Up until 2015, WeChat audio files were stored using Adaptive Multi-Rate (AMR) encoding and standard audio players, such as Windows Media Player, would play the files. Starting in 2015 and continuing to today, WeChat audio recordings appear to be stored in a format using SILK v3 encoding using a customized one-byte header offset.[1] This causes issues in the digital forensics community, as commercial forensic tools have difficulty supporting or converting WeChat audio files for playback during analysis. In some cases, customized workflows using open-source tools are required to convert the files into a standard format, such as WAV.[2] In addition, litigation review tools used by law firms in civil cases cannot play these types of audio files in their native format. This has impacted both law enforcement investigations and civil eDiscovery matters where WeChat audio files extracted from a mobile device must be examined and played back using various audio software tools

This presentation will explore WeChat audio files extracted from various mobile devices, their forensic importance in both civil and criminal cases, the SILK v3 audio codec and customized one-byte offset header used by WeChat, and recommended methodologies to examine and review the WeChat audio files outside of commercial tools.

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Digital Evidence; Apple® iOS®; Android™

C13   Validation of Image Stream Hashing: A Forensic Method for Content Verificatio

Gregory S. Wales*, Colorado Springs, CO

Learning Overview: This presentation uses five image file types transcoded with six different image conversion applications using various settings to test the image stream hashing method's "fitness for the purpose" of determining if two image file contents were identical. After attending this presentation, attendees will have gained knowledge from foundational validity testing of a forensic method to verify the digital image stream integrity when file containers may change during file handling and processing.

Impact Statement: This presentation will impact the forensic science community by providing an alternative to traditional file container hashing for data integrity verification, more precisely by verifying the digital image stream.

How does a forensic examiner know if they have altered an image stream when converting a digital image (photograph) from one codec or file container to another for subsequent forensic examination? Forensic standards and best practices recommend avoiding alteration or degradation of multimedia data during transcoding.[1-3] An image stream hashing method was recently introduced to the forensic science community to answer the question above.[4] This presentation offers an initial validation study of the image stream hashing method. The study tested the image stream hashing method's reproducibility and reliability as a forensic method. The validation study used empirical measurements of accuracy, precision, sensitivity, specificity, and error rates to evaluate the fitness of the image stream hashing method.

The validation study examined image stream hashing accuracy, precision, and reliability in predicting the contents of compared images. The study also evaluated the method for errors or limitations. In addition, the research also examined the need for image stream hashing standards controlling method operations. The quantitative research method used a quasi-experimental approach involving over 750 tests that used the image stream hashing method to compare two images to predict similar or different image contents.[4] Next, six image quality assessments provided "ground truth" between the reference and questioned images in each experiment. The results were used with a binary classification matrix to develop statistics for overall conclusion development. The research revealed that the image stream hashing method was "fit for the purpose" of determining if two image file contents were identical when using three of the five tested derivative image types. Two derivative image file types were determined not "fit for the purpose."

Additionally, using the method should only be done with implementing the proposed standards controlling the method operations developed as part of this research. Otherwise, systematic errors may develop during the method's use. Therefore, the study concluded that examiners could use the image stream hashing method for forensic science, but only if error mitigation techniques that utilize the proposed standards controlling the method operations are implemented.

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Stream Hashing; Image Stream Hashing; Data Integrity Verification

C14 **Modeling Influences on the Understanding of Digital and Multimedia Evidence (DME) by Attorneys and Digital Forensic Examiners (DFE) Within the United States Criminal Justice System**

Joseph Levi White*, United States Army Criminal Investigation Laboratory, Forest Park, GA

Learning Overview: After attending this presentation, attendees will better recognize potential influences on the understanding of DME by attorneys and DFEs, including motivation, experience, generational influences, communication, and education/training.

Impact Statement: This presentation will impact the forensic science community by providing a framework for better understanding how attorneys and DFEs approach and understand DME. The presented data may be used to identify areas for potential increased efficiency/effectiveness in DME-involved cases within the United States criminal justice system.

This presentation is based on the published dissertation research of the author/presenter.[1] As a Digital Forensic Examiner (DFE) with over 15 years of experience, the presenter has worked with attorneys on multiple cases involving Digital and Multimedia Evidence (DME). From these DFE/attorney interactions, it is believed that there is a difference between how attorneys and DFEs approach and understand DME. This research had a goal of determining potential themes that may influence the understanding of DME by attorneys and DFEs within the United States Criminal Justice System. To reach this goal, both qualitative (interviews/potential theme identification) and quantitative (questionnaires/statistical analysis) methods were utilized.

Qualitative semi-structured interviews of experienced criminal attorneys and DFEs were conducted to gather information regarding potential influences on their understanding of DME. The interview results were transcribed, and the data coded to allow for qualitative analysis. Five themes were identified from this data and are thought to play a potential role in understanding of DME by attorneys and DFEs: motivation for involvement in the criminal justice system (passion for the job, desire to work in law enforcement, monetary gain, sense of ethical obligation, and seeking justice), experience (and knowledge), generational influences (age and the CSI Effect), communication within defined roles, and education/training. A questionnaire was developed utilizing these five themes as a guide that was then distributed to attorneys and DFEs across the U.S.

Low response rates to the questionnaire were observed and will be discussed. Statistical analyses were conducted on the available survey results in relation to the five previously identified themes. Attorneys and examiners agreed on many facets of each theme. The most influential motivational factor for seeking a career as an attorney or DFE is a passion for the field. One of the most influential components to understanding DME was determined to be experience. Detractors to understanding DME included increasing age and the CSI Effect. A frequency increase in communication levels between attorneys and DFEs has the potential to affect DME understanding and case efficiency. Attorneys have higher educational levels much greater than even experienced DFEs; however, technical DME training levels (which have a greater influence on overall DME understanding) are much greater for DFEs. Attorneys tend to use online research as a primary learning method, while DFEs rely primarily on technical training. Each of the identified themes shows promise for describing influences on the understanding of DME by attorneys and DFEs within the U.S. criminal justice system and will be further discussed within the presentation.

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1. White, Joseph Levi. *The Understanding of Digital and Multimedia Evidence (DME) by Attorneys and Digital Forensic Examiners (DFE) within the United States Criminal Justice System* (Publication No. 28773290) [Doctoral Dissertation, The University of Southern Mississippi]. ProQuest Dissertations Publishing, 2021.

Attorneys; Examiner; Digital Evdence

C15   Uncovering Ethical and Legal Issues Associated With the Rising Trend of Using Chatbots—Crime Scene Investigation and Scientific Publishing

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Learning Overview: After attending this presentation, attendees will understand the ethical and legal challenges associated with the rising trend of chatbots in this era of Artificial Intelligence (AI). This presentation will shed light on the issues associated with the use of chatbots in research and some professional domains such as forensic science, law, and medical science.

Impact Statement: This presentation will impact the forensic community by showing whether attendees should rely upon ChatGPT for the investigation of a crime and other forensic examinations due to various ethical and legal challenges associated with these chatbots. Attendees will also gain knowledge about the trend of chatbots as well as the strengths and weaknesses associated with these AI tools.

In the current era, most of the work is either guided or done by algorithms or machines. The concept of AI is not new. AI emerged some time ago but currently, AI-based machines/algorithms have become more efficient and advanced. These systems are being utilized in various domains such as academics, business, government, health care, etc. Chatbots are one of the wonders of AI. These are artificial conversation entities simulating conversations over the internet.[1] These chatbots interact with humans through natural language processing. Chatbots are gradually gaining popularity across various domains due to their capacity to simulate human conversations and the need for less effort. The time taken by these chatbots to respond to every single prompt is just a matter of seconds. A chatbot was recently introduced in November 2022 by OpenAI (i.e., ChatGPT) and gained millions of users within a week.[2] This chatbot became a hot topic of discussion in the internet world. After the launch of ChatGPT, more than 200 other chatbots have been released. Chatbots can generate essays, poems, letters, email writing, streamline written language, etc. depending upon the user's query prompted in the chat interface.

The present study focused on the ethical and legal challenges associated with the use of chatbots in scientific publishing as well as in crime scene investigation. In this study, the responses obtained from ChatGPT were analyzed for every prompt given to it. After thorough analysis, the strengths and weaknesses of this chatbot have been analyzed as well as how this technology can be misused by criminals.

The results of the study indicate that the information provided by ChatGPT is not always authentic and accurate. Various experiments have been performed relating to crime scene investigation and scientific paper writing. ChatGPT was prompted to suggest a method of deciphering chassis numbers in vehicles. The responses obtained from ChatGPT were not satisfactory. In one of the experiments, when the ChatGPT was prompted to write a narrative review on advancements in forensic science, the references generated by the ChatGPT were not accurate. This may be due to the fact it has been trained on the data available on the internet such as Wikipedia, books, news articles, blogs, etc. through September 2021. We are familiar with the fact that most of the information available on the internet may be fake and may have misinformation, rumors etc. So, can this tool be used in scientific publishing and crime scene investigation? If yes, then there will be no transparency, and the circulation of misinformation will be everywhere.[3] Moreover, a human cannot differentiate whether the text is written by a human or AI.[4]

In this study, some case studies related to ChatGPT have also been discussed. Therefore, this study tends to raise awareness among forensic scientists, medicolegal experts, and the scientific community to avoid the use of chatbots for solving medicolegal cases, crime scene investigation, writing case reports, and scientific publications. This study also recommends developing some stern ethical and legal guidelines and policies for the use of AI tools such as ChatGPT in forensic research and practice.

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ChatGPT; Forensic Science; Crime Scene Investigation

C16   **The Case of the Distracted Pedestrian: The Importance of Experts in Digital Forensics**

Jason R. Lewis*, CyForce, Inc., Lakeland, FL

Learning Overview: After attending this presentation, attendees will better understand the importance of not solely relying on forensic tools and the need to validate the data being presented. Additionally, attendees will learn more about messaging apps in general, particularly Marco Polo, and what artifacts these applications leave in an iOS® environment.

Impact Statement: This presentation will impact the forensic science community by providing details on examining an iOS®-based device for evidence of the usage of messaging apps in general and Marco Polo in particular.

Recently, our firm was contracted by a law firm representing the estate of a deceased individual. This person was a well-known resident of a mid-sized city and was struck by a motor vehicle while crossing the road. The question in dispute was, while crossing the street, was this individual actively using the Marco Polo app on their iPhone® and was thus "distracted" by the phone? The local police department had examined the victim's iPhone® and "officially determined" that they were actively using a Marco Polo at the time of the collision. Thus, they were partially at fault for the collision that occurred. This question is so important because, like most states, the state where the collision occurred happens to be a "comparative fault" state, specifically a *modified comparative negligence* state, which means if the victim is found to be at fault, it could reduce the civil liability of the driver.

While examining the data, one curious fact stood out: the microphone, camera, and speaker of the iPhone® were never activated during the collision. The detailed logs and databases that make up the "pattern of life" on an iPhone® show in immense detail what is being utilized on an iPhone® and when. For example, it logs when the microphone is activated, where the output audio is sent (speaker, Bluetooth®, headphone jack, etc.), and when the camera is turned on. These logs did show that the Marco Polo was running, but it didn't show that any of the usual hardware was being utilized while it was running.

After a thorough search of the literature, it was determined that research on this application was non-existent. Still, similar applications did show the camera, microphone, and speaker activation when used to play or record video messages. This led the author to conduct a scientific experiment utilizing the same model of iPhone® and the same version of iOS® to determine if the microphone, speaker, and camera activation was recorded when using the Marco Polo application.

This presentation will show the results of the subsequent research to show what data is captured by iOS® databases and logs and how this data can be utilized to show if video messaging apps are being actively used or running in the background.

Social Media; Mobile Device; Traffic Accident

C17   The Potential for Impacting Unsolved Case Outcomes With the Inclusion of Digital and Multimedia Evidence (DME) Forensics Into Cold Case Units

Joseph Levi White*, United States Army Criminal Investigation Laboratory, Forest Park, GA

Learning Overview: After attending this presentation, attendees will understand the way technological advances may greatly impact data access and extraction capabilities in cold cases involving DME. This presentation will provide an outline of some of these advancements and provide examples of the potential enhancement of Cold Case Units (CCUs) through the incorporation of updated DME capabilities.

Impact Statement: This presentation will provide an outline of how technological advances made over time have greatly impacted data extraction/duplication capabilities, especially within mobile device forensics. This presentation will impact the forensic science community by providing insight into the potential for increasing investigative leads within CCUs through the incorporation of DME forensic capabilities.

A "cold case" may be described as an unsolved crime that is expected to remain unsolved until new evidence or investigative leads are discovered/obtained. CCUs have been developed by investigative/law enforcement agencies for the purpose of reviewing case files, conducting additional interviews, and resubmitting original evidence for additional forensic analysis. These evidence resubmissions often involve requests for analysis based on advancements in science and/or technology that have been made since the crime was committed and originally investigated. The largest focus for evidence resubmissions has been due to advancements made in DNA (deoxyribonucleic acid) analysis, such as detection/extraction capabilities, sensitivity advancements, genetic genealogy, mitochondrial DNA analysis, and the greater availability of DNA databases for comparison/familial searches. Additional advancements in forensic science traditionally highlighted as affecting cold case outcomes include anthropology/biology (such as cause of death determinations and facial feature reconstruction) and latent prints (such as advancements in latent print detection/visualization and increased database search availability/functionality). However, advancements made in DME should not be overlooked.

Personal use of mobile phones increased beginning in the 1990s. With time, mobile device (cellular phone, tablet, and laptop computer) manufacturers and software developers have implemented hardware and software security features designed to protect these devices from unauthorized access and protect the data maintained within them. These features include hardware charging/data port modifications, knowledge-based locks (Personal Identification Number [PIN] and pattern locks), fingerprint sensors, and facial recognition (face-ID). Unfortunately for forensic examiners and law enforcement, these advances often prevent access to data maintained on devices submitted for data extraction and analysis. Depending on the security features enabled on the device at the time of laboratory submission, the ability to extract usable data from the device may be severely hindered or not possible.

Therefore, DME forensics is required to constantly evolve to account for each mobile device software and/or hardware security update. Data shows that, with time, digital forensics capabilities have been able to provide methodologies for bypassing these security features and gaining access to data maintained within these devices. Technological advancements related to data extraction have made data accessible on devices that just a few years ago would be considered completely inaccessible to most digital forensic examiners. A review of the most popular mobile devices will be provided in a timeline, listing current capabilities for device data extraction for multiple device types. This information will be used to highlight the potential benefit for the inclusion of digital forensic capabilities in CCUs to assist in unsolved crime investigations.

Cold Case; Unsolved; Digital Evidence

C18 A Study of Digital Evidence (DE) Processing Efficiencies in Publicly Funded Crime Laboratories

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Learning Overview: After attending this presentation, attendees will be informed about a study to evaluate DE processing in the United States. The study data comes from DE crime laboratories and corresponding law enforcement agencies to assess allocation of resources, interagency communication, and DE evidence management and retention practices.

Impact Statement: This presentation will impact the forensic science community by explaining how, due to the rise in DE processing, crime laboratories and their law enforcement professionals are implementing strategies for triaging, processing, and managing DE.

As the usage of electronic devices rises, so does the need to process these pieces of evidence in criminal investigations. As such, the desire for data-driven evidence to advance the field of DE processing is stronger than ever. This study aimed to understand the areas in which DE crime laboratory and law enforcement professionals are experiencing their biggest challenges. Surveys were administered to DE crime laboratories and their law enforcement partners between February 2022 and March 2023. Respondents were asked about agency resources (e.g., budget, staffing), annual processing metrics of various DE types, interagency coordination, and DE submission, processing, and retention. Responding agencies included 32 crime laboratories with digital evidence sections and 24 law enforcement agencies. A purposive subset of DE crime laboratories and their law enforcement partner agencies were then selected for in-depth qualitative interviews.

Uncovered through the quantitative surveys, nearly half of DE laboratories did not have a policy for triaging DE, and about two-thirds did not have a policy on DE retention. On average, crime laboratories not accredited specifically for DE processing received 2.5 times the amount of testing requests that the DE-accredited crime laboratories did. The qualitative interviews revealed a consensus for DE-specific training and the need for more funding allocated for DE processing. Overall, these are just a few of the key takeaways from the evaluation that was conducted. Additional insights regarding submission, processing, and retention practices will be shared from the paired crime laboratory-law enforcement interviews.

This study is one of the first to examine the DE processing landscape in a multi-method approach and will serve as a foundation to uncovering the needs resulting from an incredibly high-paced evolvement in the criminal justice system. The study findings enhance our understanding of how forensic digital professionals are promoting justice and maintaining scientific integrity amidst their quickly changing field.

Crime Laboratory; Digital Evidence; Survey

C19   Standards Development Activities in Digital and Multimedia Forensic Science

Tracy Walraven*, DC Department of Forensic Sciences, Minneapolis, MN

Learning Overview: After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of digital and multimedia forensic science.

Impact Statement: This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to digital and multimedia issues. This presentation will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to digital and multimedia standards development will be discussed. These include: (1) recent standards that have been added to the OSAC Registry; (2) OSAC proposed standards; (3) published standards from the American Society for Testing and Materials (ASTM) and other authoritative bodies that have yet to go through the Registry approval process or are currently undergoing the approval process; (4) priorities for new documents or work products, and other highlights; and (5) projects undertaken by working groups within the digital evidence subcommittee, specifically the new Data Sets Library project, the newest work surrounding privacy and digital evidence. The Data Sets Library project is a catalog of artifacts that can be used as a reference for the digital forensics' community during investigations. The Privacy and Digital Forensics paper explores the line between the examiner and evidence, with the consequences for the examiner if privacy is breached. Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts. This presentation centers around the efforts of the Digital Evidence Subcommittee within the OSAC. As the Scientific Working Group on Digital Evidence (SWGDE) is now considered an SDO, the OSAC DE's efforts in combination with SWGDE is also highlighted as part of providing the best guidance for the digital evidence community.

Digital Evidence; Standards; Mobile Device

C20 What's VITAL When It Comes to Digital/Multimedia Standards? An Update From the OSAC Video/Imaging Technology & Analysis (VITAL) Subcommittee

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Learning Overview: After attending this presentation, attendees will have an understanding of the Organization of Scientific Area Committees (OSAC) Registry Process and the current work being conducted within the VITAL Subcommittee.

Impact Statement: Standards and their implementation are essential to ensuring the sound practice of forensic science across all disciplines, including digital and multimedia sciences. This presentation will impact the forensic science community by offering information on the current and upcoming OSAC standards in the areas of video analysis, image analysis, and photography.

The OSAC for Forensic Science "works to strengthen the nation's use of forensic science by facilitating the development and promoting the use of high-quality, technically sound standards by the forensic science community. These standards define minimum requirements, best practices, standard protocols, and other guidance to help ensure that the results of forensic analysis are reliable and reproducible."[1] OSAC forensic science disciplines are spread across seven major Scientific Area Committees (SACs). Video/Imaging Technology & Analysis (VITAL) is one of four disciplines that report to the Digital/Multimedia SAC (DMSAC). The three other disciplines include Digital Evidence, Facial ID, and Speaker Recognition. VITAL specifically focuses on creating and promoting standards related to methods and technologies used to analyze images. This includes all aspects from the capture of images or the acquisition of data to the processing of image or video files to the reporting of an opinion. To address the multifaceted nature of imagery, VITAL has task groups devoted to video analysis, image analysis, photography, and most recently, an exploratory task group on gait analysis.

Within OSAC, there are several processes in place to ensure relevant standards are developed and promoted. Both OSAC Proposed Standards and Standards Development Organization (SDO)/Standards Setting Organization (SSO) Published Standards are eligible for on the OSAC Registry. The processes in place for each of these types of standards will be discussed to provide an overview of OSAC operations.

Additionally, the OSAC partnerships with SDO/SSOs within the forensic community are key for the development and publication of standards. The standards within VITAL have generally come from and go to the American Society of Testing and Materials (ASTM) as an SDO. The Scientific Working Group on Digital Evidence (SWGDE) has also recently been approved as an SSO. SWGDE documents are eligible for the registry approval process and OSAC-proposed documents are able to move to SWGDE for publication. This presentation will discuss the interactions and steps needed for these organizations to work together on standards within the forensic multimedia community.

Finally, the current work products within VITAL will also be highlighted. Topics of these documents include: laboratory photography, postmortem photography, photogrammetry, image comparison opinions, digital video workflows, photography of scars/marks/tattoos, digital image management, synthetic media, image/video authentication, and relevant training for the applicable disciplines. The status of these documents, the impact they will have, and the next steps they will take will provide attendees with an understanding of what is being done to address the standards needs within image analysis, video analysis, and photography.

Reference:
[1] Retrieved from https://www.nist.gov/organization-scientific-area-committees-forensic-science on July 27, 2023.

Standards; Imaging; Multimedia

C21   The Integration of Forensic Science and Artificial Intelligence: The Implications in Crime Scene Investigations

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Learning Overview: After attending this presentation, attendees will have learned the relationship and implications of Artificial Intelligence (AI) (deep learning and machine learning) in forensic science and criminal investigation. Additionally, attendees will be able to learn the AI models (i.e., neural networks [CNN, ANN, TINN], Support Vector Machines [SVM], Linear Regression [LR], Logistic Regression [log R], and Random Forest).

Impact Statement: This presentation will impact the forensic science community and criminal investigative agencies by providing ease in the investigative process by significantly reducing the time of investigation and increasing the accuracy of various evidence analyses with the aid of AI. Moreover, this presentation will explain the AI methods to extract information to a greater extent from crime scenes and analyze the forensic evidence with different AI approaches.

From its origin in a summer research workshop at Dartmouth College, NH, in 1956 to recent intelligent machines and chatbots, AI has fueled the fields of health care and medicine, agriculture, marketing and finance, engineering, transport, cyber security, neuroscience, logical reasoning, statistical analysis, disaster management, military services, national security, etc. Additionally, these AI tools have vast applications in criminal investigation. The primary objective of forensic science is to scientifically help in crime scene and medicolegal case investigation. The recent advances in scientific methodologies of evidence analysis fulfill the primary objective of forensic science. However, the integration of AI can possibly speed up the process of identification and evidence analysis and make the work of investigators closer to a higher accuracy.[1] For instance, the identification of future possible modus operandi can be accomplished through the technique of machine learning-based "Pattern Recognition" by feeding the machine with a large dataset of previous crime patterns. Moreover, AI (deep learning) can identify the perpetrator/victim/suspect of a crime, international criminals, drug peddlers, poachers, and terrorist from the images captured in Closed-Circuit Television (CCTV) by finding the matches against the large datasets without any biasness. Moreover, "Classification Models" of supervised learning can be used in the estimation of sex, population affinity, time since death, fingerprint and impression analysis, identification of diatom, handwriting identification, classification of firearms, bullets, and drugs, etc. Additionally, regression models can be utilized in age estimation, anthropometric studies, DNA analysis, and forensic odontological research.[1-3] For instance, PredPol software identifies the risk-associated locations and time (AI-based geospatial modeling) where crime is most likely to occur. Based on datasets on type, location, time, and date of crime, risk areas are identified and patrolling officers can be advised to patrol these specific locations.[4] Yuan utilized multi-task learning (machine learning) in criminal law for judgment prediction, recommendations of relevant laws, and analysis of legal documents with high accuracy.[5] Moreover, AI has also proven powerful in restricting money laundering through the detection of unusual transactions by introducing probabilistic retina.[6]

For this presentation, scientific literature was searched from various national and international research publications using online platforms and databases. The standard indexing agencies such as PubMed®, PubMed Central®, Embase®, Scopus®, Medline®, Cochrane®, and Web of Science® were searched. The key words used to search the relevant literature and research publications include "artificial intelligence in forensic science," "artificial intelligence AND forensic science," "artificial intelligence in criminal investigation," "artificial intelligence AND criminal investigation." After thorough screening of the titles and abstract, relevant literature was included in the study.

The present communication concludes that AI holds a bright future in criminal investigation and identification. The low accuracy, reliability, and speed of evidence analysis can be defeated by the introduction of AI in forensic examinations and research. AI can overcome the error and biasness prone in human judgments and evidence analysis and can provide a more reliable criminal justice system.

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Forensic Science; Artificial Intelligence; Crime Scene Investigation

C23 Live Memory Acquisition for STM32-Based Microcontrollers with ARM TrustZone

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Learning Overview: This presentation will give an overview of a memory acquisition tool developed for STM32-based microcontrollers. Attendees will learn how ARM TrustZone technology can be leveraged to securely obtain microcontroller memory dumps for forensic analysis. Specifically, this presentation will discuss how STMicroelectronics' HAL API was leveraged in conjunction with TrustZone to send full memory dumps of the Rich Execution Environment to a server for analysis.

Impact Statement: The presentation will impact the forensic science community by presenting a reliable mechanism for obtaining memory dumps from TrustZone-enabled microcontrollers. This tool can be used on Internet of Things (IoT) and other embedded devices for forensics triaging and thorough investigation of potential compromises and malfunctions.

Most currently existing memory acquisition tools for microcontrollers, such as JLink, are hardware-based. These techniques require additional equipment such as debuggers, which can be very costly. Debuggers are also often designed for development purposes rather than security solutions, making them insufficient for memory forensics applications. On the contrary, a software-based approach opens the door to automated memory forensics tools, which can be packaged directly into products. Coupling this type of memory acquisition tool with TrustZone provides a high degree of security, eliminating security concerns of exposed hardware interfaces like Joint Test Action Group (JTAG). Nonetheless, in practice, little has been done to develop such a tool, mainly due to the challenges involved in developing tools for microcontrollers, such as the strict requirement to use low-level languages like C and Assembly to interact directly with hardware resources.

Additionally, given the close interaction with hardware, tools cannot be ported to other microcontrollers without code changes that reflect the differing hardware. Thus, the overarching objective of this research is to present a generic approach for securely obtaining memory dumps for the STM32 family of microcontrollers. In addition, this work further provides a step-by-step guide to extending our methodology for developing software-based memory acquisition tools for other MCU families by discussing the overarching process.

Our proposed memory acquisition process starts with the Rich Execution Environment (REE) relinquishing its memory to TrustZone block by block. A watchdog timer mechanism is implemented to force the REE to comply. If the REE fails to submit its memory before the timer counts down, a device reset will be performed, ridding the device of malware or malfunctions that may be interfering with the process. Once TrustZone has a block of memory, it sends it to a network resource and uses Transmission Control Protocol (TCP) to send it to a server. Each of the blocks is concatenated into a binary file as they are received on the server. At the end of this process, the binary file contains a complete memory dump that can be used for forensic analysis and/or triaging. Throughout the acquisition process, technologies like DMA and SPI are used for optimization. TCP is used to securely transmit the dumps across the network, and a message protocol was added to tightly monitor the data and protect against signal injection. Last, since this entire process is carried out by TrustZone in an isolated region of the Central Processing Unit (CPU), malicious actors cannot use the tool to obtain firmware dumps, thereby improving the overall security of the process.

Memory Acquisition; IoT; Microcontrollers

C24   Live Memory Forensics of Field Devices in Industrial Control Systems

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Learning Overview: After attending this presentation, attendees will understand the challenges of live memory forensics in field devices in Industrial Control Systems (ICS) and further explore a practical method of performing this task reliably on field devices such as programmable logic controllers to acquire and analyze volatile memory data on-demand, including process stack and firmware data structures. This presentation will also present experimental results on a real-world Programmable Logic Controller (PLC) to show the effectiveness of the proposed method.

Impact Statement: This presentation will impact the forensic community by discussing a new live memory forensic investigation method for field devices in ICS to investigate cyberattacks on power grid stations, nuclear plants, and other critical infrastructure. Physical access to the devices is required to read entire device data at the hardware level for further analysis. Proprietary engineering software provided by device vendors can also acquire device data remotely. However, this method is constrained by the engineering software functionalities not designed to support forensic data acquisition and can only acquire limited device data such as control logic and Input/Output (I/O) data; attackers can target device firmware and other parts of the memory, which are typically not accessible through engineering software.

ICS control and monitor power grid stations, nuclear plants, and other critical infrastructures. When ICS environments are under attack, the digital forensic investigation will help identify compromised field devices and understand attacker vectors.[1] Since these devices are supposed to work continuously 24/7 to control their attached physical processes in real time, they cannot be shut down. Thus, live forensics of these devices is a practical solution to perform a quick investigation while they are operational.

Programmable Logic Controllers (PLCs) run control logic in scan cycles to monitor and control physical processes continuously. These control logic programs are run as binary machine code and can be accessible and updated by design through engineering software. Our approach leverages this PLC's common design feature to develop live memory forensic capabilities at the device level.[2] Mainly, it attaches (installs) a benign code at the end of a control logic using ICS protocols to run continuously as part of the scan cycle. The code reads requested memory regions and copies them to an area accessible through PLC's network protocol. It allows accessing of any PLC memory region on request for further analysis. For instance, return-oriented programming attacks on PLCs manipulate the PLC stack.[3] Our approach can read the stack pointers and make them available to read remotely.

This presentation will provide a case study on a real-world PLC to demonstrate the proposed solution as practical and reliable. It will also discuss the scalability of the proposed approach since PLCs are heterogenous and may have different microcontrollers affecting the underlying machine instruction set for the benign code attached to the control logic, such as Schneider Electric's Midcon PLC series uses RX630, and Allen Bradley's ControlLogix uses ARM. The potential solutions to handle heterogenous instruction sets include cross compilers to have the code compiled from ARM to RX630 or MIPS to RX630. This presentation will provide examples to address scalability.

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Cyber Forensics; National Security; Artificial Intelligence

C25   Provenience-Based Cross-Verification of Digital Forensic Artifacts Applied to NTFS

Alex J. Nelson*, National Institute of Standards and Technology, Gaithersburg, MD; Sheldon L. Douglas, National Institute of Standards and Technology, Gaithersburg, MD

Learning Overview: This presentation will demonstrate a methodology for comparing the subject data exploratory coverage of two digital forensic processes that share some in-common goals in their reporting. After attending this presentation, attendees will better understand the distinction between identifiers, and identifying characteristics, when describing artifacts recovered and interpreted in the course of digital forensic analysis. Attendees will see how two metadata summary languages, Digital Forensics XML (DFXML) and Cyber-investigation Analysis Standard Expression (CASE), can implement a strategy to compare the results of two file system analysis tools and how a previous implementation of the strategy had relied on an artifact of unstable referential integrity, showing a need for using identifying characteristics with less chance of ambiguity.

Impact Statement: This presentation will impact the forensic science community by demonstrating cross-verification of digital forensic tool results and how choice of artifact identifiers can impact the ability to compare results algorithmically.

In digital forensics, file system analysis is a precursor task to event reconstruction. Often, unallocated content within a file system is content of interest to an investigation, and thus recognition, extraction, and ascription of unallocated files are typical intermediary steps en route to interpreting file system contents. The results of this general workflow form comprise a set of intermediary results worth cross-verification, due to potential impact on later interpretations of initial evidence. However, unallocated files often lack stable identifiers, presenting subtle challenges that can foil algorithmic comparison.

Unallocated content recovery requires careful understanding of the storage medium, or storage format, from which the content is recovered.[1] This work focuses on a model of a file system where an allocated file's definition comprises at least three key dimensions: the data structure housing its metadata such as timestamp and owner, often referred to as an inode; the data structure compactly housing its location within the file system's namespace, often implemented as a directory entry; and the range within the file system that houses its contents, which may be discontiguous.[2]

Prior work has used this model to implement differential analysis, both for comparing changes in a file system's state across time and across parse results when using multiple tools against the same subject image.[3,4] While the three-dimensional file model enables comparison of allocated content with seemingly little difficulty, some attempts to verify some POSIX-required characteristics of the allocated content show a weakness in the three-dimensional model that impacts interpretation of allocated and unallocated files.

We present a strengthening of the three-dimensional model, emphasizing a geometric representation of the three file dimensions as a first-order concern. This pattern extends in applicability beyond file system analysis but is presented initially in the context of New Technology File System (NTFS) file system analysis. We demonstrate corrections over a model improvement previously proposed by Casey et al. and show results from extending two independently developed open-source tools to enable geometric comparability between their NTFS results.[1] Using the tool-agnostic languages Digital Forensics XML (DFXML) and Cyber-investigation Standardized Analysis and Expression (CASE), the geometry-based identifier strategy corrects a previous measurement of unallocated content.[5,6]

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Digital Evidence; Method Validation; Chain of Evidence

C26   Testing File Carving Tools

James R. Lyle*, National Institute of Standards and Technology, Mount Airy, MD; Barbara Guttman, National Institute of Standards and Technology, Gaithersburg, MD

Learning Overview: After attending this presentation, attendees will be made aware of resources provided by the National Institute of Standards and Technology/Computer Forensics Tool Testing (NIST/CFTT) for testing file carving tools and some of the limitations and challenges for testing such tools for carving files from unallocated storage space.

Impact Statement: This presentation will impact the forensic science community by increasing awareness in the community of available resources for testing file carving tools. This presentation will aid forensic practitioners in using the test protocol and materials created by NIST/CFTT and using provided tools to develop materials that can be used for testing of file types not included in the NIST materials.

Extraction of digital evidence from digital systems is dependent on software to interpret and present relevant data. The courts need assurance that any testimony based on software is scientifically sound and reliable. The *Daubert* guidelines list testing as one of several criteria for the court to consider before deciding admissibility of evidence in court.

CFTT has been developing test methods for testing forensic tools for more than 20 years. About a decade ago, we produced a file carving methodology and applied it to ten graphic file carvers and eight video file carvers. We are releasing an updated version that adds new graphic (HEIC) and video (MKV) file types, simplified some aspects of the process, added tools for the user to create custom data sets as needed, and are incorporating the new test methodology into Federated Testing (see the CFTT web site).

The general process is to collect a set of six to ten source files of each file type to try to carve. For basic testing, two acquisition image files are created that are like an acquisition of unallocated space. The acquisition files contain content from the source files separated by varying amounts of other recognizable filler content. The layout of one acquisition file is to place each source file as one contiguous chunk followed by some amount of filler data. The layout of the other basic test image is to break each source file into several fragments with filler added between each pair of fragments. All blocks are aligned on 512-byte boundaries as a real data file would be placed by a file system. The two image files are then carved by the tool being tested and the results analyzed.

Results are analyzed by a pair of measures. The first measure is a visual inspection and classification into categories of *No Observed Defects, Minor Defects - Still Useful, Major Defects – Not Useful* and *Not viewable*. The second measure is an analysis of the source of each block of data in the carved object classified as follows: Source file, known filler, or unknown data.

Other file layouts are available for testing for scenarios such as files not aligned on sector or cluster boundaries and other file fragmentation layouts (fragments out of sequence, two files braided together or missing fragments).

Digital Evidence; Software Testing; File Carving

C27   The Latest Developments in Sailfish OS Forensics

Krassimir T. Tzvetanov*, Purdue University, West Lafayette, IN

Learning Overview: The goal of this presentation is to acquaint attendees with a mobile phone Operating System (OS) that is not very popular but has significant importance from a geopolitical standpoint.

Impact Statement: With the ongoing conflict in Eastern Europe and the tension escalating in the South China Sea, it is important to understand the technologies a belligerent is using. This presentation will impact the forensic science community by bringing awareness to a mobile phone OS that has managed to gain popularity on those markets without being noticed in America.

SailfishOS is a Linux® kernel-based embedded device OS, mostly deployed on cell phones. It is rapidly deployed in BRICS countries such as Russia, China, and India. In Russia alone, the government has mandated the replacement of all Android™ and iPhone® devices in favor of their SailfishOS version called AuroraOS. In India, the OS is deployed by a couple of the major network providers, and in China, Huawei is investigating it as an Android® replacement, as economic tensions with the United States rise.

While popularity and adoption is growing, there is no sufficient research in this space, so it is likely for investigators to encounter it in the field.

Three years ago, we presented a mapping of forensic artifacts for SailfishOS 3.2. Since then, the OS has developed and has introduced robust support for Android® applications. In this presentation, we will go over what has changed in the way data and settings are stored and show the mapping of new digital artifacts, which are pertinent to an investigation or incident response. We will cover call logs, text messages, Wi-Fi Network settings, SIM card management, cellular network artifacts, location services, address book, email, browsers, and other important artifacts, and a large number of other artifacts. In addition, we will present how this platform runs Android® software packages, how they affect the system, and what forensic artifacts they leave.

Apart from our other work, there is no other known publicly available research in this space, and the commercially available solutions for acquisition and analysis of this platform are lagging. This is a major gap, as this OS' adoption is accelerating in emerging markets on low-cost devices. This presentation documents many of the major forensics' points of interest such as call and text, log, phonebook, and web browser artifacts as well as hardware specific features.

SailfishOS; Mobile Device Forensics; MeeGo

C28 Deep Audio Analyzer: A Framework to Industrialize the Research on Audio Forensics

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Learning Overview: After attending this presentation, attendees will be familiar with various audio deep learning techniques. This presentation will attempt to present existing deep learning solutions to analyze audio with state-of-the-art deep learning models without the need to develop code via the Deep Audio Analyzer framework. Attendees will gain useful information to analyze audio in different contexts through this powerful technology.

Impact Statement: This presentation will impact the forensic science community by demonstrating the potential of the proposed tool "Deep Audio Analyzer," a framework based on Deep Learning Neural Networks, to demonstrate the importance of using recent Deep Learning models in the audio domain for conducting audio enhancement processes, automatic multilingual transcriptions, speaker verification, and voice activity detection, whose performance cannot be achieved by examiners using classical methods of audio forensics. After attending this presentation, attendees will understand how to improve specific aspects of audio analysis in the forensic domain compared to classical methods. Attendees will also learn how to employ this tool not only for stand-alone model usage, but also for combining them to achieve novel audio analysis processes that can be easily shared with the scientific community, thereby improving the reproducibility of conducted experiments.

Forensic audio, a subcategory of forensic acoustics, has taken on an increasingly prominent role in the field of law enforcement, criminal investigation, and justice over the past few decades. This specialized branch of forensic science, dedicated to the capture, analysis, and interpretation of audio recordings within the context of legal investigations, has enabled the provision of decisive evidence in an ever-increasing number of legal cases.[1]

Audio forensic investigations focus on three core aspects: authenticity, enhancement, and interpretation. Ensuring the authenticity of audio recordings is crucial as investigator deductions rely on the recording conditions. Examiners must validate the chain of custody, detect intentional tampering, and prevent accidental modifications. Audio enhancement is commonly requested to address non-ideal acoustic environments, emphasizing features of interest for court presentations. Interpretation involves reconstructing timelines, transcribing dialogs, and identifying unknown sounds, considering other evidence and testimonies.[2] Recently, applications of novel deep learning solutions to forensics investigations have experienced unprecedented growth in interest and obtained results, with many researchers developing innovative algorithms and models to solve these complex problems.[3-7]

However, reproducing published experiments and results remain a significant challenge due to the needed programming skills required. This challenge is further compounded by the lack of (or an extremely limited) standardization in the way experiments are conducted. This issue results in a significant amount of time being spent by researchers trying to get other researchers' code to work, which leads to a significant waste of resources.

The development of speech-processing technologies has been largely driven by open-source toolkits.[8-12] With the emergence of general-purpose deep learning libraries, more flexible speech recognition frameworks have emerged as well as hubs where scientists load trained models for others to download.[13-25] While it can be challenging for non-experts users to prototype new deep learning methods, it requires knowledge of coding and environmental setup.

The present study aims to fill this gap by examining what are the main Deep Neural Networks that can help the Audio Forensic field in a better way than the traditional methods. The objective of this research is to provide a tool that covers enhancement and interpretation, which are part of the three main goals of Audio Forensics and to provide a tool that integrates these new technologies and enables users to visualize audio features, evaluate the performance of pre-trained models, and create new audio analysis workflows by combining deep neural network models.

Using Deep Audio Analyzer, examiners and researchers can perform these features without the need to develop any code. The tool also provides dedicated modules to test state-of-the-art models on customized data and combine models to create a new Deep Learning audio processing pipeline, combing for tasks such as Automatic Speech Recognition, Speech Enhancement, Speaker Separation, Speaker Verification and Voice Activity Detection.

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Forensics Audio; Speech Processing; Deep Learning Audio

C28   Toward Personal Internet-of-Things User Attribution During Forensic Investigations

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Learning Overview: After attending this presentation, attendees will have gained a comprehensive understanding of the application of machine learning in the digital forensics field to facilitate user identification. Attendees will learn how device handling can be leveraged as a new and effective method of identifying users during forensic investigations. Moreover, attendees will be introduced to a novel, privacy-preserving method of achieving user identification of Personal Internet-of-Things (PIoT) device owners through machine learning and simple mandatory actions.

Impact Statement: This presentation will have a profound impact on the forensic science community by introducing a groundbreaking approach to attributing specific pieces of digital evidence to individual users based on their handling of PIoT devices. As smart phones and wearables are increasingly prevalent in criminal cases, this novel method opens up new possibilities for Law Enforcement Officers (LEOs) to ascertain whether evidence found on a device was generated by the device owner or by someone else. This crucial distinction can help prevent wrongful accusations and convictions, safeguarding innocent parties from being wrongly implicated due to device misuse or unauthorized access.

User identification, the process of recognizing individuals based on various unique characteristics, has gained prominence with the widespread use of PIoT devices. These devices collect extensive user data, enabling the creation of unique user profiles. However, further experimentation is needed in creating user profiles using IoT data. Previous research on user identification within IoT has primarily focused on continuous identification or authentication, utilizing either physiological data such as heart rate or gait or non-physiological and behavioral data such as location data, screen touches, and accelerometer, gyroscope, and magnetometer data[1,2]. These studies mainly aimed to develop an identification method that could be integrated into other technologies or take place after training with the user performing prescribed actions.[3,4] Yet, the sensitive nature of this data, such as heart rate or location history, calls for protective measures or alternative identification methods, especially during Digital Forensic Investigations (DFI), where users may be reluctant to share their PIoT data due to privacy concerns. A plausible solution lies in the use of less sensitive, non-physiological data, thus minimizing Personally Identifiable Information (PII) exposure.

Despite the numerous security and privacy issues surrounding IoT technologies, the influx of these devices continues unabated. IoT devices have become deeply embedded in all aspects of human life, including government agencies, corporate settings, domestic environments, and now, through smart phones and wearables, they have become an integral part of our personal existence. Personal IoT devices can collect incredibly intimate levels of user data. For instance, a smart watch can monitor and record metrics such as heart rate, skin temperature, physical activity, and even geographical position. Simultaneously, a smart phone can access a broad range of user-specific information, including text messages, social media activity, browsing history, and app-specific data. Given the volume and detail of data these PIoT devices capture, they have become invaluable resources during forensic investigations. Nevertheless, it is crucial for digital forensic investigators to ascertain, with a high degree of certainty, that the data procured from these devices genuinely belongs to the alleged owner and not an alternate individual.

The focus of this presentation is to highlight the effectiveness and importance of applying machine learning to Digital Forensics for the benefit of the public, law enforcement, and the judiciary. To achieve this, the current research provides an inaugural exploration of utilizing PIoT device handling for user identification, implementing machine learning models to support forensic inquiries. The study explores the use of non-physiological sensor data from PIoT devices to record users' device-handling movements about the time a smart phone is unlocked and a smart watch is attached to the wrist. The results suggest that this approach is highly effective in distinguishing device owners, exhibiting performance metrics of .9621, .9618, and .9753 in terms of accuracy, F1-score, and Area Under Curve (AUC), respectively, when applied to smart watch data. On the other hand, the models using smart phone data showed performance that was marginally satisfactory, with accuracy, F1-score, and AUC values of .8577, .8560, and .8891, respectively. With further research to build and improve on these results, this method of user identification may one day be beneficial to LEOs. Furthermore, as this method addresses an essential aspect of user identification in the context of digital forensics, it has the potential to set new standards and best practices in the field. Its implications will extend beyond individual cases, guiding the development of more robust and sophisticated methodologies for future investigations. By reducing the risk of wrongful accusations and convictions, this innovative approach could play a vital role in upholding the rights of both victims and suspects, ensuring that justice is served with greater precision and integrity.

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Identification; Cyber Forensics; Machine Learning

C29   The Impact of Multi-Camera Smart Phones on Source Camera Identification

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Learning Overview: After attending this presentation, attendees will better understand how camera identification methods perform on images taken on recent models of multi-camera phones.

Impact Statement: This presentation will impact the forensic science community by exploring the impact of multi-camera phones on the error rates of source camera identification methods. The large authenticated multi-camera phone image database used for this research will be available and free to the forensic science community sometime next year.

An investigator has a questioned image from an unknown source and wants to determine whether it came from a camera on a person of interest's smart phone. This scenario is referred to as *source camera identification*. Researchers Lukas, Fridrich, and Goljan discovered that slight imperfections in the photosites of a camera's sensor array act as an identifying feature or *camera fingerprint* in this scenario.[1] They showed that in most circumstances, these imperfections in the sensor array carry through the image-capturing process and remain in the noise level of the image's pixel values, where it is unnoticeable to the human eye but detectable through computer processing. Source camera identification methods capture reference images with the person of interest's camera, extract the *noise residuals* from each reference image, and average the noise residuals to estimate the camera fingerprint. The noise residual of the questioned image is used to estimate the unknown source camera. Then, the two camera fingerprints are compared. With only a few exceptions, previous work in source camera identification relied on image datasets comprised solely of images from Digital Single-Lens Reflex (DSLR) cameras or the main rear cameras of mobile phones. Nowadays, most major smart phone brands offer a front-facing selfie camera and two or more rear cameras.

The Center for Statistics and Applications in Forensic Evidence (CSAFE) recently purchased ten iPhone® 11 Pros, ten iPhone® 12 Pros, ten iPhone® 14 Pros, ten Samsung™ Galaxy Note 10s, ten Samsung™ Galaxy S20s, and ten Samsung™ Galaxy S21s. These 60 phones have a front selfie camera, a telephoto camera, a wide-angle camera, and an ultra-wide-angle camera. CSAFE collected 100 flatfield and 100 natural scene images from each camera on each phone to build a large, authenticated image database. This database will be made available for free to the forensic science community. We used this image database to develop and explore source camera identification methods on multi-camera phones. This presentation will focus on our insights from this research.

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Camera Identification; Digital Evidence; Smart Phones

C30   Research and Development of the Next Generation Digital Forensic Image Transmission Tool

Kevin Fairbanks*, The MITRE Corporation, McLean, VA; Mark Guido, The MITRE Corporation, McLean, VA

Learning Overview: As the state of digital media continues to evolve, so must the tools used to process, transmit, and store that data in a forensically sound manner. This presentation will present the research, development, and initial testing of a methodology for transmitting digital forensic images (e.g., phone images, hard drive images) over resource-constrained networks with the goal of optimizing usage of the available bandwidth to transmit unique data first. The presenters will review several design trade-offs and initial testing results of the prototype.

Impact Statement: This presentation will engage the forensic community in an impactful discussion of the best method, including, but not limited to, protocol selection to transmit data from one geographic location to another by presenting an early state prototype. The overall aim is to develop a better open and freely available tool that takes advantage of current technology and changes the way forensic images are transported over networks going forward.

The MITRE Image Transmission Tool (MITT) is envisioned as a novel way to send digital forensic images from one geographic location to another, particularly in resource-constrained environments. It combines techniques from Periodic Mobile Forensics (PMF) and block hashing in a client-server architecture with the aim of only sending data that is unique.[1-5]

Forensic imaging often requires accessing a device and reading its contents at the highest level of privilege that is feasible during an acquisition. In situations where it is possible to acquire a physical image of a device, the storage medium is read from beginning to end without the content being interpreted by any software or firmware on the target device. This enables the recovery of deleted file contents in many cases. In situations where it is only possible to acquire a logical image, the contents being read could be filtered through device firmware and/or Operating System (OS) software layers. This can lead to a situation where deleted contents may not be recoverable or only a partial image of a device is able to be obtained.

After imaging is completed, it may be necessary to transport the image to a different geographic location for processing either through physical or digital (i.e., network) means. Physically transporting an image can generate the most throughput as multiple petabytes of data can easily be shipped in a manner of days. The drawback to this method of transportation is the potential for a great amount of latency between when an image is created versus when it can be analyzed. Digitally transporting an image could significantly reduce the latency of receiving small images; however, transmitting massive amounts of data can actually require more time than physical transmission while saturating the bandwidth of a communications link.

PMF addresses the imaging and transmission problem by using an application of rapid differential forensic imaging to only collect and transmit information from a device that is unique. From a file layer of abstraction, this means that only unique files on a device would be collected while system files and media files previously seen on another devices (e.g., jpgs, pngs, etc.) would only be noted but not imaged or transmitted. This drastically decreases the collection time. Furthermore, cryptographic hashes can be employed to ensure that a precise image is reconstructed on the receiving side of a PMF transmission both on a per file as well as per device image basis. The PMF approach can also be extended to work on a per device or file system block level. Further research has been conducted into storage of the forensic images as sparse files to save space on a forensic server.

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Digital Evidence; Transfer; Rapid

C31   Poses and Grimaces: Challenges for Automated Face Identification Algorithms?

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Learning Overview: After attending this presentation, attendees will have learned the challenges of state-of-the-art face identification algorithms. Attendees will be acquainted with performance results obtained on images with different head poses and facial expressions.

Impact Statement: This presentation will impact the forensic science community by demonstrating the effects of two behavioral factors (head pose and facial expression) on the performance of automated face identification systems.

Forensic image identification is based on the assumption that images can convey information about a person's identifying characteristics. While any aspect of physical appearance (motion, body build, stature, clothing) can be processed, facial appearance is the most common identifying feature. Nowadays, image identification tasks are gradually being automated and are therefore the subject of many machine learning algorithms, with Convolutional Neural Networks (CNNs) being the leading strategy. From the perspective of everyday forensic expertise, automation offers several advantages. It reduces the time required to process a large number of images, increases accuracy, and eliminates the human factor. Its quantitative nature provides the necessary scientific basis, testability, and a quantifiable probability of error—all critical requirements for a method to be considered applicable in forensics.

However, the performance of automated forensic image identification is susceptible to many factors. The constantly changing behavioral characteristics of the captured subjects (e.g., pose, expression, disguise) have been repeatedly cited as major challenges. In addition, the complicated and hidden nature of automated algorithms creates the black-box problem that prevents a complete understanding of the algorithm and its outcomes unless various real-world conditions are thoroughly tested.

This study tests two state-of-the-art face identification algorithms, ArcFace and SphereFace, and examines two factors known to complicate face processing, facial expressions and head pose.[1,2]

The tested sample consisted of an array of 3D faces that varied in nine different prototype facial expressions, including a neutral face. The dataset was collected from 41 participants, 13 males and 28 females of European descent, aged between 19.96 and 50.89 years. Prior to image processing, 3D models were automatically converted to 2D color images (256x256px). For 3D faces with neutral expressions, a set of nine images/individual was created with viewpoints that varied by 5° from left to right (yaw) and from up to down (pitch). These images formed the probes. In addition, a second set of 3,610 images/individual was created with viewpoints varying in 5° increments from -45° to 45° in both head movements and with different facial expressions. These images formed the targets.

The probes were compared with each target in the pair-wise manner and a dissimilarity score was recorded. A total of 54,615,690 pairwise comparisons were computed (53,283,600 eliminations and 1,332,090 matches). The performance of the tested algorithms was evaluated using Receiver Operator Curves (ROCs). Area Under the Curve (AUC), specificity, sensitivity rates, and likelihood ratios were calculated. In addition, scores and ROCs were tested in relation to facial typicality/uniqueness, biological sex, facial expression, and head pose. To explore the effects of head rotation, angles were expressed as sine and cosine values and treated as covariates.

The results showed that minor deviations of the head from the standard position in probes had little effect on the performances. In some cases, a slight movement to the right or left even led to better results than when the probe image was in a frontal position. Dissimilarity scores were consistently higher for males than for females across all studied factors. For head poses, AUCs decreased as targets deviated from the frontal head position. In general, right-to-left movements were less influential than up and down, as was downward pitch compared with upward movements. The lowest AUC of 0.631 was obtained for upward pitch at 45°.

For upward movements, performance rates in females were consistently poorer than in males. This discrepancy starts at 15°, where the ROCs for females and males reached 0.875 and 0.988, respectively, and continues as the performance rate worsens. Of the facial expressions tested, the best performance was associated with happiness and contempt, while disgust exhibited the lowest AUCs.

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Facial Identification; Automation; Pose

D1 Technology Advances in Crime Scene Investigation

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Learning Overview: The goal of this presentation is to demonstrate the importance and utility of 3D reconstruction in crime scene investigation, to assess the manner and cause of death, and all the data required in a judicial trial.

Impact Statement: A picture says more than a thousand words! It conveys a message more clearly and leaves less room for alternative interpretation than a verbal report, where the listener has to visualize in their own mind the narrative event and has to re-adjust constantly as the report progresses. Details might easily be omitted or wrongly imagined. This presentation will impact the forensic science community by imparting that graphic presentations, in particular 3D models, certainly produce a better common basis between the viewer and the interpreter.

In the death scene investigation, the forensic pathologist or medical examiner is the official primarily responsible for a complex act to reconstruct the dynamics of a crime scene. The main objectives of forensic medicine are freezing or fixation of the crime scene, findings, collection of traces, technical assessments, biological samples collection, and records of the crime scene investigation. Traditional methods of documentation of death scene include photography, sketches, notes, electrostatic lifting, or casting as well as field forms and video footage. An alternative, supplemental method of documenting transient evidence may be the three-dimensional (3D) laser scanner. The term "three-dimensional imaging" refers to techniques that can process accurate internal 3D data by obtaining volumetric pixels (or voxels) of the measured target. 3D imaging can generate high-resolution 3D digital images; laser scanners are available in both hand-held and stationary units.

Case Series: Four significant crime scene investigations were performed with the help of the laser scanner Leica® BLK360, from the Department of Forensic Pathology at the University of Catania from January 2019 to June 2020. The reconstruction is based on a comparison of the generated, real data-based 3D models and deals mainly with the geometric evaluation of the impact situation; with subsequent reconstruction in three dimensions through the specific software dedicated Autodesk® Recap™ Pro.

Case 1: A 35-year-old man, who was working on a "carrier car" cargo ship, was hit by a truck during a storm while executing an inspection of a flooded deck.

Case 2: A 34-year-old man suffered from anxiety and depression and was found on the ground in the inner court of his building about 7:00a.m. on February 20, 2019.

Case 3: An old homicide case that was the result of a robbery in a jewelry store.

Case 4: An 86-year-old man with a history of diabetes, leukemia, and both cardiac and respiratory disease, was found dead in his apartment. He suffered from depression caused by the death of his wife and his son.

In Case 1, the use of the Leica® BLK360 laser scanner enabled in only a few hours the creation of a complete diagram scale of the crime scene, allowing the rapid restitution of the ship to the judicial police officer. In Cases 2 and 4, the clarification of suicidal occurrence instead of a homicidal one was the focus of the investigation. By the study of the open spaces, internal courtyard, and balcony's apartment of the subject (Case 2), as well as the distances between the bloodstains and the corpse (Cases 2 and 4), and the close position of the gun with the corpse (Case 4), it was possible to prove that there were no assault lesions on the body, in consideration of the circumstantial data and the findings of the judicial survey. Therefore, the study of the crime scene is essential for the investigation. In Case 3, the Leica® BLK360 laser scanner enabled the piecing together of the dynamics of the crime and, despite the obvious limitations of the case, it was possible to prove the matching of the bullet trajectory and the escape route. The 3D videos reconstruction will be presented.

Digital Evidence; 3D Technology; Crime Scene Investigation

D2 A Method for the Evaluation of Upper Rear Vehicle Structures

Chris Clarke, PE, SAFE, Goleta, CA; Brian Herbst, PE*, SAFE, Goleta, CA

Learning Overview: This presentation explores three real-world rear impact crashes resulting in intrusion into the vehicle occupant compartment and serious or fatal injuries. In addition, several testing methodologies capable of generating comparable levels of structural deformation are presented.

Impact Statement: This presentation will impact the forensic science community by exploring passenger vehicle rear impact case studies and presenting methodologies for evaluating the crashworthiness of rear structures.

In rear impact collisions, larger striking vehicles may override the rear structures of smaller vehicles even if their bumpers align. Research shows Sports Utility Vehicles (SUVs) are twice as aggressive as passenger cars, while large vans and pickups are three times as aggressive.[1] These larger vehicles comprised less than one-quarter of vehicles produced in the 1970s and have grown to currently encompass over two-thirds.[2]

There are no regulations for structural performance or occupant protection in rear impacts. The government implemented a rear impact crash test for fuel system integrity in 1976. This test was conducted using a rigid barrier at 30mph with full overlap of the impacted vehicle.[3] The standard was upgraded in 2004 with an increased energy level, a deformable barrier, and only 70% overlap.[4] While the upgrade was a significant improvement, this regulatory test is limited in scope to the fuel system, which is located at or below the level of the bumper. Neither version of the standard addresses override, intrusion, or occupant protection. Some manufacturers have relied on internal rear impact testing requirements dating back decades that include offset barrier and vehicle-to-vehicle impacts to ensure occupant protection.[5] Despite this, a safety gap in the vehicle structures above the level of the bumper exists in many vehicle designs.

Three case studies have been evaluated, and each studied vehicle was found to have no significant energy-absorbing rear structures above the level of the bumper.

Case 1: A 2010s sedan was rear-ended by an SUV. The right rear occupant sustained fatal injuries as a result of intrusion. The structural members at the bumper level were overridden and relatively undeformed.

Case 2: A 2000s sedan was rear-ended by an SUV. The right front passenger sustained fatal injuries as a result of intrusion into the occupant space. This collision had a lesser degree of override.

Case 3: A 2000s coupe was rear-ended by an SUV. The right rear passenger sustained permanent injuries as a result of intrusion.

Quasi-static testing was utilized to evaluate the upper rear vehicle structures of the vehicles involved in Cases 1 and 2. A platen was pushed 46 inches into the upper structures of the test buck, generating a peak force of 21,000lb and a crush energy of 39,000ft-lbs. The damage pattern was similar to the Case 1 vehicle. Another test buck was modified with the addition of high-strength steel cladding and structural void-filling foam to reinforce the structures above the rear bumper. These modifications created an additional structural load path through the upper rear structures above the level of the bumper. When subjected to the same quasi-static crush test, the modified test buck absorbed 41,000ft-lb of energy with 22 inches of displacement. The stiffness of the modified test buck was more than double that of the production design. In addition, the intrusion was generally confined to the structures aft of the occupant compartment, resulting in minimal intrusion.

A full-scale rear impact test pair was conducted on a production vehicle and a second vehicle modified with the same reinforcements used in the quasi-static testing. Each vehicle was subjected to a 50mph 50% overlap rear impact with a sedan. The production test generated minimal override but resulted in 11 to 12 inches of intrusion at the rear seatback. The modified test vehicle sustained just 4 inches of intrusion measured at the rear seatback. In addition, the reduction of intrusion demonstrates the alternative design structure improves crash performance in rear impacts with compatible vehicles in addition to reducing susceptibility to override.

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Rear Impact; Accident; Crash

D3   Rear Impact Crashworthiness Defects Proven by Forensic Analysis Combined with Static and Dynamic Testing: The Failures of Seats, Head Restraints, and Belts

Mark C. Pozzi*, Sandia Forensic Sciences, Edgewood, NM

Learning Overview: The goal of this presentation is to demonstrate how and why a catastrophic child head injury was caused in a moderate rear impact with all vehicle occupants seated upright and properly restrained. The mechanics of the collision, the predictable failure of the front seats, head restraints, and belts, and the biomechanical trauma consequences for the front-seated adults and rear-seated children are demonstrated. Forensic analysis of physical and medical evidence, static seat testing, and dynamic sled testing are combined to prove how and why these injuries occurred. Safer alternative seat and belt designs from the same automaker are compared with Original Equipment Manufacturer (OEM) seats and belts, proving that the failures and injuries were readily preventable.

Impact Statement: This presentation will impact the forensic community by providing specific insight into not only how to properly investigate such vehicle crashes, but also the predictable failures of weak seats, unsafe head restraints, and slackened seat belts that are rendered useless. The biomechanics of catastrophic head injuries to children due to these predictable seat and belt failures are demonstrated. A series of dynamic sled tests show the significant improvements in occupant protection that were ignored by the automaker, despite the availability of their own safer alternative designs. The testing proved that the automaker did not meet their own internal corporate safety standards for seats and belts in a rear impact.

In moderate to severe rear impacts, predictable safety defects often cause restrained vehicle occupants to be significantly more vulnerable to catastrophic injury and death than in vastly more severe frontal impacts, all else being equal. This is due to no vehicle rear impact occupant safety regulations, and the "flawed and inadequate" Federal Motor Vehicle Safety Standard (FMVSS) 207 that allows weak seats that predictably fail, while also causing the front seat belts to slacken and fail. FMVSS 208 requires that vehicle occupants be protected from significant injury in frontal impacts of 30mph barrier equivalent. Since 1979, the New Car Assessment Program has tested vehicles at 35mph frontal barrier equivalent, and Insurance Institute for Highway Safety (IIHS) has tested vehicles in 40mph offset frontal crashes. The results of these far more severe crash tests are published, and automakers have responded by making significant improvements in frontal and side crashworthiness, to maintain sales. There has been no corresponding improvement in rear or rollover crashworthiness, despite the rear impact vector being the easiest in which to protect vehicle occupants, but only if the seat remains upright and maintains the occupant within their designated upright seating position.

This is a case study involving forensic accident reconstruction, biomechanical injury evaluation, and vehicle crashworthiness analysis, as well as static and dynamic testing to prove how and why a restrained child incurred catastrophic head injury in a readily survivable rear crash. A compact SUV stopped in traffic was struck in the rear by a medium truck, resulting in a moderate 21mph rear collision with a peak average crash pulse of 11-12G. Two adult females were seated upright and properly belted in the SUV front seats, and two children were seated upright and properly belted in child seats in the second-row seats. The 4-year-old left rear child incurred significant head injury when struck by the head of the driver, when she catapulted rearward because of predictable failures of the driver seat and vehicle-anchored belt. There was a corresponding laceration to the left occipital-parietal area of the driver's head. The 6-year-old right rear child incurred moderate head injury when struck by the detached head restraint, and/or probable contact by the head of the right front passenger as she catapulted rearward due to front seat, head restraint and belt failure.

The predictable failures of both front seats, slackening of belts, and detachment of one head restraint caused the front occupants to ramp rearward head-first into the rear occupant survival space, with no effective restraint by the slackened belts. Absent the front seat failure and concurrent belt slackening, there would have been no inter-occupant contact, let alone significant injury, to any vehicle occupants in this collision. The front seat belts were equipped with pretensioners, which did not activate in rear impact.

The SUV rolled to a stop with no occupant compartment intrusion. All injuries occurred solely due to loss of rear survival space caused by front seat and belt failure. The automaker claimed that the seats "could not have failed because its corporate seat performance test was met, therefore there could be no defects in the vehicle." The corporate sled test supposedly required there be no inter-occupant contact between a belted 50%-ile male and a 3-year-old child in the rear seat, in a "25mph Delta V, 15 G crash pulse," which is significantly more severe than the subject collision. The forensic investigation and testing proved that the vehicle clearly did not meet its own corporate crash test requirements. It was also proven that the automaker already produced far safer alternative design seats in similar vehicles of the same and older vintage.

Rear Impact; Failure Analysis; Head Trauma

D4   Mapping Vehicle Headlights to Identify the Threshold of Human Visual Performance in a Nighttime Visibility Study

Jorge Mendoza*, Litigation Animation, Inc, San Jose, CA

Learning Overview: After attending this presentation, attendees will understand how we mapped a vehicle headlight pattern in the field that defines the threshold of human visual performance. Attendees will also learn how a computer light simulation was performed to define diffuse lighting in a nighttime environment. Attendees will see how real-world light specifications (Illuminating Engineering Society [IES]) are used to illuminate a 3D model and how reflectance and transmittance material properties scatter and diffuse the light throughout the scene using a computer-rendering algorithm called radiosity.

Impact Statement: This presentation will impact the forensic science community by explaining the relationship between finite element analysis heat transfer algorithms and light simulation algorithms.

A nighttime visibility study was performed involving a pedestrian struck by a vehicle entering a parking spot near the back entrance to a big box store. The pedestrian was struck by the left front corner panel of a mid-size Sports Utility Vehicle (SUV) truck. Rainfall was light, and there was minimal lighting in the parking lot. The main source of light was from the turning vehicle's headlights. The collision reconstructionist provided the vehicle dynamics for the turning maneuver to the point of impact. An exemplar vehicle was obtained to perform headlight light measurements. An isolated environment was located to identify the headlight profile at 0.3-foot candles (the threshold at which human visual performance degrades).[1] The headlight profile was mapped at each of three elevations: zero feet, three feet, and six feet above grade. From these readings, a 0.3-foot candle distribution profile for the vehicle's headlights was created. At 0.3-foot candles, there is degradation in contrast sensitivity, color recognition, depth perception, visual acuity, and conspicuity. Acuity and contrast sensitivity are critical for visual recognition.[2]

The incident site was laser scanned and a model of the building, vehicle, and pedestrian was constructed in 3DS Max. VRayIES was also used to perform a photometric light simulation of the environment. Photometric lights use Illuminating Engineering Society (IES) files that contain the lights distribution profile. The IES file contains complete specifications of real-world light bulbs. VRayIES can accurately replicate physical lighting conditions that can be calibrated to real-world light measurements. The real-world materials' reflectance properties were measured with a light meter and applied to the corresponding materials contained within the models. The IES light specifications for each light in the scene were documented and applied to the corresponding lights. The VRayIES light simulation was then examined to evaluate the overall light levels from the driver's position.[1]

The 0.3-foot candle headlight distribution profile of the truck was combined with the photometric simulation within 3DS Max to produce an animation/simulation exhibit that showed the movement of the pedestrian from the sidewalk to the point of impact. The animation exhibits demonstrated what was available to be seen by the driver pulling into the parking stall, and that the pedestrian was struck outside the 0.3-foot candle headlight distribution profile. The results of the nighttime visibility analysis show that the pedestrian was struck in an area where the driver's visual performance was degraded.

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3D Technology; Simulation; Visualization

D5   An Experimental Analysis of How Non-Nominal Sitting and Pyrotechnic Seat Belt Pretensioners Interact

Adam Goodworth*, Westmont College, Santa Barbara, CA

Learning Overview: After attending this presentation, attendees will be informed about non-standard sitting and how this can impact forensic analysis and injury. Attendees will learn about the standard pyrotechnic pretensioner that is deployed in many crashes (physical pretensioners will be shared with attendees). Attendees will appreciate how 3D motion capture can be used to track kinematics in humans. Finally, attendees will understand how a pretensioner physically impacts a forward-leaning occupant and how body size and belt fit may play a role.

Impact Statement: A better understanding of the interaction between an occupant and their seat belt is important for understanding injury mechanisms in many real-world crashes. The pretensioner is ubiquitous, but to my knowledge, no research study has yet described the kinematic responses following deployment in live subjects. The pretensioner has the potential to physically move an occupant and change their posture early-on in a crash event, which could have a direct effect on the type of injury that is sustained. Moreover, the forensic science community will benefit from understanding how body size and belt fit impact the response to a pretensioner deployment.

When analyzing an occupant's injury from a vehicle crash, the starting posture and interaction with a seat belt is often an important factor in the analysis. Occupants (especially passengers) may adopt non-standard sitting postures. Recent research indicates non-standard sitting may be very common and associated with a heighted injury risk.[1-5] Therefore, the study experimentally investigated the interaction of one common non-standard sitting posture (forward leaning) with a seat belt pretensioner deployment. Seat belt pretensioners are installed on nearly all vehicles. When deployed, they pull out about 8–15cm of slack from the belt very quickly. Pretensioners are typically deployed at the same time as airbags, and many vehicles also deploy pretensioners in rear crashes independent of airbags. While previous studies tested pretensioners on cadavers and dummies, it is observed that pyrotechnic deployment on live subjects has not been published. This presentation will first describe the inner working of pretensioners, outline non-standard sitting, and summarize experiments where the hypothesis was tested that a dual pretensioner could reposition forward-leaning subjects and characterized the influence of body size and initial belt fit.

Nine young and active subjects were tested because a pyrotechnic pretensioner provides a large force to the pelvis and torso. Subjects ranged from 50-110kg. Six were male. The D-ring location was adjusted for each subject to normalize the belt geometry across subjects. Each subject donned the belt in their typical manner. The subjects then leaned forward 26cm from C7 to the seat. At a semi-unexpected time, the outboard anchor pretensioner and retractor pretensioner were deployed. The subjects' kinematics were quantified through reflective markers on 12 bony landmarks. 3D kinematics were reconstructed with a nine-camera motion capture system. To explore the effect of initial belt tightness, a subset of four subjects were also tested with the belt pulled tight across the pelvis with removal of as much slack as possible prior to the deployment. The test order (typical vs. tight) was random for these four subjects.

For all nine subjects during the normal belt fit condition, there was a stereotypical pattern initially following deployment. During the first 50ms, the trunk (measured at C7) moved backward at a velocity that was dependent on body size (i.e., larger subjects moved significantly slower than smaller subjects). The subjects moved backward about 7cm (range of 4.6-8.1cm) at about 160ms. During this period, trunk rotation in the transverse plane occurred from asymmetrical loading of the belt onto the trunk. Specifically, the right shoulder of the passenger (nearest to the D-ring) was initially pulled back 4cm farther than the left shoulder. After the initial pull-back motion (about 160ms), all subjects slightly rebounded. After this 160ms, there was high inter-subject variance in responses. In the subset of four subjects where belt tightness was explored, the tight belt was associated with less inter-subject variance (more stereotypical responses) during the initial trunk movement (tight=8.7% coefficient of variation vs. typical=26%). Due to the small sample size, no metrics were significantly different between the tight and typical belt tests, although all four subjects had about 20% lower peak chest velocity (measured at the sternum) during the tight belt test.

The results show that the average forward-leaning person was pulled back about 7cm with the dual pretensioner deployment in the current study. The stereotypical pattern during the first ~160ms provides investigators new information about how current pretensioners influence head and trunk motion when occupants are leaning forward. Moreover, these results may help in validating crash simulations where pretensioners are included and may encourage other researchers to test pretensioners with live humans.

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Motor Vehicle; Engineering; 3D Technology

D6 **Photogrammetry Software Used to Survey Site, Map Tire Friction Marks, and Recreate the Driver's View in Support of Alternative Collision Scenarios**

Kurt D. Weiss*, Case Study Collision Science, LLC, Santa Barbara, CA; Jorge Mendoza, Litigation Animation, San Jose, CA

Learning Overview: The goal of this presentation is to share with the forensic science community how the photogrammetric analysis of collision scene photographs was used to correctly determine vehicle impact positions, assist in the creation in exhibit animation, and support alternative driver actions that were argued could have mitigated injuries sustained in a high-speed tractor-trailer versus a Sports Utility Vehicle (SUV) collision.

Impact Statement: This presentation will impact the forensic science community by stimulating thought while presenting an approach to collision analysis when tire friction marks are not fully documented on scene by law enforcement investigators when performing a comprehensive traffic collision reconstruction.

The driver of an SUV was stopped at the limit line of a T-intersection, awaiting traffic to clear before commencing a left turn onto a rural highway. Approaching from the left was a fully loaded tractor-trailer weighing nearly 78,000 pounds. Believing there was sufficient time to clear approaching traffic, the SUV driver accelerated across the near-side lane.

The tractor driver observed the stopped SUV. Anticipating its forward acceleration, he tapped the brakes to deactivate the cruise-control that was set to 57.5mph. When the SUV suddenly accelerated forward, the tractor driver applied the brakes, but also steered to the left. The SUV was struck when it was nearly straddling the highway center line.

The SUV was salvaged before the Event Data Recorder (EDR) could be imaged; however, the tractor's Engine Control Module (ECM) was interrogated. The ECM Hard Brake data was used to determine speed and position of the tractor up to impact. Tractor tire marks confirmed leftward steering before impact, however, the SUV's post-impact tire marks, while visible in several photographs, were not fully documented by law enforcement.

Three photogrammetric software packages and techniques were used to determine the SUVs movement and position from impact to rest. The collision site was documented with a drone, and Pix4D photogrammetry software was used to create an optical scan. PF Tracker software was used to track the camera movement of a video camera mounted at the eye height of the tractor driver.[1] The collision scene photos were analyzed using the survey in 3D Studio Max to reconstruct the collision scene camera's focal length, position, and orientation. Next, the reconstructed cameras and collision scene photos overlayed on the survey were used to locate the tire friction marks on the survey and rest positions of both vehicles.[2] 3D Studio Max compiled the results, and with ECM data, an animation of the collision event from the tractor-trailer driver's perspective pre-impact to rest was also created. This included aerial views of the incident.

The collision reconstruction accurately determined the collision pair's time-position history to impact. Four other collision scenarios based on the tractor driver's alternative actions were presented: (1) holding steering wheel straight, (2) steering to the right, (3) applying brakes sooner, and (4) reducing travel speed to 55mph, resulting in a favorable settlement.

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ECM; Skid Marks; Photogrammetry

D7   Recreational Vehicle Crashworthiness: Enhanced Injury Caused by Unsafe Vehicle Interior Structures Combined with Predictable Seat and Belt Failures

Mark C. Pozzi*, Sandia Forensic Sciences, Edgewood, NM

Learning Overview: The goal of this presentation is to introduce a forensic field investigation that has been conducted to identify the cause and effect of common, and dangerous Recreational Vehicle (RV) designs as well as static and dynamic testing that discovered the failure mode of seats and belts, as well as the predictable extreme hazards of unpadded interior structures. Safer alternative seat, head restraint, and belt restraint designs were discovered that would have prevented hazardous occupant contact with the unpadded force-concentrating structures. Dangerous force-concentrating structures should be reduced in lethality and should not be located where occupant contact will likely occur in otherwise survivable crashes.

Impact Statement: This presentation will impact the forensic science community by showing that these severe hazards commonly exist in RVs, why they exist, how they create enhanced injury, and how those injuries can be prevented using lightweight, economically and technologically feasible means to protect vehicle occupants. Vehicle crash investigators and pathologists will learn how to identify recreational vehicle seat and belt failure, vehicle interior occupant contact witness marks, and associated forensic evidence that will allow more accurate assessment of occupant injuries in such circumstances.

Seat and belt failure in rear impact has been known to cause severe injury and death to both front and rear seat occupants. Unpadded rigid interior structures located within predictable occupant contact zones exacerbates these hazards. A case of predictable enhanced head injury was discovered in a recreational vehicle where a multi-position seat without head restraint and lap-only belt failure was combined with rigid, unpadded force-concentrating structures in the vehicle occupant compartment. The causation of these enhanced injuries was proven by a combination of accident reconstruction, human surrogate testing, and dynamic sled testing. Prevention of injury by lightweight, technologically and economically feasible means was also demonstrated.

It has been known since the 1940s that lap-only belts can cause fatal injuries, and lap-only belts have been banned in Australia and much of Europe since 1972. It has been known since 1958 that human rear impact tolerance of 83G without significant injury can be achieved if a crashworthy seat and head restraint is utilized. Head impact into narrow, force-concentrating structures can result in skull fracture, brain injury, spinal injury, and death at much lower load levels than if those same structures were adequately padded, contoured, or otherwise modified to reduce their lethality. To reduce or eliminate such injuries, Federal Motor Vehicle Safety Standard (FMVSS) 201, Occupant Protection from Interior Impact, has been part of the Code of Federal Regulations related to vehicle crashworthiness since 1967, with updates in the 1990s. It has required certain interior structures within the range of occupant contact zones to be effectively padded, flattened, rounded, and otherwise made less lethal when contacted by vehicle occupants, whether belted or unbelted. Therefore, dash panels and other vehicle interior trim is padded, and this is why narrow, projecting controls and other structures have been eliminated in the forward areas of passenger vehicles for decades. These requirements are not applicable to the rear occupant seating areas of RVs, however, due to loopholes in very lax safety regulations.

Several contradictions to the well-known foregoing occupant protection principles have been discovered in late-model recreational vehicles, in which unpadded, force-concentrating structures have been placed into the predictable path of vehicle occupant heads and upper torsos that are allowed to catapult rearward or sideways because of obvious, predictable seat and belt design deficiencies and failures. RV safety regulations permit lap-only belts and do not require head restraints for anything other than the front outboard seats. They also allow side-facing seating while the vehicle is in operation. These dangerous conditions have resulted in enhanced catastrophic injuries to vehicle occupants that would otherwise not have occurred if the seat had been in a forward-facing position, was crashworthy enough to remain reasonably upright, was equipped with an effective head restraint, and had an effective belt restraint system. Unless the seat remains upright, the belts and head restraints are not effective at protecting the occupant. The absence of energy-absorbing padding and the multitude of rigid, force-concentrating structures in the rear of the recreational vehicle interior exposes occupants to these extreme hazards even while wearing the ineffective lap-only belts while seated in low-back seats.

The reason that these dangerous conditions are occurring in late-model recreational vehicles is that there are no effective occupant protection standards for such vehicles, especially because there are no dynamic seat or belt performance standards for rear impact, and there are no requirements for equivalent padded structures in the rear of vehicle interiors like those found in the front seating areas per FMVSS 201.

Rear Impact; Head Trauma; Failure Analysis

D8   Visibility Study Uses Camera Tracking Software to Analyze Dashcam Video in a Bicycle Collision

Jorge Mendoza*, Litigation Animation, Inc, San Jose, CA

Learning Overview: After attending this presentation, attendees will know how to identify a High Dynamic Range (HDR) environment and the steps necessary to perform a visibility analysis. Attendees will also learn how to use match-moving software to track dashcam video and reconstruct the vehicle's   movement.

Impact Statement: This presentation will impact the forensic science community by providing a better understanding of how illumination, reflectance, and luminance are related and their impact on a visibility study.

A cyclist was traveling downhill on a southbound, two-lane road in the bike lane. Tall trees casting shadows across the roadway camouflaged the rider. The downhill grade was 7%, and the cyclist's speed was between 25–30mph. At the bottom of the hill, a northbound vehicle approached the left-turn pocket in direct sunlight. The illumination in the bike lane averaged 152 lux, whereas the illumination in the turn pocket averaged 10,500 lux, producing a medium HDR.[1] As the cyclist approached the bottom of the hill, two vehicles passed in the number one lane. At the same time, the northbound vehicle entered the left-turn pocket, slowed to allow the two vehicles to pass, and without stopping, turned left in front of the cyclist. The cyclist impacted the right side of the vehicle and was critically injured.

The assignment included conducting a line-of-sight visibility analysis, focused on what was available to be seen by the northbound driver and the cyclist leading up to the collision.

The scene was laser scanned and 3D models of the site, vehicles, bicycle, and cyclist were constructed using 3D-Studio-Max. The physical evidence examined included the vehicle, bicycle, dashcam video, clothing, and helmet worn by the cyclist. Reflections of the dash on the windshield were also investigated.[2]

Using the laser scan model in 3D-Studio-Max, the sun position (altitude of $19.6°$ and azimuth of $255.9°$) at the time of the collision was simulated. Next, a scaled 3D model of the vehicle and driver was positioned in the left-turn pocket (sun visor down) to demonstrate that the driver's eye height and position were not in direct sunlight to be affected by sun glare.

The incident vehicle was equipped with a dashcam, which captured the cyclist seconds from impact. The dash cam was mounted under the rearview mirror in direct sunlight and produced an under-exposed video in the area of the approaching cyclist. An exemplar vehicle and dashcam were obtained for analysis and testing at the collision site under similar lighting conditions. Test results showed that the dashcam video of the collision was not representative of the lighting conditions available to the driver.

The dashcam video was also processed using Camera Tracking software to generate the necessary scene and motion data to reconstruct the collision from the perspective of both the driver and cyclist.[3] A focal length of 50mm was used on the real-world cameras to match the human central angle of view between 40–60.[4]

An examination of the scene revealed that the direct sunlight falling on the left-turning vehicle and the shadows covering the bike lane created a medium HDR condition, making it difficult to see the approaching cyclist from the turn pocket. The effects of different colored materials, reflective clothing, and a blinking light on the bike were also evaluated. Green reflective clothing and a bike light increased the cyclist's conspicuity. Illumination light measurements were made in critical areas of the scene. The luminance of the reflected clothing was also measured.

In conclusion, had the driver stopped and carefully examined the environment allowing the eyes to adapt to the low light condition, the cyclist's movement through the shadows would have been available to be seen.

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3D Technology; Accident; Multimedia

D9   Slip and Fall: Methods of Simulation and Validation of a Formula

Darren Franck*, Advanced Engineering Associates, Inc., Charleston, WV; Harold Franck*, Advanced Engineering Associates, Inc., St. Petersburg, FL; Vincent D. Franck, WVU Tech, South Charleston, WV; Alexander Franck, WVU Tech, South Charleston, WV

Learning Overview: Attendees of this presentation will gain an understanding about the underlying physics during a fall.

Impact Statement: This presentation will impact the forensic community by presenting more accurate depictions of slip-and-fall accidents.

This case study involves a dummy used to model a real slip-and-fall accident. Using a grid background, its trajectory can be mapped as it falls to the ground. The data can be used to determine its velocity upon impact. This data will then be compared to a theoretical equation that calculates a person's impact velocity based on their height.

The current understanding of predicting impact velocity does not accurately simulate real-world conditions, so part of this investigation entails an improvement to this understanding via the aforementioned simulation. The current methods assume that someone falls directly downward once the fall begins, a situation that can easily be modeled by equating potential and kinetic energy. This situation is not an accurate depiction as it neglects the fact that people experience rotational movement upon slipping. Since a person's fall is not purely rotational nor translational, it is believed that the velocities will be somewhere between the values calculated based on pure rotation and those from pure translation. The investigation will also account for the buckling of the knees, which expands on the model of a slender rod.

An equation has been derived based on these fundamentals, which will be proven or disproven based on the tests with the dummy. The dummy height of 5 feet 3 inches will be used to predict its impact velocity using the equation. It will impact a solid surface with no cushioning, so the dummy can only decelerate upon the compression of the skin. During the tests, the head of the dummy will be instrumented with load cells and accelerometers that will measure the g-forces acting on the dummy at the point of impact. A high-speed camera will be used to describe its trajectory and measure its impact velocity. The testing will involve multiple types of falls, which will yield varying degrees of translational and rotational movement. This procedure can also be used to model the forces that injure other parts of the body, such as the elbow, knee, and shoulder.

Fall From Height; Blunt Force; Accident

D10 **Comparing Statistical/Machine Learning Approaches to Identifying Authorship**

Carole E. Chaski, Institute for Linguistic Evidence, Georgetown, DE; Francesco Gabriele Strafforello*, Bethesda, MD

WITHDRAWN

D11   Counterexamples for the Use of Spectrogram Comparison as a Method of Forensic Speaker Recognition by Law Enforcement and in Courts as Evidence

Jeffrey Nathaniel Holmes*, Boles Holmes White LLC, Birmingham, AL

Learning Overview: The goal of this presentation is to urge caution in using spectrogram comparison as a method of speaker identification and to provide counterexamples that show visually similar or identical spectrograms produced from different speakers.

Impact Statement: This presentation will impact the forensic science community by urging caution in using spectrogram comparison as a method of speaker identification.

The practicum of speaker recognition entails the specific identification of a person by voice. There are three methods: listening, viewing spectrograms, and automatic speaker recognition.[1]

The predominant method used in courts and in investigations is the spectrogram comparison method. The notion that a spectrogram can be used for speaker recognition began with research conducted at Bell Laboratories in the 1940s, which suggested that a spectrogram produces a unique "voiceprint" analogous to a fingerprint.[2] This method has been used in the American legal system since the 1960s and has been used in many cases around the world.[3]

The idea behind the spectrogram as a voiceprint notion is that the acoustic signals of speech, such as energy, time, and frequency, can be recorded graphically on a spectrogram and visually analyzed by humans who can then determine or exclude the identity of the speaker by comparing it to the spectrogram of a known speaker.

This method is sometimes excluded as evidence in cases due to accuracy concerns. This research argues that this is a correct result. However, upon information and belief, there are no United States jurisdictions outright barring its use. In other words, it is feasible that an individual qualified by the court as an expert may be able to proffer speaker identification evidence using the spectrogram method in all jurisdictions in the United States, which indeed occurs regularly. Additionally, the spectrogram method is used in countries outside the United States, including China, where it is recommended by the Ministry of Justice and the Ministry of Public Security Guidelines.[4] These spectrogram comparison services are performed by forensic consulting firms that tout the reliability of their methods, analysis, and procedures.

Spectrogram comparisons are also used by law enforcement and often performed by members of the agency, instead of by a third-party consulting firm. According to the most recent Organization of Scientific Area Committees (OSAC) Speaker Recognition Committee Current Practices in Forensic Speaker Recognition, spectrographic analysis is used by the United States government in its investigation of crimes.[5]

Within the legal jurisdictions in the United States, there are no uniform standards on the admissibility of speaker identification evidence. Similarly, there are no standards or best practices worldwide. Moreover, much of the practices of law enforcement internationally is classified, which presents interesting and legally complicated issues on the fundamental rights of self defense versus the competing interest of the government's duty to enforce the law and public policy interest in preserving safety. This issue is beyond the scope of this research but incumbent on lawmakers to resolve.

This research identifies counterexamples where different speakers produce visually similar or identical spectrograms. The methods used are as follows. First, select a large audio dataset with thousands of speakers and identify instances in which different speakers spoke identical sequences of words. This research used sequences of identical words that are of equivalent audio length to the segments that are compared by forensic examiners, in accordance with their methods. Second, generate spectrograms of the identical sequences. Third, extract features from the spectrograms using a Convolutional Neural Network (CNN). Fourth, reduce the dimensions of the CNN's output vector using linear discriminant analysis. Finally, with this new low-dimensional data, split the data into classes of the same sequence of words by the same speaker and the same sequence of words by a different speaker and using Probabilistic Linear Discriminant Analysis (PLDA), train the PLDA to maximize the probability of identifying when the same person says the same sequence of words. From there, identify counterexamples by showing instances where the model still fails.

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Spectrogram; Evidence; Machine Learning

D12   Using Statistical Analysis to Assist with Writer Identification

Mike Wakshull*, Q9 Consulting, Inc., Temecula, CA

Learning Overview: After attending this presentation, attendees will have learned a new method of handwriting identification using statistical process control for decisions of writership.

Impact Statement: This presentation will impact the forensic science community by introducing a new method of handwriting identification that advances the scientific foundations of questioned document examination.

Identification of handwriting relies on the document examiner's ability to discover the variability of the subject's known writing. Although qualitative assessment is prone to judgment error by the document examiner, variability in forensic document examination is often presented in qualitative rather than quantitative terms. Qualitative analysis is typically neither repeatable nor reproducible. A quantitative approach to writer identification can and should be used to reduce bias and errors in document examiners' opinions.

This presentation applies aspects of statistical process control to determine whether a writer of a known document is a potential author of a questioned document, based on such variability. Further, this presentation presents the results of validation testing using ground truth handwriting data where the writership is known and the writers were not copying but producing spontaneous handwriting.[1] Using this dataset, the method was applied blindly, and the statistical control decisions for identifying writers was used to calculate error rate of the method.[2]

Literature shows that variability of the proportions of height and width of a person's written letters and words remains stable within common cause variability across writing sessions.[3,4] The ratio of height and width and slant angles are established ideas in forensic handwriting identification.[5,6] In this method, the ratio of the height of lower case letters extending into the upper zone and height of lower case letters remaining in the middle zone is calculated. The ratios of the length of lower case descenders to the height of middle zone letters are calculated. For each calculated ratio, a run chart is created for the ratios of the known writing. The same is performed for the slant angle of a given letter.

Tests are made for comparison of letters in the same order. As an example, the words [may] and [yam] contain the same letters. Studies have demonstrated that the structure of a letter depends on the order relationship with the letter with which it is being compared. [7,8]

The standard deviation of the ratios or angles are computed to one, two, and three sigma. These measurements are plotted to create a control chart. The measurement of the angle or ratio of the questioned writing is plotted on the control chart to determine how the questioned writing compares to the known writing with respect to the variability of the known writing. The number of standard deviations from the mean of the known writing is explored to determine writer identification and create a threshold for the decision.

If the ratio of the given letters in the questioned writing falls beyond 2.5 standard deviations from the mean, the questioned writing is evaluated as having been written by a person other than the known writer (i.e., the decision is different writers). If the ratio falls below the 2.5 standard deviation threshold, the questioned writing is evaluated as having been written by the writer of the known sample (i.e., the decision is same writers). The result is an indicator of authorship rather than a conclusive determination, in relation to the error rate.

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Metric Analysis; Forgery; Quality Control

D13 Linguistics, Computer Vision, and Handwriting Identification: HANDLER

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Learning Overview: After attending this presentation, attendees will understand how language structure and patterning affects handwriting identification, and how linguistics—the study of language structure and patterning—can serve as a foundational science for handwriting identification with tools coming from computer vision.

Impact Statement: This presentation will impact the forensic science community by showing a new method of handwriting identification grounded in linguistics and computer vision.

Like other pattern-matching techniques in forensic investigation, handwriting identification originated from the layman's ability, rather than originating from an underlying, established science. Due to this origin, handwriting identification has not benefited from the standard operating protocols of a normal science, and instead it has struggled to create and sustain a research agenda and to formulate protocols and standards that are empirically validated. In 1996, a meeting to develop a scientific foundation and research agenda was hosted by the National Institute of Justice (NIJ) at the National Institute of Standards and Technology (NIST), involving all federal laboratories as well as NIST researchers.[1] The Technical Working Group on Digital Evidence (TWGDOC), (then the **Scientific Working Group** for Forensic **Document Examination** [SWGDOC], then the Organization of Scientific Area Committees Questioned Documents [OSAC QD]) was born out of this meeting, but these projects have not developed methods that are grounded in established science. This presentation shows how handwriting identification can be grounded in linguistics and computer vision, and how methods can be developed and tested on ground-truth data of spontaneously written texts by known authors.[2-7]

Linguistics provides principles, while computer vision provides tools of measurement. There are several reasons for deploying linguistics as a foundational science for handwriting identification, including iconicity, anticipatory co-articulation, phonotactic frequency effects, morphological segments, and function word frequency effects. Linguistics is foundational to handwriting: handwriting systems are intimately associated with the languages they represent through iconicity. For example, the strokes in Korean hangul indicate tongue position (or the articulatory phonetics) for the sounds represented by the symbol. Even the Roman alphabet has some symbols that are iconic to articulatory phonetics, such as [i] for [+high +front - round] and [o] for [+round]. Anticipatory co-articulation, a feature of acoustic phonetics, appears at the graphemic level in Roman cursive handwriting, when letter shapes are modified in anticipation of the following letter. Each language has phonotactics, or allowed sound sequences, which are reflected in the frequency effects of letter sequences. Morphological segments affect handwriting when a handwriter copies, because the handwriter typically pauses at morphemic edges. Finally, almost all languages have function words (or function morphemes) that signify grammatical relations rather than entities or actions (e.g., prepositions, determiners, conjunctions, quantifiers). Function words are a closed set of words, and they are very frequent in any text.

Likewise, Computer Vision provides tools of measurements. Manual measurement has been available for decades from Sirchie®, but this is extremely time-consuming and subject to errors from human fatigue.[7] Some computational tools are proprietary, such as Adobe®, but others are open source, including JMIcroVision, ImageJ, and OpenCV.[8-10]

When measurements based in computer vision are combined with principles of linguistics, it is possible to create a new, theoretically grounded method of handwriting identification that is testable, explainable, statistical, and objective, known as ALIAS HANDLER. In particular, handwritten function words are extracted and quantified from known documents, with measurements, then statistically analyzed by discriminant function analysis, logistic regression and random forest classifiers. Results of applying ALIAS HANDLER on a ground truth dataset of spontaneously handwritten are presented, with error rates for the method reported.

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Linguistics; Computer Vision; Handwriting Identification

D14   Presenting Model Uncertainty to Decision Makers

Willem A. Schreuder*, Principia Mathematica, Inc., Evergreen, CO

Learning Overview: After attending this presentation, attendees will understand that model predictions presented as uncertainty distributions are difficult for decision makers to grasp. This can lead to incorrect decisions because uncertainty is misinterpreted.

Impact Statement: This presentation will impact the forensic science community by presenting lessons learned in terms of how model uncertainty can bolster a case or lead to a complete misinterpretation of the science.

All model predictions have a degree of uncertainty associated with it. In the case of simple models such as the Johnson and Ettinger Vapor Intrusion Model, it has been demonstrated that associating an uncertainty distribution to each input parameter can lead to a model that produces an output uncertainty distribution that closely matches the distribution of observed data.[1] In such an instance, the model uncertainty can be presented in a manner similar to how the variation in measurements can be presented as the error rate of a method.

However, when dealing with more complex models, it is much more difficult to explore the range of uncertainty in the calibration and predictions.[2] The uncertainty is often over-simplified such as a fixed percentage, regardless of the prediction. This is often based on, for example, the measured uncertainty in data used to calibrate the model.

Even when using formal uncertainty analysis such as Null Space Monte Carlo or an Integrated Ensamble Smoother, the range of uncertainty is limited to that permitted by the model, while structural contributions to uncertainty are silently ignored. This can lead to instances where updates to the model lead to non-overlapping uncertainty predictions that undermine the confidence in the model.

In some instances, the uncertainty in one prediction is inappropriately transferred to another prediction, such as when the uncertainty derived for a regional model is applied to a highly localized prediction.

In an adversarial process such as tort litigation, uncertainty can be attacked as a weakness. It is also difficult to convey to decision makers how to apply standards such as "more probable than not" or "preponderance of the evidence" to a result presented as a probability distribution.

The work presents three case studies involving models: a simple analog model, a complex fate and transport groundwater model used in toxic tort litigation, and a groundwater flow model used to evaluate water rights. For each model, we discuss how uncertainty was presented to and perceived by decision makers, and how uncertainty was abused in achieving desired outcomes

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Error Rate; Modeling; Court

D15   Graph Theoretic Techniques for Image Comparisons

Gautham Venkatasubramanian*, Iowa State University, Ames, IA; Alicia Laura Carriquiry, CSAFE, Ames, IA

Learning Overview: This presentation will demonstrate the application of maximum cliques, a standard concept from graph theory, in comparing images that arise in forensic science applications. This presentation will focus on comparing shoeprint images based on corner points present in the pattern of the shoe outsole, but the general technique is applicable to any point cloud or structure-based comparison: for example, fingerprint images can be compared by constructing a maximum clique to match the minutia points.

Impact Statement: This presentation will impact the forensic science community by demonstrating the potential of a novel shoeprint comparison technique that is easy to describe visually, is extensible to other image-based comparisons, and is computationally tractable. The goal is to complement examiners' workflow with automatic comparison methods using graphical and quantitative descriptions of the automated methods.

Shoeprints are commonly found at the scene of a crime and can sometimes help link a suspect to the scene. Recent methods to compare shoeprints use maximum cliques, a standard concept from graph theory, to align the images and calculate a similarity score.[1,2] To reduce the dimensionality of the problem, most proposed approaches rely on interest points (such as corners of shapes or centers of circles) obtained from the images, align the images to minimize comparison errors caused due to rotational and translational differences, and calculate a similarity score. The similarity score provides a quantitative assessment of the resemblance between a crime scene print and a reference print obtained from a shoe of interest.

In real life, however, crime scene images may be photographed with a camera at a variety of heights, while reference impressions are photographed with the camera at a known height. Other differences between the methods used to capture an image in the field and in the lab may result in a pair of images that differ in terms of location, rotation, and scale. Transformations that can eliminate differences in location and rotation are readily available.[3] However, easily implemented transformations that can also correct for differences in the scale at which images were obtained have not been developed.

This study explores how the maximum clique-based comparison method can be extended to handle differences in rotation, translation, and capture height differences between shoeprint images. A small dataset of shoeprint images was collected with photographs taken at different heights. Interest points, like corners, were marked on the shoeprints. These images were then aligned using maximum cliques. Finally, similarity scores for known matching and known non-matching pairs were calculated to demonstrate the viability of this new method. This study also examines the possible application of the proposed alignment approach when comparing fingerprints using examiner-identified minutiae.[4]

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Footwear Evidence; Fingerprint; Automation

D16   **Quantitative Matching of Forensic Evidence Fragments Using Fracture Surface Topography and Statistical Learning**

Ashraf F. Bastawros*, Iowa State University, Ames, IA; Ranjan Maitra, Iowa State University, Ames, IA; William Meeker, Iowa State University, Ames, IA; Lauren Claytor, Virginia Department of Forensic Science, Richmond, VA

Learning Overview: After attending this presentation, attendees will be informed about: (1) fracture mechanics foundation for comparative evidence match of fractured articles; (2) the identification of proper scales for observation and analysis of fragments; (3) 3D imaging and analysis of fracture fragments; and (4) the basis of statistical analysis to provide a statistical expression of the comparison and express the likelihood of a match between patterns in statistical terms that support the qualitative and quantitative judgment of the examiner.

Impact Statement: This presentation will impact the forensic science community by making attendees aware that: (1) the proposed technique has the potential to provide a new investigative machine-based analysis with quantified error probabilities that can be applied in performing physical matches for a variety of materials: (2) the analysis protocol is expected to enhance the ability of forensic scientists to capture, visualize, and analyze fracture patterns and to express the likelihood of a match between patterns in statistical terms that support the qualitative and quantitative judgment of the examiner; and (3) this study has the potential to provide a technique for examining continuous fracture pieces with relatively few gross macro features in metals and plastics, where visual physical matching may not be possible.

Fractured fragments with jagged and irregular surfaces, discovered at crime scenes, are identified as "being a match" using comparative microscopy and physical pattern analysis. The surface topography of said fragmented pieces, as measured by 3D microscopy, is utilized to establish a quantitative basis for declaring a match, complete with quantified probability and error rates. The comparison scale is configured to capture the transition of fracture surface topography from self-affine to non-self-affine (surface roughness that is independent of the observation window). At this transitional scale, fracture surfaces display distinctive roughness characteristics, determined by intrinsic material properties, microstructure, and exposure history to environmental and external forces.

In the case of the examined class of hardened alloys, which are common in cutlery and tool steel, the identified scale is approximately two times the grain diameter. This scale closely resembles the characteristic distance necessary for the initiation of cleavage fractures in semi-brittle and hardened metallic alloys. Consequently, the imaging scale required is approximately 20 times the grain diameter. For each pair of fractures, six overlapping images were recorded, with an overlap ratio of 50%. The acquisition of spectral representations for various wavelengths and critical features on the fracture surface was accomplished using the mathematical Fourier Transform. Subsequently, quantitative topological descriptions were devised for the image pairs by performing correlation comparisons on two spectral bands encompassing the transitional fracture scale. These frequency bands are bounded by frequencies corresponding to 2–4 and 4–8 grain diameters. Consequently, each set of fracture pairs under examination yields a total of 12 correlation values. A statistical learning tool was then formulated, employing multivariate statistical analysis methods to classify the fracture pairs based on this collection of 12 topological descriptors. This classification offers a foundation for establishing the uniqueness of forensic comparisons. The efficacy of the proposed statistical learning methodology was assessed using a robust training dataset and validated with a set of 38 distinct broken pairs, encompassing knives fractured in bending and stainless-steel rods with comparable grain sizes broken under either tension or bending. The versatility of this framework was also examined across various loading conditions by applying it to a set of nine twisted knives until failure. Remarkably, all broken pairs were accurately classified. This framework establishes the groundwork for forensic applications involving quantitative statistical comparisons across a wide spectrum of fractured materials, characterized by diverse textures and mechanical properties.[1,2]

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3D Technology; Pair Matching; Trace Analysis

D17   Innovation Fundamentals: The Prelude for Next Generation Advances in Forensic Sciences

David Pienkowski*, University of Kentucky, Lexington, KY; Jacklyn N. Ady, University of Kentucky/Brunswick Billiards Group, Georgetown, KY

Learning Overview: This presentation reviews the basics of innovation and sets the foundation for subsequent topics in this special section. After attending this presentation, attendees will gain a new appreciation for the people, activities, and outcomes attending the processes of innovation.

Impact Statement: This presentation will impact the forensic science community by assisting forensic professionals to better understand the innovation process and hopefully, in turn, facilitate the development and implementation of new forensic technology.

We relentlessly pursue novel devices, methods, or materials to address the current myriad of technological challenges and gain competitive advantage. Such novel advances result from innovation, an esteemed process, yet one seldom adequately understood. Innovation is a purposeful activity focused on creating value from nothing or disparate entities, solving problems, and doing new things.

A common myth regarding innovation is that "it's all about the light bulb moment" occurring in the mind of the innovator. Innovation is not simply thinking new things. Innovation is a three-stage process consisting of: (1) idea formulation (a.k.a. "the light bulb moment"); (2) development of the idea through repeated research and testing (including numerous and repetitive failures); followed necessarily by (3) application of the developed idea. Remove any of these three elements and innovation vanishes.

Innovators are a special breed who doggedly exercise their cognitive abilities, pursue advancements using a creative mindset mixed with science and technology, and are receptive to serendipity. Innovators acquire their talents chiefly by immersion amid others actively engaged in the art of innovating. For example, Edison, Ford, and Goodyear were friends who collaborated to solve problems in their respective fields through innovations based on established science and manufacturing processes.

Stage one of innovation (i.e., idea formulation) is often an individual activity; however, stages two and three (development and application) are group efforts that culminate in the concept successfully penetrating the commercial marketplace. Success of group innovation depends upon the environment in which the group operates. While each organization has their own recipe for nurturing group innovation processes, all share common elements that include leadership, structures, processes, technology, people, metrics, rewards, and recognition. Persistence of the group effort is essential because innovation stages two and three are time- and effort-consuming repetitive processes demanding perspiration following the stage one act of inspiration. Innovators must also be adaptable and accept change as required to navigate the ambiguities that invariably arise during the processes of creating something novel.

Innovative efforts are driven by many factors, but chiefly powered by passion and funding. Sources of funding for innovation vary and the objects of these efforts have responded in kind. Funding generally, but not always, reflects the category (incremental, extraordinary, or disruptive) of the resulting innovation. These innovation categories may best be communicated using examples such as Tide® laundry detergent, Otis' passenger elevator, and Alfred Nobel's explosive invention, respectively.

Numerous current innovations resulted from "fortuitous accidents." Such accidents led to transformative discoveries because of the unique attributes of the innovator and the mindset that persistently sought answers to questions such as: why did this occur, what can be learned, and how can this finding be exploited. Louis Pasteur said it best: *Chance favors only the prepared mind.*" Thomas Kuhn similarly comments in *Scientific Revolutions* that the ability to detect anomalies or unexpected results is often crucial to innovation, because this ability enables the innovator to search for explanations outside the paradigm in which he or she is currently working.

Our discipline has witnessed transformative innovations that changed the practice of forensic science. It often accepts innovations from an established discipline, for example DNA analysis entering forensic science through molecular biology and paternity testing.

The following talks illustrate innovations for solving traditional forensic problems, and this panel ends with suggestions for enabling forensic science to nurture and adopt innovations.

Engineering; Handwriting; Interdisciplinary

D18   What Researchers and Practitioners Should Know About the Forensic Innovation Ecosystem

Rebecca L. Shute*, RTI International, Research Triangle Park, NC

Learning Overview: The goal of this presentation is to provide more context on what innovation is, what an "innovation ecosystem" is, and what circumstances enable or hinder innovation.

Impact Statement: This presentation will impact the forensic science community by helping attendees to understand strategies to enable innovation in forensic science by being informed by lessons learned in successes and failures.

Innovation in forensic science advances new technologies and techniques to improve the scientific foundation of the field as well as the objective collection, analysis, and interpretation of evidence. Innovation is often described using the biological ecosystem concept, including resources, relationships, competition, and collaboration. The forensic innovation ecosystem comprises a network of multiple stakeholders, including researchers, funding agencies, development partners, legal and policy professionals, accrediting bodies, and criminal justice end users, working together to develop, validate, and implement forensic technology transition solutions. Successful innovation ecosystems have resources and members where individual efforts are encouraged and ideally coordinated at the community level, and the overall health of the ecosystem is demonstrated by the adoption of technology supporting changing forensic needs.

The forensic innovation ecosystem has successfully adopted several technologies offering significant or even transformative value to day-to-day workflows. For example, Terrestrial Light Detection And Ranging (LiDAR) Scanning (TLS), adopted from alternative metrological disciples, emphasizes circumstances that were right and ready for adoption of innovative technology (e.g., open communication with vendors). However, for most researchers, moving innovation from research to operation is often fraught with uncertainty and barriers. Realities in the current forensic ecosystem make the process of transition inefficient, as motivations vary between community members, and collaboration and communication channels are limited. Incorporating technologies and knowledge into practice requires strategic and committed community engagement.

This presentation will leverage case studies, such as TLS, to illustrate the concept of an innovation ecosystem and identify conditions that foster innovation. Conversely, this presentation will cover examples where conditions in the innovation ecosystem hindered meaningful engagement. These insights are informed by the efforts of the Forensic Technology Center of Excellence, a program of the National Institute of Justice (NIJ) run by RTI International, to assist NIJ-supported researchers through technology development and knowledge dissemination. Attendees will also learn about specific resources and organizations that aim to promote collaborative partnerships that enable innovation.

Innovation; Technology Transition; Collaboration

D19   The Use of STL Technology and 3D Processing in Forensics: New Tools and a Multidisciplinary Approach

Gennaro Baldino*, PhD, University of Messina, Messina, Italy; Filippo Cucinotta, Messina University Engineering Department, Messina, Italy; Cristina Mondello, University of Messina, Messina, Italy; Fabio Salmeri*, Messina University, Messina, Italy; Marcello Raffaele, Messina University Engineering Dept., Messina, Italy; Alessio Altadonna, University of Messina, Engineering Dept., Messina, Italy; Elvira Ventura Spagnolo, University of Messina, Messina, Italy; Alessio Asmundo, Messina University, Messina, Italy; Daniela Sapienza*, University of Messina, Messina, Italy; Vincenzo Cianci, Messina, Italy

Learning Overview: After attending this presentation, attendees will understand the contribution of new technologies, including 3D printing methods and Stereolithography (STL) data manipulation, to forensic sciences in the analysis of complex cases, where it is currently mandatory to provide for a multidisciplinary approach.

Impact Statement: This presentation will impact the forensic science community by highlighting a case series of criminalistic interest in which the use of new analytical and productive technologies linked to STL, with the creation of 3D models, have allowed an easier resolution with an explanation of the causes and dynamics of the crimes.

Forensic medicine looks with growing interest at the innovative and technological resources of biomedicine, aiming at prospects for the creation and use of products that could be used both in the field of investigation and in forensic diagnostics, temporally following the application of 3D drawings in other clinical-therapeutic medical sectors. Recently stereolithography and 3D printing, as well as other technologies that can be included within the vast panorama of so-called bioengineering applied to medicine, have aroused interest as auxiliary applications to forensic diagnostics.[1,2] The work, starting from a collaboration with the engineering department of the University of Messina, analyzes the possibilities connected to the manipulation of Digital Imaging and Communications in Medicine (DICOM) and STL data, transforming computed tomography files into stereolithographic ones, then in 3D models, with subsequent and consequential forensic applications.

In this regard, a case series, emerging from personal investigations, is presented analyzing the use of new technologies and the creation of 3D virtual reconstructions as a support for autopsy and histo-immunopathological traditional investigations. Visual representations of 3D data added value to the presentation and understanding of complex evidence, producing a physical model of evidence that was easier to understand and evaluate the case. Based on the typical forensic investigation (crime scene analysis and autopsy) and through a collaboration with the group of forensic engineers, as foreseen by a broader multidisciplinary methodological approach, the reconstruction of 3D models has made it easier for an audience of jurists to understand, becoming real, effective evidence in judicial processes.

In the future, it will be important to establish the accuracy of the 3D evidence that determines its validity, an essential prerequisite for all evidence admitted in court, especially in relation to a criminal proceeding, where the error rate must be known and quantifiable. Thus, the process would benefit and there would be more demand for manufacturing and 3D printing business.

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Stereolitography; Forensic Engineering; 3D Technology

D20   Rule 702: Origins and Implications for Engineers and Attorneys

Gail Groy*, Groy Law Firm, S.C., Fitchburg, WI; David Pienkowski, University of Kentucky, Lexington, KY

Learning Overview: This presentation reviews past and current standards for admissibility of expert testimony and provides information regarding new Federal Rule of Evidence 702 legislation. After attending this presentation, attendees will better understand the history of expert-qualifying standards and current rules for the preparation of analytical forensic engineering reports and testimony.

Impact Statement: This presentation will impact forensic engineers and litigation attorneys by: (1) increasing understanding of the historical basis of regulations affecting expert testimony, (2) bolstering the rigor of forensic engineering methodology, (3) enhancing the scientific merit of forensic engineering reports and courtroom testimony, and (4) complying with recent legislative amendments to Rule 702 for experts' testimony to qualify.

This presentation consists of two sections. The first recounts the historical legal evolution of legislative and caselaw standards used to qualify experts and the basis for current Rule 702 amendments. The second provides practical information assisting practicing forensic engineers to comply with recently implemented Rule 702 standards.

It may be said that the amendments to Rule 702 are intended to align expert witness endeavors more closely to those used by the scientific community. The scientific method is based upon formulation of hypotheses to explain observations, empirical tests evaluating these hypotheses, data collection and analyses, drawing conclusions and hypothesis acceptance, rejection or modification and reformulation. Standardization of empirical test and analytical methods are essential for validation and independent verification. Forensic applications of the scientific method require acceptance by non-scientists and, therefore, necessitate standards by which courts, justices, and attorneys can qualify the information based on a preponderance of evidence, of the scientific principles related to the facts of the case on point.

Criteria embodied in *Frye, Daubert,* and Rule 702 were introduced to assist the courts in systematizing the methodological standards used for technical forensic testimony.[1-3] Briefly, the "general acceptance" standard of *Frye*, introduced in 1923, *Frye V. United States* held that expert testimony could be admitted into court if the evidence was widely accepted in the scientific community. This standard was challenged and then superseded by the 1993 case, *Daubert v. Merrell Dow Pharmaceuticals*. *Daubert* replaced the *Frye* standard in federal court. The United States Supreme Court in *Daubert* provided that as a standard expert testimony could be accepted without the need for peer review, provided that the judge acts as the gatekeeper to ensure that the expert witness was qualified, scientifically reliable, relevant, and could assist the trier of fact to understand the evidence or to determine the facts of the case on point. Specifically, "The trial judge must ensure that any and all scientific testimony or evidence admitted is not only relevant, but reliable". Recognizing the variability extant within the federal courts for implementation of these rules and for clarification, Federal Rule of Evidence, Rule 702 proposed amendments were unanimously approved in May 2023 and go into effect, after the statutory period, on December 1, 2023.

Compliance with current Rule 702 regulations will be assisted by efforts which: (1) state the forensic investigative objectives; (2) list the evidence and information used; (3) note each hypothesis evaluated; (4) describe the test procedures used for the engineering analyses; (5) list the empirical data obtained; (6) show the qualified statistical methods used to accept or reject each hypothesis; (7) state the data-supported conclusion that satisfies the stated objective; and (8) relates to the facts scientifically in the stated objective, to the case on point.

References:
1. *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923).
2. *Daubert v. Merrell Dow Pharmaceuticals, Inc*, 509, U.S. 579, 589 (1993).
3. 28 USC App Fed R Evid Rule 702: Testimony by Experts.

Report Writing; Expert Testimony; *Frye*

D21   A Study on the Application of the Genetic Algorithm for Fall Analysis

Chan-Seong Park*, Division of Forensic Safety, Wonju-si, Kangwon-do, Republic of Korea

Learning Overview: After attending this presentation, attendees will understand the necessity of an optimization program for the analysis of falls from height.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of a key aspect of investigations of falls from height. Specifically, it augments the traditional means of investigation in a systematized format via an optimization program using the genetic algorithm.

In general, falls are analyzed to derive the initial conditions of the victim such as posture and velocity. The information of fall height, distance of the first impact and the resting ground position of the victim after the fall, and bodily injuries determined from an autopsy is considered for the reconstruction of a fall. A fall simulation is performed using the trial-and-error method until it meets this given information. However, there are difficulties in the trial-and-error method because the fall simulation programs such as PC-Crash and MADYMO using a multibody model do not adopt an optimization algorithm. Consequently, a large number of cases are simulated until they meet the given information.

This presentation introduces the application of an optimization algorithm in a fall simulation. A simple program was developed to study the possibility of applying an optimization algorithm. Because there are many variants in a fall simulation, the genetic algorithm, which does not result in an exact solution but rather provides an approximate solution, was applied. The first impact and the resting ground position, the resting posture of the victim, and the first impact point of the body were considered for optimization. In the developed optimization program, a multibody model that allows change of the weight and height was adopted and point cloud data acquired by a 3D laser scanner were imported to model the geometric conditions of the fall site. The optimized initial posture and velocity were automatically listed considering the ranking of deviation error based on the difference in the resting posture between the multibody model and the victim.

Two fall cases having autopsy reports and recorded Closed-Circuit Television (CCTV) files were analyzed using the developed optimization program in comparison with the results of PC-Crash. The overall falling process shown in the CCTV was analyzed using the developed optimization program in comparison with the PC-Crash 14.0 Multibody model. The moment when the victim impacted the ground was also modeled by a human model, Total Human Model for Safety (THUMS®), and simulated thereafter by the LS-Dyna program in comparison with the postures shown in the CCTV. The body injuries from the THUMS® simulation results were also compared with those identified in the autopsy

From this investigation, it was concluded that the overall reconstructed falling process using the developed optimization program satisfies the given information shown in the CCTV. In addition, the THUMS® human model accurately simulated the body injuries in comparison with the autopsy results.

Fall From Height; Optimization; Genetic Algorithm

D22   Comparing Event Data Recorder (EDR) Pre-Crash Data With UTC Time Codes in Cell Phone Records and Pre-Crash Data and UTC Data in Front Camera Modules (FCMs)

Billy S. Cox, Jr.*, Billy Cox Group, Navasota, TX

Learning Overview: The goal of this presentation is to share with the forensic science community how the analysis of EDR pre-crash data, combined with the Front Camera Module (FCM) pre-crash data and cell phone data can assist an investigator's evaluation of a driver's pre-crash behavior.

Impact Statement: This presentation will impact the forensic science community by using a real-world crash to illustrate how to examine EDR pre-crash data, in conjunction with FCM pre-crash data and cell phone data to demonstrate driver behavior in a comprehensive traffic collision reconstruction.

Speed at impact and velocity change are important collision parameters often cited in a comprehensive traffic collision reconstruction. Knowing vehicle speed at impact can help determine if traffic laws were violated. However, pre-crash dynamic time-series data commonly recorded by EDR, including but not limited to, percent braking, precent accelerator pedal, and steering wheel angle, also allows the investigator to interpret the vehicle operator's behavior several seconds before impact. Some late model vehicles also have an FCM that captures pre-crash, crash, and post-crash images, along with the typical vehicle parameters captured in the EDR and Universal Time Code (UTC) data.

Following a crash in Jasper County, TX, the FCM was removed from a 2022 Chevrolet® Equinox® SUV due to the extensive damage sustained. The FCM was imaged on a work bench using the Bosch® Crash Data Retrieval (CDR) 900, software version 23.0.2 and direct-to-module cable ID#856. The FCM was powered with a 12-volt power source. The FCM is capable of recording more than one event. This module had two event records. Event Record 1 was defined as an SIR event—Airbag Deployment Event, and Event Record 2 was defined as a "Pedestrian Warning."

Event Record 1 was related to the subject collision. Event Record 2 was not related to the subject collision. UTC is the basis for local times worldwide. UTC Date at Event was 5/9/2022 and the UTC Time at Event was 14:05:17 hours. The UTC time in Jasper County, TX, was UTC-5 at the time of the collision. The local time was calculated to be 09:05:17 hours.

Figures 1-3 are the images captured by the FCM of the Chevrolet® Equinox® SUV.[1]

Figure 1: FCM Image, "Pre-Event."

Figure 2: FCM Image, "At-Event."

Figure 3: FCM Image, "Post-Event."

Figure 4 is the FCM Image, "Pre-Event" enlarged to show the Ford® F350 pickup in clear view. This image was taken approximately 4.0 seconds prior to the impact.

Figure 4: FCM Image, "Pre-Event" with Ford® F350 pickup visible (446 feet from impact).

The cell phone record shows text messages at UTC 14.04.05, 14.04.22, 14.04.36, and 14.04.37. Thus, the last text data is 40 seconds prior to the event. Based on the characteristics of the recording characteristics, it appears that the event could be up to 30 seconds or more earlier than the FCM recorded event time of UTC 14.05.17. The last text recorded in the cell record, UTC 14.04.37 may also be reported slightly later than the moment when the actual text was sent.

Based on the crash reconstruction, it is obvious that the driver of the Equinox® was distracted because there was no evasive maneuver, including braking, before impact. The comparison of the FCM data with the cell phone records would seem to support that.

References:
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Crash; Reconstruction; Vehicle Speed

D23   Using EDR Pre-Crash Data to Evaluate Driver Behavior in Traffic Collisions

Kurt D. Weiss*, Case Study Collision Science, LLC, Santa Barbara, CA; Billy Cox*, Billy Cox Group, Navasota, TX; Robert Anderson*, Biomechanics Analysis, Tempe, AZ

Learning Overview: The goal of this presentation is to share with the forensic science community how the analysis of Event Data Recorder (EDR) data can assist an investigator's evaluation of a driver's pre-crash behavior.

Impact Statement: This presentation will impact the forensic science community by using real-world case studies to illustrate how to examine EDR pre-crash data and how it is applied to a comprehensive traffic collision reconstruction.

Speed at impact and velocity change are important collision parameters often cited in a comprehensive traffic collision reconstruction. Knowing vehicle speed at impact can help determine if traffic laws were violated. Likewise, since the magnitude of the collision force is related to the velocity change of a vehicle during a collision, then vehicle velocity change, or Delta V, may be used to define collision severity and to evaluate an occupant's risk of injury. However, pre-crash dynamic time-series data commonly recorded by EDR, including but not limited to percent braking, precent accelerator pedal, and steering wheel angle, also allows the investigator to interpret the vehicle operator's behavior several seconds before impact. This behavior demonstrated by the time of, or changes in pre-crash data, often confirmed using other sources of case-related information, allows the investigator to better understand the driver's pre-impact actions.

This presentation explores the additional benefits of pre-crash data using cases studies, including: (1) a pre-impact startle response in an attempt to avoid a collision; (2) expected versus aggressive braking force by comparing brake oil pressure data; (3) pre-impact steering data recorded during an inadvertent lane change, that later illustrates what is considered the driver's evasive steer back into the traffic lane; (4) examination of wheel speed sensor data in the case of sudden loss of control that resulted from wet road hydroplaning; (5) driver's awareness of the traffic signal interval illustrated by reasonable vehicle approach speed, then acceleration and hard braking indicating an attempt to beat the traffic light: (6) pedal error/unintended acceleration documented with surveillance camera video of a driver who described their vehicle accelerated while braking; (7) inattention of a driver who failed to apply the brakes before an impact with another vehicle that had been stopped for an extended period of time; and (8) reckless driving using acceleration data and corresponding vehicle speed in several high-speed crashes.

Pre-Crash; EDR; Driver Behavior

D24   Understanding the Limitations of EDR Data for Crash Reconstruction

Brendan Bourdage*, Owner, RPSAVA, Williamsburg, VA; John Smith, RPSA, Parker, CO; Peter Alexander, Raymond Smith & Associates, Aurora, CO

Learning Overview: The goal of this presentation is to review studies that assess the accuracy of Event Data Recorder (EDR) data from real-world incidents, identify causes for concern when using EDR data for collision reconstruction, and to suggest methods for increasing the usefulness of EDR data in collision reconstruction.

Impact Statement: This presentation will impact the forensic science community by illuminating problematic assumptions made by collision reconstruction experts with respect to EDR data and suggesting a path forward for validation of EDR information.

Title 49 Code of Federal Regulations (CFR) Part 563 specifies standards for EDR data recording, including requirements for accuracy.[1] The CFR states that vehicle speed must be accurate to within +/- 1kph, and instantaneous change in velocity and maximum change in velocity (lateral and longitudinal) must be accurate to within +/- 10%. However, this standard does not require O**riginal Equipment Manufacturer**s (OEMs) to disclose the precise variance in their EDR data for each vehicle, making it difficult for reconstruction experts to know exactly how accurate a given EDR is in a specific collision. Additionally, a 2022 study of rear impacts compared EDR Delta-V values to the National Highway Traffic Safety Administration's (NHTSA's) Crash Investigation Sampling System (CISS) database showed differences that ranged from -4.6% for full impacts to -10.1% for moderate impacts.[2] In a 2014 study of EDR accuracy for NHTSA Side Impact New Car Assessment Program (SINCAP) side impacts with a movable deformable barrier, researchers found an average lateral Delta-V error of 15.9%, with a maximum reported error of 37.6%.[3]

Several explanations for discrepancies between EDR data and test data have been cited in other research. For instance, in a literature review published in 2016, researchers pointed to wheel slippage during braking and wheel sideslip due to rotation of the vehicle around the vertical axis as sources of error.[4] Also, a 2013 study that reported a longitudinal Delta-V error of -6.6% for high severity full frontal crashes revealed that the values were "clipped" at a threshold of 50g's and that many manufacturers use an acceleration bias of 1g to prevent unnecessary airbag deployment, which can lead to underreporting of Delta-V.[5]

Conclusion: If EDR data accuracy could be ascertained under a variety of collision conditions, it could help collision reconstruction experts calculate important values like change in velocity and pre-crash speed. Current research does not provide the necessary validation. Until EDR data has been proven to be repeatable under various conditions, reconstruction experts should use caution when providing opinions based primarily on EDR data.

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2. Watson et al. (2022). Comparison of rear impact crash reconstructions to Event Data Recorders in the Crash Investigation Sampling System database. *SAE Technical Papers*, 2022-01-5069. DOI: 10.4271/2022-01-5069.
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5. Tsoi, A. et al. (2013). Variation of Event Data Recorders in high severity full-frontal crash tests. *SAE International Journal of Transportation Safety, 1*(1), 76-99. https://www.jstor.org/stable/10.2307/26169208.

EDR; Validation; Accident

D25   Vehicle Wheel Speed: Did Tire Wear Lead to a Sudden Loss of Control on a Wet Road?

Billy S. Cox, Jr.*, Billy Cox Group, Navasota, TX

Learning Overview: The goal of this presentation is to share with the forensic science community how the analysis of Event Data Recorder (EDR) pre-crash data, specifically the brake activity, steering input, yaw rate, and wheel speed can assist an investigator's evaluation of a driver's pre-crash behavior.

Impact Statement: This presentation will impact the forensic science community by using a real-world crash to illustrate how to examine specific vehicle performance characteristics contained in Chrysler® EDR pre-crash data, in conjunction with an inspection of the vehicle tires, to yield clues to driver behavior and vehicle dynamics in a comprehensive traffic collision reconstruction.

A Chrysler® vehicle was traveling eastbound on a wet two-lane rural road, approaching a curve. The collision occurred fully within the westbound lane. The owner of the vehicle purchased two new tires in the days preceding the crash. The tire store installed the new tires on the steering axle (front) and rotated the worn front tires to the drive axle (rear).

The investigation centered on the role that the worn tires played on the drive axle (rear) in the loss of control. The EDR data contained 5.0 seconds of pre-crash data, collected in 0.1-second intervals. The uniform tread depth on the left and right rear tires exceeded 3/32 inches. In addition to the typical data (speed, accelerator pedal %, brake on/off), Anti-lock Braking System (ABS) activity (anti-lock braking), steering input, yaw rate, and wheel speed for all wheel positions was recorded.

According to the EDR data, the Chrysler® vehicle was traveling 71mph about 5.0 seconds before the recording threshold was met. The vehicle slowed to a speed of 69mph at 2.2 seconds prior to the recording threshold being met. At this point, the vehicle "stability control" was "engaged." Steering input increased from -7 degrees to -162 degrees at 1.7 seconds prior to the recording threshold being met. There was hard braking at this point as the ABS activity began as the first brake application. The steering input, ABS activity, yaw rate, and wheel speed were analyzed up to the point of impact. The right front wheel speed of the Chrysler® vehicle slowed from 733rpm to 5rpm over the next 0.6 second period.

A review of the data showed that the right front tire drove through standing water, significantly reducing the tire speed. As the wheel speed went to very near zero, the counterclockwise rotation of the vehicle rapidly increased. Along with the steering input of the driver, the vehicle entered the oncoming westbound lane, whereupon a collision took place.

Crash; Reconstruction; Vehicle Speed

D26   The Detection of Blood Stains on Natural, Semi-Synthetic, and Synthetic Fabric Exposed to Extreme Environmental Conditions Using ATR/FTIR Spectroscopy

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Learning Overview: The goal of this presentation is to explain the detection of blood stains on different types of fabric (natural, semi-synthetic, synthetic) using Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) and to study the stability of blood evidence on fabric exposed to extremely hot weather

Impact Statement: This presentation will impact the forensic science community by shedding new light on the analysis of blood on different types of fabric (stimulating a relevant forensic scenario) where the effect of the substrate is thoroughly looked into in terms of its influence on the ATR/FTIR detected signal. Furthermore, the effect of extreme hot weather (temperature above 50° Celsius), humidity, and dust on the stability of blood evidence traces is also investigated, which can help gain a better understanding of the capabilities of ATR/FTIR and the limitations of having blood evidence on different types of fabric.

Detection of peripheral blood in forensic investigations is of paramount importance to successfully identify its origin in sexual and violent assault, murder, and trauma cases. Herein, the use of non-destructive and powerful ATR/FTIR spectroscopy for the detection of whole peripheral blood stains deposited on different white fabrics, namely cotton, wool, satin, linen, organza, polyester, modal, and chiffon is proposed. Both natural and synthetic fabrics contaminated with blood were investigated by obtaining the blood from different female donors and investigating the effect of substrate (fabric) types on the detection of the blood stain.

The analysis showed promising results in terms of probing the two main bands related to blood on all substrates: amide I and amide II positioned approximately at $1,640cm^{-1}$ and $1,533cm^{-1}$ in blood-stained fiber.[1] The type of fabric highly influenced the detection of blood; for example, detection on wool fabric was challenging, which could be due to its highly absorbent nature and thickness. The best detection results were obtained with cotton and modal, which were subsequently employed for the detection of blood stains after being exposed to extreme hot weather.

Kuwait is characterized by very hot weather during the summer, particularly during the months of June, July, and August, where temperatures can reach up to 55ºC.[2] Therefore, further investigation into the effect of hot summer weather on the stability of blood on cotton and modal was investigated. These samples were placed in a homemade glass box with holes to allow aeration of the sample and direct exposure to sunlight. Similarly, control experiments were carried out by the same donors on the same surfaces and were placed on a bench exposed to light and under air conditioning. Initial assessment of the samples using ATR/FTIR showed the common spectroscopic signature of the whole blood with the main two bands of blood on both substrates—amide I and II—positioned at $\sim16,40cm^{-1}$ and $\sim1,535cm^{-1}$, respectively. Thus, given the proven track record of ATR/FTIR in forensic analysis, it is a promising strategy to expand the application of ATR/FTIR for detection of peripheral whole blood on natural and synthetic fabric substrates.

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2.   Al-Kandari, N.M., Singh, Jaipaul, and Sangar, Vikrant Chandrakant (2016) Time-Dependent Effects of Temperature and Humidity on Quality of DNA in samples of Human Saliva, Blood, and Semen in Kuwait. *International Journal of Pharmaceutical Sciences and Research*, 7 (7). pp. 2852-2873.

Bloodstains; Age Assessment; Spectroscopy

D27 **Fluorescent Carbon Quantum Dots Produced From Natural Resources for Sweat Latent Fingerprint Recognition**

Amarnath Mishra*, Amity University Uttar Pradesh, Noida, India; Nisha Yadav, Amity University Uttar Pradesh, Noida, India; Vivek Mishra, Amity University Uttar Pradesh, Noida, India

WITHDRAWN

D28   **The Discrimination of Smokers and Non-Smokers Based on the Analysis of Fingermarks Using ATR/FTIR Spectroscopy and Chemometrics**

Mohamed Amin*, Kuwait University, Khaldyia, Al Asimah, Kuwait; Entesar Al-Hetlani, Kuwait University, Khaldyia, Al Asimah, Kuwait; Igor K. Lednev, University at Albany, SUNY, Albany, NY

WITHDRAWN

D29   The Influence of Taphonomic Changes on the Persistence of Serrations in Stab Wounds

Sarah V. Hainsworth*, University of Bath, Bath, England, United Kingdom; Sophie A. Stanley, Manchester Royal Infirmary, Manchester, England, United Kingdom; Guy N. Rutty, University of Leicester, Leicester, England, United Kingdom

Learning Overview: Following this presentation, attendees will understand the ways in which striations in wounds caused by bladed weapons persist following taphonomic changes and the optimum way of analyzing these types of marks.

Impact Statement: This presentation will impact the forensic science community by contributing to improved quantification and validation of tool marks from bladed implements on wounds in skin. The analysis will show how accumulated degree days of decomposition change the data and how this may impact on the validity of the conclusions from forensic evidence.

Stabbing with kitchen knives or other serrated blades is a common method of homicide. The serrations on the edge of the blade can leave tool marks or striations in the soft tissues. Striations can also be found on bones as a result of the weapon that was used to cause injury. In the National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, published in 2009, tool mark identification was one of the areas highlighted where forensic methodology needs improved quantification and validation.[1] Thus, the persistence and study of striations for weapon identification is an important area of study.

This study investigated the effect of different taphonomic alterations and the different imaging modalities on the recording of striations in porcine skin. Samples were studied either fresh or after decomposition (mummification and burning) or being waterlogged in either fresh or brine water. The samples that were exposed to water were either exposed to tap water from the main water supply in the Leicester Royal Infirmary or from brackish water obtained from the lock gate area at Boston Docks, Lincolnshire, United Kingdom. The brackish water was collected at half tide (ebbing). The wounds were made with four different knives: three with serrated edges and one with a plain edge. Wounds were made using a drop-tower method to stab the tissue. Samples were subsequently excised and imaged by photography, stereo-optical microscopy, and micro-computed tomography. Four independent observers (all medical students) were asked to analyze different images of the striations that were prepared in advance. The observers were independent (i.e., they did not know what the others' findings were) and were blinded to the knife used to create the wounds that had been imaged. They were asked to state whether an image showed striations or not.

Striations were recorded more frequently in wounds made with serrated blades, but were also observed with wounds made using a plain-edged blade. This is a confounding false-positive that means that the observation of striations does not simply allow the conclusion that the wound was made with a serrated blade, although striations were observed more frequently in stabs made with serrated blades.

Serrations were found to degrade following decomposition to the level seen in stabs made with a plain-edged blade. There was an inverse linear relationship between accumulated degree days of decomposition and the number of striations identified.

Dehydration by mummification and burning was found to make serrated blade wounds and plain-edged blade wounds indistinguishable. Striations were possibly rendered indistinguishable owing to tissue contraction and other artifacts as the tissue changed owing to exposure to the temperature.

Wounds exposed to tap water and wounds exposed to brackish water were found to differ in the preservation of the striations. The proportion of striations identified following exposure to tap water gradually fell to the level seen in wounds made with the plain-edged blade. The proportion of striations seen in fresh samples and those left in brackish water was similar up to 68 days. Thereafter, the proportion of striations fell to the levels of the non-serrated blade wounds.

Photography, stereo-optical microscopy, and micro-Computed Tomography (micro-CT) were all capable of imaging striations for all samples exposed to the different taphonomic conditions. Stereo-optical microscopy was found to be the optimum technique from which observers were most consistently able to distinguish the striations. This technique is also more readily available in a laboratory setting, is easy to use, and is a low-cost route to imaging the samples.

Reference:
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Stabbing; Striations; Taphonomy

D30 **Environmental Forensic Microscopy Considering Assemblages for World Trade Center Dust and Wildfire Smoke**

James Millette*, Millette Technical Consulting, Stone Mountain, GA

Learning Overview: The goal of this presentation is to present to the forensic community information regarding the use forensic microscopical analysis to determine particle assemblages in the source identification of particulate material.

Impact Statement: This presentation will impact the forensic science community by helping attendees gain an understanding of the considerations necessary when using microscopy-determined particle assemblages to the source of particulate populations. Examples of assemblage analysis will include 9/11 World Trade Center (WTC) dust and wildfire smoke contamination.

According to Crutcher, 2007, ssemblage analysis involves the grouping of objects or features in combination that establishes a fact or context not established by any individual feature or object.[1] This scientific grouping is typically referred to as an "assemblage" with the understanding that the objects or features are related in some specific manner. Forensic microscopy uses assemblage analysis to determine a particle assemblage signature that infers the origin of a population of particles.

In 2002, Region 2 of the United States Environmental Protection Agency (US EPA) was considering how best to ascertain which residences in lower Manhattan had been contaminated by "World Trade Center dust" resulting from the building collapse following the 9/11 terrorist attack. Several laboratories analyzed the dust using multiple microscopy techniques and found similar sets of particles. MVA Scientific Consultants reported the dust to contain approximately 35-40% mineral wool (also called Manmade Vitreous Fibers [MMVF]), 25-30% gypsum, 10-15% calcium/cement, 5-10% cellulose, including soot, 6% silica, < 1-3% asbestos, and < 1% other material, including lead particles.[2] One laboratory proposed an assemblage of ten particle types as a positive WTC dust signature: chrysotile asbestos, mineral wool, glass fibers, pulverized gypsum, vermiculite, soot, both metallic and alumino-silicate spherical particles, and lead particles. After reviewing the work of many laboratories, including that of the United States Geological Survey, the EPA concluded that no particle assemblage could be used as a signature for WTC dust contamination.

In 2018, the American Industrial Hygiene Association (AIHA) published a technical guide for wildfire impact assessments for occupational, environmental, health, and safety professionals.[3] It described the use of microscopy tests of particles found in buildings where assemblage analysis could be applied to determine if a building had been affected by wildfire smoke and also suggested that the assemblage could infer a particular wildfire as a source. The 2018 AIHA document said, "In the case of a wildfire, the assemblage consists of charred wood/plant indigenous to the area where the fire took place, fire retardant, carbon/soot coated quartz or mineral grains, burned or heat altered clay minerals from the soil, and pyrolyzed calcium oxalate or silica phytoliths from various plants and trees."

A review of the forensic microscopy techniques used in particle analysis for source determination involving WTC dust and wildfire smoke illustrates the difficulties in validating an assemblage signature.

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Microscopy; Trace Analysis; Wildfire

D31   Noxious Gas Inhalations—A Lethal Occupational Hazard While Cleaning Effluent Treatment Plants in India: A Forensic Analysis

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Learning Overview: After attending this presentation, attendees will have a better understanding of the workings of an Effluent Treatment Plant (ETP) or Sewage Treatment Plant (STP) in an industrialized or residential establishment and various reasons that lead to the release of noxious gases like hydrogen sulfide and methane. Inhalation of the noxious gases that emanate from an ETP may claim the lives of people who are engaged in cleaning ETPs manually, more so in the absence of protective gear. Invariably, people who belong to an extremely low strata of the society, or who are migrants from the neighboring state of New Delhi, the capital of India, in search of earning a livelihood, usually get engaged in this high-risk job.

Impact Statement: This presentation will impact the forensic science community by showing that ill-managed ETPs may produce noxious gases like hydrogen sulfide and methane, which may prove fatal. The forensic science community will enhance its knowledge by watching a video of a typical ETP setup and operation. A three-minute video will be shown to explain the workings of a typical ETP.

A typical industrial ETP has an inlet pipe that carries all the waste generated at the site of the commercial or residential setup that reach to the tank via an inlet pipe with help from a propelling electric motor. When the waste reaches the tank, an appropriate amount of chemicals like alum and lime are added for the treatment of waste material. After this, the waste is discharged in the main sewage drain via an outlet pipe. A regular cleaning of the inlet and the outlet pipes is essential to prevent the clogging of the effluent treatment system.

Presented here are two incidents, one that happened in a residential set up that claimed five lives and the other in an industrialized set-up that claimed two lives. In both the cases, inhalation of the hydrogen sulfide gas was the causative factor for the ensuing fatalities.

In the first incident, two workers entered the residential ETP to remove the sludge and fell unconscious. The other three workers who were waiting outside then entered the ETP to rescue their co-workers, and also fell unconscious. All five workers died on the spot.[1]

In the second incidence that happened in the industrialized set-up, when four workers entered the ETP plant to clean the sludge, they fell unconscious. Two workers died on the spot and the others were rushed to the hospital.[2]

During the examination of the ETP where the incidence occurred, two tanks were found completely filled with sludge. On further inspection, it was found that there was inadequate ventilation of the ETP.

At autopsy, the bodies of the deceased were found covered in a thick layer of black-colored sludge and greasy particles. Blood samples, trachea, tissues of the lungs and stomach and its contents, loops of the intestines, the liver with gall bladder, bilateral kidneys, and samples of sludge were preserved during the postmortem examination for toxicological analysis. During the analysis of samples collected from the ETP plant and biological samples preserved at autopsy, hydrogen sulfide was detected in all the samples.

Residential and industrial set-ups generate tremendous amounts of waste, most of which ends up in the ETP. Regular cleaning of the tank and inlet and outlet pipes is required for the optimum functioning of an ETP. In most cases, a flawed design and inadequate volume of the ETP adds to the problems. Furthermore, using huge amounts of chemical cleaners like alum and lime may destroy helpful bacteria that break down organic waste and can lead to ETP malfunction.[3] ETP gas poisoning occurs due to an accumulation of the toxic gases, harmful fumes, and vapors in enclosed the ETP chambers that also contain incompletely degraded waste and sewage water. This may result in a potentially hazardous generation of hydrogen sulfide gas.

Hydrogen sulfide is produced from the breakdown of organic matter by bacteria in the absence of oxygen, such as in sewers and effluent of the ETP; by a process that is commonly known as anaerobic digestion, which is done by sulphate. It is easily soluble in water and dissolved in sewage inside the sludge and silt that forms the gas pockets in the ETP. Exposure to hydrogen sulfide may cause irritation of eyes, dizziness, and even death.[4]

India is a developing country where impoverished youths jump into high-risk jobs without knowing the potential hazards. Safety gear that should be essential while working are hardly being provided by the employer to these poor workers.

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ETP; Hydrogen Sulfide; Death

D32 The Ruggedness of a Kinetic Model to Predict Chromatograms of Gasoline Under Different Evaporation Conditions

Hannah Jewell LaVoie*, Michigan State University, Okemos, MI; Victoria L. McGuffin, Michigan State University, East Lansing, MI; Ruth Waddell Smith, Michigan State University, East Lansing, MI

Learning Overview: This presentation will describe the effect of different evaporation conditions on the accuracy of a kinetic model to predict chromatograms of evaporated ignitable liquids. After attending this presentation, attendees will understand the benefits of the kinetic model in fire debris analysis.

Impact Statement: This presentation will impact the forensic science community by demonstrating the accuracy and ruggedness of a kinetic model to predict evaporation of ignitable liquids for use in forensic fire debris analysis.

Forensic fire debris analysis focuses on identifying the chemical class of any ignitable liquid present in submitted samples. Such samples are typically analyzed by Gas Chromatography/Mass Spectrometry (GC/MS), and the chromatograms are compared to reference ignitable liquids that have been evaporated to various levels. Often, reference collections are produced in-house which, depending on the volatility of the liquid, can be quite a time-consuming process.[1]

Previously, a kinetic model was developed in our laboratory to predict evaporation rate constants as a function of retention index.[2] The model can be applied to the chromatogram of an unevaporated liquid and used to predict the total fraction remaining (F_{Total}) of the liquid, which is related to the evaporation level. As such, chromatograms can be predicted corresponding to any evaporation level of the liquid. In our previous work, liquids representing different chemical classes, including the gasoline, isoparaffinic, and petroleum distillate classes, were experimentally evaporated to different levels. The model was used to predict chromatograms corresponding to these levels and the predicted and experimental chromatograms were then compared. Across all chemical classes and evaporation levels, the predictive accuracy of the model was demonstrated with strong correlation between the predicted and experimental chromatograms.[3]

In continuing to evaluate model accuracy, one of the next steps is to investigate the effect of evaporation conditions. Specifically, in this presentation, the effect of evaporation vessel and agitation will be discussed. In any evaporation process, molecules in the bulk liquid must diffuse to the surface for evaporation. In our previous experimental evaporations, a 10mL aliquot of liquid was evaporated in a graduated cylinder and agitated via nitrogen sparging and stirring. Based on the measuring cylinder dimensions, molecules in the bulk liquid must travel a maximum distance of 8cm to reach the surface for evaporation.[4]

In the current work, 10mL aliquots of fresh gasoline samples were transferred to measuring cylinders and evaporated to different levels with sparging and with or without stirring. In addition, 2mL aliquots of gasoline were evaporated in a petri dish to approximate evaporation from a thin film. In this case, molecules in the bulk liquid must travel a maximum distance of only 0.1cm to reach the surface for evaporation.[4] For each condition, gasoline was evaporated in replicate to nominal F_{Total} = 0.7, 0.5, 0.3, and 0.1, which correspond to evaporation levels of 30, 50, 70, and 90%, respectively. All evaporated samples, along with unevaporated gasoline, were analyzed in replicate by GC/MS. The kinetic model was then applied to the chromatogram of unevaporated gasoline to predict chromatograms corresponding to these fraction-remaining levels.

Predicted and experimental chromatograms for each evaporation were compared using Pearson Product-Moment Correlation (PPMC) coefficients to evaluate the degree of similarity between the chromatograms. For all F_{Total} levels, strong correlation was observed between predicted and experimental chromatograms. However, as the F_{Total} level decreased, corresponding to a greater extent of evaporation, the PPMC coefficients decreased but remained greater than 0.9 in all cases. Further, the predictive accuracy of the model was similar, irrespective of evaporation conditions. For example, for a nominal F_{Total} = 0.5, the mean PPMC coefficients for comparison of predicted and experimental chromatograms were 0.961 ± 0.008 for evaporation from the measuring cylinder with sparging and stirring, 0.982 ± 0.004 for evaporation from the measuring cylinder with sparging and no stirring, and 0.965 ± 0.004 for evaporation from the petri dish. In each case, PPMC coefficients demonstrate strong correlation between predicted and experimental chromatograms.

In this presentation, these data will be discussed in more detail and the accuracy of the kinetic model in predicting evaporation under different conditions will be demonstrated.

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Fire Debris Analysis; Gas Chromatography/Mass Spectrometry; Kinetic Model

D33   Data Collection of Forensically Feasible AI-Generated Texts: The ILE AI-Generated Corpus

Colton B. Wolk*, Institute for Linguistic Evidence, Monmouth Junction, NJ

Learning Overview: After attending this presentation, attendees will understand why forensically feasible data is required in forensic science and how it can be collected from generative-Artificial Intelligence (AI) sources in a procedure called prompt engineering.

Impact Statement: This presentation will impact the forensic science community by showing how to collect data from generative-AI sources and how linguistic prompts work.

With the launch of OpenAI's ChatGPT at the end of November 2022, the general public became aware of "generative artificial intelligence."[1] OpenAI and its competitors use the "Large Language Model" (LLM) approach to language. LLMs exploit the large amount of text available on the web, taking as its primary principle that language is a string of words that can be determined and described by the probability of the next word in a sequence, given all previous words. Two claims from OpenAI and its competitors are forensically intriguing. First, OpenAI and its competitors claim that their LLMs— and chatbots like ChatGPT that are based on those models—can be used to author human-like texts. Second, OpenAI and its competitors claim that their LLMs can be prevented from producing harmful texts. This presentation discusses the second claim, and addresses this issue: Can ChatGPT and other LLM-based chatbots and models be used to generate forensically feasible texts of sufficient and specific length, even including harmful texts that could be used in criminal or civil violations? In particular, this talk describes the Institute for Linguistic Evidence (ILE) AI-Generated Corpus (ILEAIC), which has been designed and created specifically for forensic research and development.

When texts are needed for testing forensic methods, the texts should be as close as possible to texts that are actually found in cases, in both content and length. This principle is known as "forensic feasibility", and is highlighted by the following examples: in developing a method for authenticating suicide notes, actual and independently authenticated suicide notes must be used; in developing a method for handwriting identification, spontaneous rather than copied handwriting must be used (unless the issue is forgery); and in developing a method for determining authorship, documents with known authorship must be used.[2-4] Further, when control texts are needed in experiments, the control texts must provide communicative purposes and contexts that enable the author to write in various genres (communicative purposes) and registers (communicative contexts).[5] This is needed because in forensic casework, the exemplar documents will generally not be in the same genre and register as the questioned document. The first collection of documents for experiments in forensic authorship identification was created in 1995 and used ten linguistic prompts to obtain samples of authorship that include multiple genres and registers.[6] These ten prompts were published and subsequently deployed to collect a forensically feasible Spanish corpus.[7,8] Among the ten prompts, three stimuli could be considered harmful: (1) write an angry letter; (2) write a threatening letter to someone you know; and (3) write a threatening letter to a celebrity or public official. Last, empirical evaluations of techniques for authorship identification have shown that the most robust classification results occur when the known documents meet the guidelines of 2,000 words or 100 sentences.[9]

According to OpenAI and its competitors, the prompts listed above should not elicit responses and procure texts, at least through chatbots. But by using the underlying LLM and prompt engineering, all ten linguistic prompts, including the potentially harmful ones, elicit texts. Additionally, although these LLMs struggle to produce long responses or those of a particular length, careful prompt engineering overcomes these limitations and allows responses of specified word count.[1] The techniques for model selection and prompt engineering are explained, and general characteristics of data in ILEAIC are presented.

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Artificial Intelligence; Data; Linguistics

D34   The Lexical Characteristics of AI-Generated Texts

Colton B. Wolk*, Institute for Linguistic Evidence, Monmouth Junction, NJ

Learning Overview: After attending this presentation, attendees will understand lexical differences and similarities among different sources of text generated by Artificial Intelligence (AI).

Impact Statement: This presentation will impact the forensic science community by showing characteristics of machine-generated texts on the lexical level of analysis.

With the launch of OpenAI's ChatGPT at the end of November 2022, the general public became aware of "generative artificial intelligence."[1] OpenAI and its competitors use the Large Language Model (LLM) approach to language. LLMs exploit the large amount of text available on the web, taking as its primary principle that language is a string of words that can be determined and described by the probability of the next word in a sequence given all previous words. Two claims from OpenAI and its competitors are forensically intriguing. First, OpenAI and its competitors claim that their LLMs— and chatbots like ChatGPT that are based on those models—can be used to author human-like texts. Second, OpenAI and its competitors claim that their LLMs can be prevented from producing harmful texts. This presentation discusses the first claim and addresses this issue: Can texts generated by ChatGPT and its competitors be identified by characteristics at the lexical level?

Lexical analysis is the description of words in language. From detailed descriptions and measurements of words in language, textual relationships can be determined. For example, word-based measurements are very good for determining the topic and associating documents from different authors writing on the same topic.[2] Several computer scientists and a few forensic linguists have suggested that lexical analysis could also be used for authorship identification, but the accuracy rates for methods that focus on words is low, ranging from far less than 50% to around 80%, with one recent report showing only 54% accuracy on classifying known documents in the case to the actual known authors.[3-5]

This work presents a series of experiments in which texts from the Institute for Linguistic Evidence (ILE) AI-Generated Corpus are analyzed for lexical features.[6] For analysis, the linguistic analysis toolkit Text Analysis Toolkit Toward Linguistic Evidence Research (TATTLER) is used. TATTLER enables non-linguists to perform lexical analysis in various ways, including comparative analysis using the same methods from different industrial algorithms. Lexical analysis methods include Term Frequency-Inverse Document Frequency, relative frequency, distribution of function and content word classes, word similarity using algorithms from Rosette and ALIAS Technology, n-grams of lemmas, function and content words using spaCy and Stanza lemmatizers, with n-grams from size 2 to 12.[7-12]

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Artificial Intelligence; Document Analysis; Data

D35 Semantic Characteristics of AI-Generated Texts

Carole E. Chaskim PhD*, Institute for Linguistic Evidence, Georgetown, DE

Learning Overview: After attending this presentation, attendees will understand semantic differences and similarities among different sources of Artificial Intelligence (AI) -generated text.

Impact Statement: This presentation will impact the forensic science community by showing characteristics of machine-generated texts on the semantic level of analysis.

With the advent of OpenAI's ChatGPT (Chat Generative Pre-Trained Transformer) at the end of November 2022, the general public became aware of "generative artificial intelligence."[1] OpenAI and its competitors use the Large Language Model (LLM) approach to language. LLMs exploit the large amount of text available on the web, taking as its primary principle that language is a string of words that can be determined and described by the probability of the next word in a sequence given all previous words. Two claims from OpenAI and its competitors are forensically intriguing. First, OpenAI and its competitors claim that their LLMs—and chatbots like ChatGPT that are based on those models—can be used to author human-like texts. Second, OpenAI and its competitors claim that their LLMs can be prevented from producing harmful texts. This talk discusses the first claim and addresses this issue: Can texts generated by ChatGPT and its competitors be identified by characteristics at the semantic level?

Semantic analysis is the description of meaning in language. From detailed descriptions and measurements of meaning in language, texts of different types can be classified. For example, suicide notes and control texts can be differentiated by machine-based measurements and statistical analysis, with an average 88% cross-validated accuracy.[2] Likewise, threatening communications can be differentiated from related documents such as simulated threats, angry letters, and love letters with accuracies over 90%.[3] Olsson suggested that semantic analysis could also be used for authorship identification.[4]

This talk presents a series of experiments in which texts from the Institute for Linguistic Evidence (ILE) AI-Generated Corpus are analyzed for semantic features.[5] For analysis, the linguistic analysis toolkit TATTLER is used.

TATTLER enables non-linguists to perform semantic analysis in various ways, including comparative analysis using the same methods from different industrial algorithms. Semantic analysis methods include topic extraction using Latent Semantic Indexing, Latent Dirichlet Analysis, Hierarchical Dirichlet Process, all from Gensim algorithms, topic modeling using BERT, Semantic Categories using an algorithm from Babel Street's Rosette and an algorithm from ALIAS Technology, Named Entity Recognition and Sentiment Analysis using algorithms from Babel Street's Rosette, Spacy, Stanza, and FLAIR, and Semantic Vectors at various levels using GloVE, FastText, Rosette, and FLAIR available through GitHub.[6-12]

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Artificial Intelligence; Semantics; Natural Language Processing

D36   The Syntactic Characteristics of AI-Generated Texts

Samuel Olanrewaju*, Institute for Linguistic Evidence, Ilorin, Kwara, Nigeria

Learning Overview: After attending this presentation, attendees will understand the syntactic differences and similarities among different sources of Artificial Intelligence (AI) -generated text.

Impact Statement: This presentation will impact the forensic science community by showing characteristics of machine-generated texts on the syntactic level of analysis.

With the launch of OpenAI's ChatGPT at the end of November 2022, the general public became aware of "generative artificial intelligence."[1] OpenAI and its competitors use the Large Language Model (LLM) approach to language. LLMs exploit the large amount of text available on the web, taking as its primary principle that language is a string of words that can be determined and described by the probability of the next word in a sequence given all previous words. Two claims from OpenAI and its competitors are forensically intriguing. First, OpenAI and its competitors claim that their LLMs—and chatbots like ChatGPT that are based on those models—can be used to author human-like texts. Second, OpenAI and its competitors claim that their LLMs can be prevented from producing harmful texts. This talk discusses the first claim and addresses this issue: Can texts generated by ChatGPT and its competitors be identified by characteristics at the syntactic level?

Syntactic analysis is the description of cumulative relations among words that create phrases and sentences in language. Syntax is the primary reason that language is not a list of words (or bag of words), but rather a cognitive system for packaging meaning into sound. From detailed descriptions and measurements of syntax in language, authorship of known and questioned documents can be classified. For example, by machine-based measurements and statistical analysis, known documents can be classified to the correct author with an average 98% cross—validated accuracy.[2-4]

This talk presents a series of experiments in which texts from the Institute for Linguistic Evidence (ILE) AI-Generated Corpus are analyzed for syntactic features.[5] For analysis, the linguistic analysis toolkit Text Analysis Toolkit Toward Linguistic Evidence Research (TATTLER) is used. TATTLER enables non-linguists to perform syntactic analysis in various ways, including comparative analysis using the same methods from different industrial algorithms. TATTLER also enables linguists to check the results of syntactic analysis for accuracy. TATTLER's syntactic analysis methods include part-of-speech tagging and chunking into phrases using algorithms from Babel Street's Rosette, ALIAS Technology, Spacy, Stanza, NLTK, and FLAIR.[6-11]

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Artificial Intelligence; Linguistics; Authorship Identification

D37 A Statistical Authorial Modeling of AI-Generated and Human-Generated Texts

Carole E. Chaski, PhD*, Institute for Linguistic Evidence, Georgetown, DE; Colton Wolk, Institute for Linguistic Evidence, Monmouth Junction, NJ; Samuel Olanrewaju, Institute for Linguistic Evidence, Ilorin, Kwara, Nigeria; Annabelle K. Bland, BS

Learning Overview: After attending this presentation, attendees will understand differences and similarities between human-generated and Artificial Intelligence (AI) -generated text that contribute to statistical modeling of authorship for both kinds of text.

Impact Statement: This presentation will impact the forensic science community by showing how to differentiate human- and machine-generated texts.

With the advent of OpenAI's ChatGPT in November 2022, the general public became aware of "generative artificial intelligence."[1] OpenAI and its competitors use the Large Language Model (LLM) approach to language. LLMs exploit the large amount of text available on the web, taking as its primary principle that language is a string of words that can be determined and described by the probability of the next word in a sequence given all previous words. Two claims from OpenAI and its competitors are forensically intriguing. First, OpenAI and its competitors claim that their LLMs (and chatbots such as ChatGPT) can be used to author human-like texts. Second, OpenAI and its competitors claim that their LLMs can be prevented from producing harmful texts. This talk discusses the first claim, and addresses this issue: Can texts generated by Chat-GPT and its competitors and text generated by humans be differentiated by statistical models of authorship using a multi-level linguistic analysis? OpenAI has already discontinued its "ChatGPT Detector,", as widely reported in the news, because its accuracy rate was so low, with only about 26% of AI-generated texts identified as such and with 9% of human-generated text identified as AI-generated.[1]

There are three main approaches to identifying authorship: (1) stylistic or error matching; (2) lexical overlap; and (3) syntactic.[2-4] The syntactic approach has been the most accurate at determining authorship, with an average of over 95% cross-validated accuracy on a set of 30 demographically matched authors writing business and personal documents.[5]

This talk presents a series of experiments in which texts from the Institute for Linguistic Evidence (ILE) AI-Generated Corpus are compared to human-generated texts from the Chaski Writing Sample Database under two conditions: matched for register and genre, and not matched for register and genre.[6,7] Lexical, semantic, syntactic features are used in quantifying the texts as well as syntactically classified punctuation.[4,8-10] Statistical models of authorship for AI-generated and for human-generated texts are presented.

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Artificial Intelligence; Authorship Identification; Machine Learning

D38   Bone Injuries in Nursing Home Residents

David Pienkowski*, University of Kentucky, Lexington, KY

Learning Overview: This presentation reviews the fundamental composition and structure of human bone and shows how age-related biological changes affect bone material properties and reduce fracture thresholds. After attending this presentation, attendees will have gained insight regarding the diminished mechanical integrity of older adult bone.

Impact Statement: This presentation will impact the forensic science community by assisting forensic nursing efforts to understand the etiology of bone fractures in nursing home residents and may foster new ideas for preventing low-energy skeletal injuries in this population.

Bone fractures in the elderly often entail serious morbidity and mortality concerns enduring for as long as ten years post-fracture, pose challenges to current health care technology, and have substantial economic consequences.[1-3] The Centers for Disease Control and Prevention estimated that approximately 34,000 deaths occur annually as a result of falling; many of these deaths occurred in long-term care facilities. The likelihood of sustaining a bone fracture from falling increases with age. Furthermore, the prevalence of bone fractures in residents of long-term care facilities may increase due to the growing population of aging baby boomers and the increased usage of long-term care facilities.

The solution to any vexing technological challenge optimally begins by problem identification beginning at the level of the fundamental science(s) involved. Based on that perspective, it is important to understand that the human bone is a living organ in a constant state of biological change. Understanding bone function is complicated by the fact that it answers to two simultaneous (and sometimes conflicting) masters: calcium ion storage and release as well as the mechanical load-bearing demands of the activities of daily living. The dynamic processes that occur in this constantly changing composite material (i.e., bone) can be likened to an active checking account, except that deposits and withdrawals occur continuously (not discreetly) as a result of various ongoing cellular activities focused on homeostasis. Bone mechanical integrity and fracture resistance depend on the quantity and quality of bone. While age-related bone quantity changes (e.g., bone loss due to cessation of ovarian function affecting mechanical integrity are generally known) bone quality losses affecting mechanical integrity are largely unappreciated yet contribute to "weakened" older adult bone. Further complicating matters are undiagnosed or incorrectly treated conditions that also affect bone mechanical integrity.

This presentation will show that forensic analyses of nursing home-related bone fractures need consider the: (1) mechanics of the fracture incident to quantify the forces acting; (2) patient-specific bone fracture thresholds modified by various factors, including patient history, lifestyle, medication usage, and others; and (3) probability that the incident forces exceeded patient and bone-specific fracture thresholds. These principles also apply to injuries involving other musculoskeletal tissues or organs. The approach noted underscores that forensic analyses of nursing home injuries are best accomplished by collaborative engagement of engineers, biostatisticians, and forensic nurses.

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Bone; Engineering; Forensic Nursing

D39   Electrical Injury Causes: Part 2—Evidence Retention

Helmut G. Brosz*, Forensic Science International Group, Markham, ON, Canada

Learning Overview: After attending this presentation, attendees will appreciate that forensic electrical engineers and nurses play an important role in reconstructing an electrical injury matter partly based on injury evidence and physical clothing evidence collected by forensic nurses, doctors, and pathologists upon admission. Clothing evidence can assist forensic engineers. Forensic nurses, doctors, pathologists, and lawyers thus play an important role in determining if the injuries are consistent with the evidence. Also, some electrocutions without visible markings may have a criminal aspect that was perpetrated with the use of electricity, either intentionally or unintentionally.

In this presentation, pictorial electrical injury and electrocution reconstructions will be presented with various outcomes and sequelae. Electrocution generally means that current has passed through the heart and death ensued. Through investigation, one may discover whether this death was caused intentionally, by criminal negligence, accidentally, or by misadventure.

Impact Statement: This presentation will impact the forensic science community by contributing to an important understanding for forensic nurses and others involved in an investigation of electrical injury causes and by assessing the importance of evidence retention such as clothing or other artifacts.

Electrical injuries can be multifaceted, and in some cases high voltage and high current can cause devastating trauma to the victim's body. In some low voltage electrocution cases, there is no visible trauma at all. The severity of electrical trauma can range from gross loss of limb and tissue due to sustained high voltage and high current contact causing sixth-degree burns. Low voltage and low current contact may not leave any marks on the body depending on contact area, duration, and other environmental factors; however, nerve damage can lead to delayed sequelae. Since some electrical injuries involve marks on the body, there can be challenges in accurately assessing the pathway(s) of current and the modes and circumstances of contact. When an electrocution is being examined, forensic electrical engineering investigators inspect the scene and examine any clothing or other artifacts that may have been collected and preserved by nurses or first responders before reaching a conclusion.

In some cases, it can be difficult to determine whether the incident was deliberate, an accident, or if an electrocution even occurred due to lack of evidence. For example, if a victim were to be electrocuted by fallen electrical wires, there are many possibilities of what caused the wires to fall in the first place. One possible situation is an electrical worker who installs wires in such a manner that they could foreseeably cause an electrocution; then, such a case could be considered negligent homicide or manslaughter. Another situation is that the electrical system was sabotaged in some way, and if so, this would be intentional electrical homicide. An incident could also be the fault of the victim, such as electrical suicide or auto-erotic misadventure. Natural causes such as floods, winds, or a storm bringing down power lines and poles, as well as lightning, are some other causes of electrocution.

It is also important for emergency department nurses and doctors to preserve a victim's clothing. The clothing artifacts such as shirts, boots, watches, and gloves will assist the forensic electrical engineer in reconstructing the electrocution event, and thus are likely to play a key role in future litigation.

Accident; Injury; Unknown

D40 Powered Two-Wheeler Safety: An Experimental Analysis of Injuries and Injury Mechanisms Using Instrumented PMHS

John H. Bolte IV*, The Ohio State University, Columbus, OH; Amanda M. Agnew, The Ohio State University, Columbus, OH; Yun-Seok Kang, The Ohio State University, Columbus, OH

Learning Overview: After attending this presentation, attendees will have an appreciation for interdisciplinary research in skeletal trauma analysis, specifically focused on injuries to occupants of Powered Two-Wheelers (PTW) involved in a crash with a motor vehicle. Attendees will learn how to conduct experimentally driven biomechanical validation of skeletal trauma for forensic engineering purposes and understand the importance of utilizing experimental data to better understand the injury mechanisms behind the occupant's injuries.

Impact Statement: This presentation will impact the forensic community by demonstrating the need for interdisciplinary biomechanical analyses of skeletal trauma to better understand injury mechanisms related to PTW crashes. Increased understanding of the mechanisms of trauma will lead to better safety for PTW occupants.

There are over 350,000 PTW fatalities each year around the world.[1] To date, the majority of PTW research has focused on analyzing crash data to document typical crash configurations and common occupant injuries.[1-4] While this research is helpful in determining occupant injuries due to PTW crashes, it does not determine the mechanisms of each injury, which are critical to create wearable safety equipment or altering safety components on PTWs. To obtain critical injury mechanism data, this study conducted three repeatable impacts involving a PTW with an instrumented Postmortem Human Subject (PMHS) occupant into a sedan to mimic a real-world crash.

Each test included a 50th percentile male PMHS seated on a 2022 KTM® 390 Duke® that was propelled into the side of a stationary 2011 Honda® Accord®. Instrumentation on each PMHS included strain gauges on the head, both anterior and posterior ribs, sternum, cervical spine, pubic rami, and both the upper and lower extremities. In addition, six Degree-Of-Freedom (6DOF) motion blocks (6DXPro® DTS) were placed on the sternum, posterior nasal bone, C4, C6, T4, T12, S1, as well as the right and left humeri, femora, and iliac wings. Data were analyzed to determine how the PMHS interacted with the PTW, the Accord®, and the ground. Strain and strain rate were used to identify fracture timing, whereas the 6DOF motion blocks were used to analyze occupant kinematics and potential injury mechanisms. High-speed cameras were placed surrounding the impact zone and onboard the PTW cart to allow for additional analysis, including aligning the video to strain data to provide context for investigating injury mechanisms. After each test, a detailed anatomical dissection identified PMHS injury location and severity, and data were analyzed to determine injury mechanisms.

Data analysis revealed similarities between both whole-body kinematics and injuries across all tests. The PTWs impacted at an average speed of 50.0kmph and had approximate contact timings of 52ms for the pelvis with the gas tank, and 120ms for the head of the PMHS with the vehicle. All tests resulted in severe injuries to the pelvis, thorax, and thoracic spine. The installed instrumentation suite allowed for the timing of skeletal fractures to be identified, thus helping better understand mechanisms of each injury. For example, strain data helped identify the time of pelvis injury as being early in the event from forward movement of the pelvis into the fuel tank. Injuries observed in this study were consistent with the most commonly injured body regions documented in literature.

Thousands of PTW fatalities occur annually across the world, and there is a current gap in knowledge when it comes to how to better protect PTW riders. These biomechanical data and injury mechanisms will be useful in improving current PTW safety tools, which will ultimately help improve the safety of PTW occupants.

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Motorcycle; Injury; Cadaver

D41   What Is Known About Pregnant Occupants and Fetal Risk in Car Crashes

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Learning Overview: After attending this presentation, attendees will understand the most common injury mechanisms underlying fetal death from vehicle crashes. Attendees will learn the difference in relative outcomes of fetuses vs. mothers subjected to the same crash event and will understand how seat belt use affects fetal and maternal outcomes. Finally, attendees will be exposed to the various methods researchers have used to investigate fetal injury from vehicle crashes in the past 40 years: experimental animal studies, simulations, Anthropometric Testing Devices (ATDs), case studies, and statistical approaches.

Impact Statement: It has been estimated that about 160,000 pregnant women are in a vehicle crash each year and estimates of fetal deaths from vehicle crashes range from about 750 to 4,800 per year.[1-3] The upper estimates are alarming. By comparison, about 28,000 occupants in vehicles died in 2021, excluding pedestrians, bicycles, and motorcycles.[4] Importantly, any fetal injury that does not cause death can still result in long-term life impairments.[5] Together, these statistics suggest the forensic science community take note of fetal injury during vehicle crashes by having a better understanding of the injury mechanisms and factors associated with an increased likelihood of injury. This knowledge may help educational programs, general awareness, and possible solutions.

Trauma affects between six and seven percent of all pregnant women, with the leading cause of trauma during pregnancy being from vehicle crashes.[6,7] Pregnant women and non-pregnant women are as equally likely to get into a vehicle crash and have similar injuries.[8] But what about injuries to the fetus? Using a PubMed® search and references from identified articles, over 50 research articles were extracted that present data on the prevalence of fetal death, the mechanism of injury, and/or the effect of seatbelt use.

In the systematic review, 36 articles were found that directly quantified maternal and her fetus outcomes when the two were involved in the same crash between 1980 and 2023 (11 additional articles prior to 1980 were found but excluded from the analysis due to the high differences in seatbelt availability and usage). Articles included single case studies, case series, and population-based statistical studies. Across all articles, 147 fetal deaths and 31 maternal deaths were documented for an overall ratio of 4.74 to 1 fetal to maternal deaths. Ratios were also determined for case studies and cases series since 2000, which resulted in a ratio of 3.6 to 1 fetal to maternal death. With seatbelt use in the past 20 years, the ratio was 5.4 to 1 fetal to maternal death and only 2.0 to 1 for unbelted. For unbelted occupants, the ratio is lower because it is more likely for both the mother and fetus to die in the event of a crash. When available, maternal injuries were also examined in crash events where the fetus died. The articles provided detailed maternal outcomes on 141 crash events since 1980. Maternal injuries were categorized as "none" or "minor" in 46% of fetal deaths, "moderate" in 17%, "major" in 17%, and "fatal" in 20%.

Many studies point out that the most common mechanism of fetal death is placental abruption. Placental abruption is the separation of the placenta from the uterine wall prior to birth, leading to the disconnection of oxygen, food, and waste removal from the mother to fetus. Researchers have measured the mechanical properties of the placenta and uterus and often used 60% strain in computer simulations as an injury tolerance for the uterus-placenta-interface.[9] Other injury mechanisms include uterine laceration and direct fetal contact with the mother's internal structures.[3,10] Poor outcomes are exaggerated with improper belt fit; however, it should be emphasized that population and case series consistently demonstrated more death and injuries to the fetus and mother without a 3-point seatbelt and crashes at higher speeds.[11]

In summary, fetuses have died in vehicle crashes at a rate of 3.6–4.7 times more than mothers, with placental abruption as the most common cause. When a fetus dies, the maternal injuries have been reported as minor or none about 46% of the time and major or fatal 37% of the time. Fetal injury is also important because long-term impairments can result from insult to the developing brain. While belt use and reduced driving speeds are key factors in reducing injuries, other steps may include development and testing of new belts and increased awareness and education about limiting vehicle exposure.

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Motor Vehicle; Pregnancy; Injury

D42   A Child Fatality in a Vehicular Rear Impact: A Forensic Analysis and Static Testing to Scientifically Prove Seat and Belt Failures

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Learning Overview: This study shows some aspects of forensic field investigations and testing that have been conducted to identify the predictably dangerous weak seats that eject occupants rearward out of slackened belts. This presentation will show in detail the evidence found in various vehicles because of seat and belt failures, as well as static and dynamic testing that discovered the random failure modes of the seats, head restraints, and belts, as well as how to improve designs to prevent such injuries. Alternative safer seat and head restraint designs were incorporated in the static and dynamic testing to prevent any occupant contact with other occupants or interior structures.

Impact Statement: This presentation will impact the forensic community by showing that these severe vehicle safety hazards have existed for decades, why they continue to exist, how they create enhanced injury to children and adults, and how those injuries can be prevented using lightweight, economically and technologically feasible means. Vehicle crash investigators, medical examiners, and law enforcement will learn how to identify vehicle seat and belt failure, vehicle interior occupant contact witness marks, and associated forensic evidence that will allow more accurate assessment of occupant injuries in such circumstances.

Predictable front seat and belt failure in survivable vehicular rear impacts have been known for over 60 years. Weak seats and slackened seat belt failures have predictably caused severe injury and death to thousands of properly belted front and rear seat occupants, including children, every year. This is due to the lethal combination of no rear impact occupant protection requirements and weak seats allowed by the grossly outdated Federal Motor Vehicle Safety Standard 207, which was declared "flawed and inadequate" by the National Highway Traffic Safety Administration (NHTSA) in 1996, because it only requires approximately 275 pounds of static load capacity for seats. In contrast, Federal Motor Vehicle Safety Standard (FMVSS) 210 requires seat belts to meet a minimum of 6,000 pounds static load in frontal impacts. Since 1996, NHTSA and automakers have warned parents to place children in rear seats to avoid front airbag hazards, despite these known seat and belt defects. Subsequent crash statistics published in 1997 have proven that rear-seated children are safer in front and side crashes, but they are 61% more likely to incur catastrophic injury or death in rear crashes.

This forensic study concerns recent moderate rear vehicle impacts where weak front seat and slackened seat belt failure resulted in rearward ramping of belted front occupants into the rear seat survival space, causing predictable fatal injuries to children properly restrained in rear seats. Causation of these enhanced injuries was proven by a combination of scientific accident reconstruction and static testing, combined with comparative review of dynamic testing conducted for FMVSS 301 fuel system integrity compliance and other similar incidents. Prevention of injury by use of lightweight, technologically, and economically feasible crashworthy seats was also demonstrated via scientific testing.

In 1958, human volunteer tolerance to rear impact exceeding 83G was proven, but only if a crashworthy seat and head restraint is utilized that will not collapse under predictable inertial loading by the vehicle occupant. Racing rear impacts of over 200mph into fixed concrete barriers have been repeatedly survived without injury. Crashworthy, belt-integrated seats have been commercially available since 1965. Many automakers produced crashworthy seats and rear structures in the Department Of Transportation's (DOT's) Experimental Safety Vehicle and Research Safety Vehicle programs between 1970 and 1980, which were capable of withstanding greater than 50mph rear-moving barrier impacts without significant injury to occupants, no inter-occupant contact, and no loss of intended head restraint or seat belt performance.

Despite voluminous knowledge on how to protect motorists in rear crashes, these dangerous conditions have continued to occur in most vehicles, due to the lack of meaningful safety standards. Automaker engineers have testified it would cost "about a dollar" per vehicle to provide crashworthy seats and belts that would not fail in a severe rear collision. Contrary to automaker claims, and as proven in this study, there is no need for seats to "yield" into the survival space of other occupants. Such loss of occupant survival space violates every ethical concept of crashworthiness, biomechanics, and vehicle occupant protection, especially of children in the rear seats. During these forensic investigations, one automaker's engineers testified that their so-called concept of "yielding seats" was nothing more than a meaningless "metaphor," which had no engineering specifications or scientific justification. Federal court rulings in our cases declared that automakers are no longer allowed to make such specious false claims when defending seat and belt failures in rear collision incidents.

Rear Impact; Failure Analysis; Death Investigation

E1 Is a Nurse Also a Forensic Nurse? Gaining a Forensic Insight: Empowering Nurses in Addressing Interpersonal Violence

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Learning Overview: After attending this presentation, attendees will learn why it is so important to provide nurses with forensic nursing basics in their daily work, which topics on Interpersonal Violence are not/rarely known from the perspective of nursing professionals, and the appropriate offerings and their contents on forensic nursing basics for nurses.

Impact Statement: This presentation will impact the forensic science community by informing attendees that an appropriate access to the important aspects of forensic nursing for nurses and other professionals with direct contact to persons affected by violence is important to enable all involved professional groups to provide professional care as first contact. These events and trainings are designed to raise awareness and educate nurses but are not a substitute for specialized and advanced training in forensic nursing.

In Switzerland, advanced educational programs in Forensic Nursing have been available for almost ten years. These differ in duration, degree, and curriculum. Nurses who want to gain in-depth expertise in Forensic Nursing can, for example, complete a Certificate in Advanced Studies in Forensic Nursing at the University of Zurich.[1] There are also non-university course offerings in Switzerland, which also take one year. The offer of a profound education in Forensic Nursing is available, but what about nurses who cannot complete such a specialized education and who are nevertheless regularly exposed to situations of interpersonal violence in their daily work? Can they recognize situations suspicious of violence and respond to them in a professional and timely manner? How do they come to their knowledge on this topic? What are their professional and organizational resources?

To close this gap and provide all nurses with access to relevant aspects of Forensic Nursing, the Swiss Association Forensic Nursing has set itself the goal of developing appropriate training programs for nurses: education, awareness, and networking of nurses are the keywords here—for the nursing professionals and in order to professionally care for those affected. At the end of February 2023, the Interest Group Forensic Nursing of the Swiss Professional Association for Nurses began a survey on the current state of knowledge as well as on the need for further education of nurses regarding the treatment of persons affected by violence. A total of 83 nursing professionals participated in the survey. The results showed: more than half of the respondents had been exposed to cases of interpersonal violence (suspected or confirmed) in the past six months or several times a month. Almost half of all respondents feel uncertain in their interaction with people affected by violence. An impressive result was presented by the question whether the respondents would welcome a regular training and further education offer from the interest group Forensic Nursing of the Swiss Professional Association for Nurses on the topic of "interpersonal violence": almost 93% agreed with this question.

Based on the results, the Interest Group Forensic Nursing (2) developed various public events and further training courses for nurses focusing on "Recognition and documentation of situations of suspected violence," "Violence Against Older Persons," and "Interfaces and partner organizations around the topic of violence" as well as in the area of violence prevention. The first event for nursing professionals is planned for fall 2023.

If we raise awareness of all treating, caring, and consulting professionals on the topic of '"preventing and addressing interpersonal violence," support them, and establish and expand networking with and among each other, we will succeed in de-tabooing and achieving an improvement in the recognition, documentation, and sensitization with the topic of violence. Whether in providing long-term care or in an emergency setting, nurses are on the front line and must be able to act in a professional and timely manner. It is time to integrate the topic of "addressing interpersonal violence" into the nursing practice.

The findings and outcomes of nursing professional events on Forensic Nursing aspects will be presented.

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Forensic Nursing; Education; Interdisciplinary

E2 The Progress of Forensic Nursing in Japan

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Learning Overview: The goal of this presentation is to introduce the progress of forensic nursing in Japan. The Japanese Association of Forensic Nursing (JAFN) was established in 2014 and became a general incorporated association in 2020. Violence and health have become global issues these days, and this presentation will clarify further issues by describing the development of forensic nursing in Japan.

Impact Statement: The presentation will impact the forensic community by showing the process to gradually spread the concept and effect of forensic nursing.

The purpose of JAFN is to promote the clinical and academic development of forensic nursing, conduct various projects such as dissemination of knowledge, and thereby contribute to the academic improvement of members, the prevention and care for the victims of violence and abuse, and the improvement of people's lifelong health and welfare. At the time of its launch, there were 113 members, and by the end of fiscal 2022, there will be 335 members. Main activities include holding an academic conference once a year, publishing an academic journal, and conducting various training and study sessions. In addition, since 2020, we have been certifying sexual violence nurses. The English name is "Sexual Assault Nurse Examiner-Japan," abbreviated as "SANE-J," and is different than **Sexual Assault Nurse Examiner** (SANE), which has created a certification exam. In the past three years, 116 SANE-J certified persons have been created.

So far, we have held nine academic meetings. As a forensic nurse, I have been pursuing various themes such as addiction, disaster, crime victims and perpetrators issues, and sexual violence in the public space. The most recent theme for 2022 is "Toward a society that responds to complaints about sexual abuse," and the president of the conference, Jun Yamamoto, a SANE-J, has contributed to revolutionary major reforms related to rape laws. She set up a Citizen's Council for Law Reform, became a member of the Legislative Council, and has been an active speaker. In June 2023, it became a crime of non-consensual sexual orientation. "Japan changed its definition of rape, raised the age of consent to 16 from 13 on Friday, bringing the country's sex crimes law closer in line with the United States and Europe."[1] Therefore, forensic nursing is required to contribute to victim response and evidence collection. Based on these, we would like to be able to make grounded proposals in basic nursing education and the overall health care system and to link them to concrete forensic nursing practice. In the future, we would like to play a unique role within the Japanese society.[2]

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Nursing; Sexual Crimes; Sex Offender

E3 **Community-Based Forensic Nursing: The Development of Fully Functioning Forensic Programs in a Rural State**

Kacie Parrish*, Mercy, Rogers, AR; Abigail Ellis, Northwest Arkansas Forensic Nurse Team, Prairie Grove, AR

WITHDRAWN

E4   Creating a Clinical Preceptor Course for SANE Pediatric/Adolescent Nurses

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Learning Overview: This project utilized the International Association of Forensic Nurses (IAFN) education guidelines to establish a hands-on clinical preceptor course for nurses who wish to become certified pediatric/adolescent Sexual Assault Nurse Examiners (SANE-P®).[1] When attempting to create a hands-on course for pediatric care, we found no course throughout the United States where nurses could gain this vital hands-on experience unless they were able to work with nurses and other providers in a Child Advocacy Center, which is not feasible for most nurses, especially in rural and underserved areas. The establishment of such a course presents many challenges as there are no options for nurses to practice the exam with a live pediatric/adolescent standardized patient. Utilizing pediatric standardized patients is not an option for obvious reasons. The goal of this initiative is to increase the national workforce of SANE-P® to improve health care access and outcomes for pediatric victims of sexual abuse or Interpersonal Violence (IPV), thus providing equitable medical forensic care for all populations.[2] Our objective is to develop and implement a Pediatric Model for the Clinical Preceptor Course (CPC) adapted to the successful Adult Model CPC by utilizing the currently available simulators.[3]

Impact Statement: This presentation will impact the forensic science community by attempting to identify alternative experiences using simulation versus live standardized patients as are used in the adult population. Over the years, it has presented much difficulty for nurses to gain the clinical practice experiences needed to care for the pediatric/adolescent victim of sexual assault because it is not feasible to use live pediatric standardized patients in these courses.

According to the IAFN, there are currently only 744 board-certified pediatric SANE-Ps® in the entire United States.[4] Providing an educational opportunity to increase the number of Pediatric SANEs will begin to close the gap in pediatric medical-forensic examination services in areas without access to a child abuse clinician.[5]

In response to the issues involved, nurses who have been awarded a Department of Health and Human Services Health Resources and Services Administration (HRSA) Advanced Nurse Education (ANE) Sexual Assault Nurse Examiner (SANE) grant collaborated to identify an alternate education component that could provide quality training for the new pediatric SANE. It is projected that collaborative efforts among grantees will result in the creation of a Tool Kit that other educators can use to train new pediatric SANEs. This collaborative group of forensic advanced practice nurses undertook the significant task of creating a procedure and ultimately a toolbox for other nurses to utilize in addressing this clinical gap.

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Pediatric; Intimate Partner; Forensic Nurse

E5   **One Voice, One Vision: A System Approach to Sustaining a Best Practice Model With Forensic Nursing for Quality Patient Care, Community Safety, Credible Judicial Outcomes, and Staff Retention**

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Learning Overview: After attending this presentation, attendees will identify strategies to sustain forensic nursing practice resulting in improved quality patient care, community safety, and credible judicial outcomes. This commitment is further enhanced by focusing on staff retention, stakeholder partnerships, and identifying fiscal resources to support the long-term viability of forensic nursing programs.

Impact Statement: This presentation will impact the forensic science community by raising awareness of the challenges faced to sustain forensic nursing, a best practice model. Forensic nursing programs have demonstrated improved health outcomes, higher reliability of forensic evidence preservation, and factually influencing judicial outcomes with trial testimony.

The national nursing shortage has had a significant impact on the field of forensic nursing.[1] In response to the COVID-19 pandemic, numerous forensic nursing programs across the nation were suspended and have yet to be reinstated, as some hospitals deemed them non-essential services. This situation is currently affecting victims of physical and sexual violence and hospitals nationwide, giving rise to a range of issues such as compounded trauma experienced by patients and strained community law enforcement relationships due to compromised evidence preservation resulting in hindered judicial outcomes.[2] The forensic nurse effectively addresses these challenges by executing precision in evidence preservation, maintaining evidence integrity, and providing crucial trial testimony.[3] Forensic nurses, specialized health care providers, employ a trauma-informed, compassionate care approach that is intricately linked to the well-being and healing of the patients we serve.

Memorial Hermann Health System, as a leading health care organization, has taken significant strides to address these issues. Forensic Nursing Services has been established in all 11 acute care Memorial Hermann Hospitals to provide comprehensive trauma-informed compassionate care for survivors of sexual violence. By providing the necessary tools and processes, such as designated care areas and specialized equipment to enhance the patient experience, forensic nurses can confidently perform their duties during what may be the worst day of a patient's life. Building strong community relationships, particularly with law enforcement agencies, is highlighted as a vital component in the success of community safety.[4]

This strategic approach focuses on structure and sustainability, ensuring that Memorial Hermann remains the high-reliability institution for forensic nursing services across the Greater Houston area. Additionally, grants and programmatic support are cited as essential elements in the long-term fiscal viability of the program. Through the establishment of a fellowship program and fostering partnerships with academic institutions, we are developing a robust system pipeline to support ongoing forensic nursing recruitment with efforts to increase enrollment in advanced nursing education opportunities. Finally, the Nursing Institute at Memorial Hermann is committed to enhancing the research platform in this area, further advancing the forensic sciences field.

The insights provided on resource allocation, unified leadership vision, and emphasis on the moral obligation to support front-line nursing staff as well as the broader community will empower health care institutions nationally and globally to implement proactive measures to establish forensic nursing practice and ensure the availability of these valuable resources.

By prioritizing training for forensic nursing staff, embracing leadership perspectives, and ensuring community engagement, hospitals will enhance patient care, strengthen community safety, foster credible judicial outcomes, and promote staff retention.[5] Through initiatives like fellowship programs, academic partnerships, and robust research platforms, health care institutions can cultivate a sustainable environment for forensic nursing, ultimately benefiting patients who have experienced inflicted violence. This presentation aims to equip attendees with best practice strategies while offering guidance on the importance of sustaining and expanding forensic nursing programs.

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Forensic Nursing; Community Safety; Credible Judicial Outcomes

E6   Tracking Sexual Assault Kits (SAKs) and CODIS-Eligible Profiles

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Learning Overview: This presentation will provide quantitative data from the Texas Department of Public Safety (DPS) on SAKs from facilities in Texas that conduct medical forensic examinations. Facility characteristics with the lowest and highest weighted averages of SAKs with Combined DNA Index System (CODIS) -eligible DNA profiles will be compared. Subsequent to this presentation, attendees will be more aware of the next steps to be taken to improve evidence collection methods and the need for further education for care providers.

Impact Statement: This presentation will impact the forensic science community as this presentation puts great focus on the evidence collection methods of Sexual Assault Nurse Examiners (SANEs) and the factors that impact the number of CODIS-eligible profiles at each site for future improvement in the field.

It is currently unknown how a medical provider's education, background, or evidence collection method impacts whether a SAK will yield a CODIS-eligible profile. According to the Texas Forensic Evidence Collection Protocol, the recommended evidence collection process for collecting a vaginal sample is to use a speculum to open the vagina and swab the cervical os, surface, and posterior fornix with two swabs simultaneously.[1] Nurses who are not SANE educated are typically not authorized by their facility to use a speculum. They may perform a "blind swab" of the vagina, without using a speculum. Providers in rural hospitals without SANE programs may use a speculum but not have knowledge or training on the recommended process for collecting vaginal swabs. This poses the question of whether lack of adequate training affects the quality of vaginal samples and subsequent likelihood of obtaining a CODIS-eligible profile. The purpose of this study was to describe the proportion of SAKs that yielded CODIS-eligible profiles in facilities collecting SAKs. Results can be used to identify facility characteristics associated with the need for further education on evidence collection procedures.

We examined data from the Texas Department of Public Safety (DPS) electronic SAK tracking program A public information request was submitted to the Texas DPS for data on the total number of SAKs submitted at each medical facility in Texas and number of kits that yielded CODIS-eligible profiles. Requested dates were from January 1, 2020, to June 26, 2023. Using data for facilities that collected a minimum of 30 SAKs in the study period, we calculated weighted averages of CODIS-eligible profiles based on the number of SAKs collected during the time period. The lowest and highest ten medical facilities were further analyzed based on if the facility is listed as a SAFE-ready facility, which is defined by Texas law as having or contracting with SANEs, the size of the facility, and the county population.

For non-Sexual Assault Forensic Exam (SAFE) -ready facilities, the patients can request a medical forensic examination for sexual assault; however, the exam is conducted by a nurse or physician with limited training.[2] Due to their lack of education in performing high-quality medical forensic examinations, these healthcare providers could potentially overlook important evidence that might yield a CODIS-eligible profile. The size of the medical facility was measured upon the number of staffed beds. Based on the data, a trend is present as larger, more urban medical facilities tend to have higher weighted averages compared to smaller facilities. The lowest ten facilities had fewer than 50 cases during the data analysis period. Results emphasize ways in which the forensic science field can further improve collection methods, along with increasing the training for health care providers conducting medical examinations.

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Forensic Nursing; Sexual Crimes; Statistics

E7   **Who Doesn't Report to the Police? A Quantitative Analysis of Victim, Offender, and Assault Characteristics for Non-Report Sexual Assault Medical Forensic Examinations**

Jessica Volz*, Adventist HealthCare Shady Grove Medical Center, Rockville, MD; Rachael Goodman-Williams*, Wichita State University, Wichita, KS

Learning Overview: After attending this presentation, attendees will be able to describe which victim, offender, and assault characteristics predict a sexual assault victim's decision to report to law enforcement after Sexual Assault Evidence Kit (SAEK) collection. Attendees will gain insight into the concerns that sexual assault survivors may have about reporting to the police and come away with ideas on how to supportively respond to those concerns.

Impact Statement: Since the Violence Against Women Act (VAWA) Reauthorization of 2005, the forensic science community is committed to providing forensic evidence collection for sexual assault victims whether or not they choose to engage with law enforcement. The forensic science community, therefore, has a vested interest in understanding what forensic evidence collection means to victims in the absence of a police investigation so they can provide the best holistic care to this patient population. Understanding the concerns of this patient population may help guide forensic nurses and the collaborative partners they work with to improve systems.

Prior to the mid-2000s, sexual assault victims in many communities could only access a Medical Forensic Examination (MFE) and SAEK collection with law enforcement approval.[1] Practically, this meant that sexual assault victims could only obtain forensic evidence collection if they reported their assault to law enforcement and if law enforcement believed their report had merit.[2,3] This changed with the introduction of the VAWA Reauthorization of 2005, which mandated that sexual assault victims have access to an MFE and SAEK regardless of whether they participate in a law enforcement investigation.[4] Often called "non-report SAKs" or "Jane/John Doe Kits," these alternative reporting options vary by jurisdiction, but all require that victims be able to have forensic evidence collected and have that evidence stored for a period of time, during which they can decide whether they want to report the assault.[5]

The current research analyzed 12 years of medical forensic records from a hospital-based medical forensic unit in the mid-Atlantic United States. The goal of the study was to determine whether victim, offender, or assault characteristics predict whether victims who have an SAEK collected choose to report the assault to law enforcement and release their kit for testing or instead choose to have a non-report SAEK collected and stored. The data set contains the total number of medical records, for all non-report SAEKs collected from 2010–2021 and a systematic random sample of full report SAEKs during the same time period.

These data build upon data presented at AAFS 2023, in which the authors presented results of a qualitative study exploring the reasons why sexual assault victims who choose the non-report SAEK option do not wish to report to police at that time. In conjunction with that prior analysis, these results will provide insight into sexual assault victims' decisions regarding forensic evidence collection and reporting to police after engaging with forensic nurses.

Preliminary results used chi-square analyses to determine whether there was a significant bivariate relationship (defined as $p < .05$) between assault characteristics and whether a sexual assault patient chose a non-report SAK or a full report. In contrast to the general reporting literature, which typically finds that being assaulted by a stranger predicts reporting, we did not find a significant relationship between being assaulted by a stranger and choosing a full report over a non-report SAK, regardless of whether "stranger assailant" was operationalized as someone they had never seen before (i.e., complete stranger) ($p = .27$) or someone they did not know well (i.e., complete stranger or someone known by sight/nickname) ($p = .71$). Excessive force (defined as the patient reporting physical blows, strangulation, being injured by a weapon, or being burned) predicted reporting behavior ($p = .03$) such that 60% of patients whose assaults involved excessive force chose a full report compared to 50% of patients whose assaults did not involve excessive force. Voluntary alcohol or drug use prior to the assault was also predictive ($p < .001$) with 58% of patients who reported no voluntary alcohol or drug use prior to the assault choosing a full report, compared to 44% of patients who endorsed voluntary pre-assault alcohol or drug use. Collectively, these results indicate that while some of the factors related to whether patients choose a full report SAK or a non-report SAK are similar to what has been found in the reporting literature more broadly, there are unique aspects, as well, in need of further study.

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Sexual Crimes; Victim-Offender Relationship; Examination

E8 **Lock-Downs Causing Lock-Ins: The Impact of the COVID-19 Pandemic on Domestic and Intimate Partner Violence Trends Observed by Forensic Nurse Examiners**

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Learning Overview: After attending this presentation, attendees will understand how certain COVID-19 pandemic timepoints affected the patient demographics, assault types, and severity of violence seen by the Forensic Nurse Examiner (FNE) Program at the University of Colorado Hospital (UCH).

Impact Statement: This presentation will impact the forensic science community by serving as a key point of reference and a case example for what types of trends and issues to anticipate in the event of world crisis or pandemic for those who seek health care after experiencing violence. This presentation has important implications for the forensic science community, particularly underscoring how local epidemiological trends in violence type and severity during the COVID-19 pandemic may translate to other areas of forensic science, preparedness, and response.

Background: During the COVID-19 pandemic, worldwide upticks in patient volumes and trends in assault types and severity were observed by the forensic nursing community, advocates, and community service providers.[1] The FNE Program at the UCH is no exception, as their specialized program of care for patients who are experiencing acute and long-term health consequences associated with violence reported being inundated with high volumes of severe cases of intimate partner violence, sexual assault, and physical assault cases.

Methods: To explore these trends quantitatively and how certain COVID-19 timepoints varied in their patient dynamics, this study consists of a secondary, longitudinal analysis of medical records pulled from the FNE Program at UCH between 2018–2021. Multilevel mixed-effects modelling was used to analyze the number and severity of consultations performed by the FNE team at UCH during the COVID-19 pandemic, with random intercepts and slope testing to examine random and fixed effects over time.

Results: Patients in heterosexual relationships seen during/after the second lockdown (7/1/20–12/1/22) report higher Danger Assessment (DA) scores than those before COVID; however, the COVID timepoint did not predict changes in same-sex DA scores. The COVID timepoint predicted the odds of a patient getting an FNE consult for sexual assault. Those who came to the Emergency Department (ED) during the first lockdown had 48% lower odds of being consulted for Sexual Assault (SA). Those who came to the ED during the second lockdown had 55% higher odds of being consulted for SA. Those who came to the ED after the second reopening (2021) had 36% higher odds of being consulted for SA. The COVID timepoint predicted the odds of a patient getting an FNE consult for Drug-Facilitated Sexual Assault (DFSA). Those who came to the ED during the first reopening (after the first lockdown) had 18% higher odds of being consulted for DFSA. The COVID timepoint also predicted the odds of a patient getting an FNE consultation for strangulation. There were no significant differences in the number of human trafficking FNE consults between COVID timepoints; however, the COVID timepoint did impact the odds of a patient getting an FNE consult for elder abuse.

Conclusion: This longitudinal analysis with a robust dataset over multiple timepoints has potential to not only contribute to inform what emergency services can expect during public health emergencies and lockdowns, but also contribute to scientific evidence around who is most affected or at risk for certain types of assault or violence in the region, help seeking behaviors, and trends over time.

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COVID-19; Forensic Nursing; Statistics

E9 Stay at Home, Stop the Virus, Stay Safe? The Impact of the Coronavirus Pandemic on Intimate Partner Violence in England and Wales for Heterosexual Female Victims

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Learning Overview: This presentation will explore the impact of the coronavirus pandemic on the devastating crime of Intimate Partner Violence (IPV). It analyses the effects on the highest-risk population of heterosexual female victims located in England and Wales to understand the impact of specific lockdown regulations. After attending this presentation, attendees will be able to clearly understand five key impacts the pandemic had on IPV: increased control and coercion; anxiety and fear; the mental health of victims; the inability to access valuable services, including forensic examinations and support charities; and increased physical abuse.

Impact Statement: This presentation will impact the forensic science community by increasing awareness of the hidden "IPV pandemic" and attempting to understand and discuss the constant challenges in the global combat against IPV. Forensic scientists and governing bodies must tackle IPV together to ensure the protection of victims and the effective adaption and preparation for future pandemic circumstances.

To date, one in three women globally experience IPV in all forms of abuse, equating to approximately 736 million victims.[1] Sadly, this statistic is on the rise. With the introduction of the Coronavirus Pandemic in 2020 and the lockdown regulations in England and Wales of "stay home, stop the virus, stay safe," there was grave concern about the millions "trapped" in abusive households. In order to protect those suffering and gain understanding for future pandemic circumstances, it is essential that the impact of the pandemic regulations in England and Wales on IPV is explored.

Methods: To analyze the overarching aim—to determine the overall impact of the Coronavirus Pandemic on IPV for heterosexual relationships with female victims living in England and Wales—a strict search strategy using four well-regarded databases and strict inclusion criteria were implemented to analyze the impact. This search strategy enabled the inclusion of nine research papers from different locations in England and Wales and, with a variety of methods, enabled an overall picture of IPV to be analyzed. From this analysis, five key themes were determined to highlight the impact of the Coronavirus Pandemic on IPV for this high-risk population.

Results: Five key themes were determined across all of the included literature: isolation and control and coercion; anxiety and fear by the victim; the mental health of the victim; access to services; and physical abuse and tension. Key results included the use of lockdown regulations by perpetrators to further isolate the victim, an increase in the frequency of abusive events, and an inability to access valuable services, thus silencing the victims.[2,3] Furthermore, victims expressed devastating impacts to their mental health as a result of constant exposure to perpetrators and a lack of "escape routes," alongside the feeling of being trapped to suffer behind "closed doors." In addition, the pandemic introduced a number of additional factors, including increased financial stress, alcohol consumption, and unemployment, which further increased the likelihood of abuse during the pandemic.[3] Furthermore, the effectiveness of services, for example, police, health care, and forensic examinations, were questioned during this challenging time. For example, the ability to recognize signs of abuse from the remote nature of services and the accuracy in collection of evidence by the requirement for victims to use self-swabbing techniques, alongside the ability to effectively provide care and treatment as a result of pandemic regulations.[4] With the National Healthcare System (NHS) not surprisingly focused on the Coronavirus and the underfunding to women's refuges, there was nowhere for victims to seek the help they desperately required, thereby highlighting the immediate need for action to uphold society's duty to protect those suffering from IPV.

Conclusion: The Coronavirus Pandemic in England and Wales had a number of effects on IPV by increasing the prevalence, trapping the victim with a lack of support services, and concerning impacts to victims' mental and physical health. The pandemic impacts have created further difficulty in the ongoing uphill battle to combat the IPV pandemic and to protect those suffering from the devastating crime of IPV.

In the academic field today, there is a clear lack of research into specific countries, particularly England and Wales. Therefore, there is a significant lack of understanding of the impact of specific regulations on IPV. This research is paramount for professionals to prepare for potential future lockdowns and ensure practice is conducted at the highest standard. It is of great importance for all to become aware, adapt, and successfully tackle IPV in England, Wales, and worldwide, whatever the circumstances.

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Intimate Partner; Domestic Violence; Pandemic

E10 We Can Help Health Care Professions Understand and Do More: The Survivors of Intimate Partner Violence in Israel Talk

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Learning Overview: After attending this presentation, attendees will understand that knowledge is fundamental and vital for health care teams, especially nurses, to understand the physical and psychological symptoms and signs of abuse coming from the survivors. The women's stories (with Israel as a case study) will strengthen the knowledge of health care professions worldwide.

Impact Statement: As a part of the forensic science community, this presentation can make an impact by educating other health care professionals to pay more attention to women in distress.

Background: Violence against women is a pervasive phenomenon that affects all sectors and socio-economic layers of society. Domestic violence, particularly violence against women, exacts a significant emotional and physical toll on the victims and the health care system. The consequences of violence against women have both short-term and long-term effects. Killing women due to their gender, often referred to as femicide, represents the extreme form of violence. Unfortunately, despite increased awareness and public discourse on the subject, cases of violence and femicide still occur in Israeli society and worldwide.

Aim: Breaking the cycle of violence by identifying behavioral patterns, warning signs, and similar characteristics to those women for medical professions in an attempt to prevent future cases of violence.

Method: Qualitative research, with in-depth interviews with women who experienced violence from their partners who agreed to participate in the study and share their personal stories. Four main themes emerged from the analysis of the interviews.

Results: The first theme explores the type of household in which the woman grew up, whether she experienced violence during childhood, and whether she had family support. Women who grew up in complex households where they experienced violence also experienced violence from their partners in adulthood. Additionally, those women who lacked familial support found it more difficult to leave the abusive relationship. However, women who grew up in healthy households without experiencing violence still faced violence from their partners, but the familial support they received helped them leave the abusive relationship more easily. The second theme focuses on the tactics employed by the abusive partner, including initial isolation from family and social connections, disconnection from familiar and loved ones, severing supportive ties, and cutting off access to individuals who could shed light on the abusive relationship. The third theme focuses on the behavioral patterns of the abusive partner, the warning signs that women recounted and later recognized, realizing that if they had known them, they may have left the relationship much earlier, even before it began. Examples of behavioral patterns include total control in the relationship, obsession with the partner, and a desire for the relationship to progress rapidly. The fourth theme addresses the process of leaving the relationship, what prompted the woman to rise and leave, and how she suddenly realized that she was in an abusive relationship based on the evidence she received from various sources. An example is a lactation consultant who witnessed one of the women in an abusive situation, established contact despite the woman's resistance, and offered her assistance, which led the woman to stop and realize that she was indeed in an abusive relationship. Another example involves a public health advertisement that portrayed violent situations, prompting another woman to realize that the situation depicted on television was a daily reality in her own home.[1-11]

Conclusion: Professional knowledge is fundamental and vital for health care teams, especially nurses, to understand the physical and psychological symptoms and signs of abuse. They need to be familiar with the phenomenon of intimate partner violence, and femicide. Professional knowledge enables us to identify early signs of abuse and intervene efficiently to stop the next tragedy.

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Femicide; Survivors; Intimate Partner Violence

E11 Forensic Nursing in the Balkan Region

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Learning Overview: While forensic nursing exists as a recognized specialty in the United States, the United Kingdom, and other countries, it is not yet recognized in Balkan countries. The Balkan Academy of Forensic Sciences (BAFS) developed a project aimed at transforming "forensic nursing" into a recognized career for nurses. For this purpose, we formulated a systematic, certified, and four-level training program. The goal of this presentation is to discuss the present efforts related to the establishment of forensic nursing in the Balkan region and present the curricula that has been developed.

Impact Statement: This presentation will impact the forensic science community by introducing the current forensic nursing practice in the Balkans and by sharing the basics of the developed curricula harmonized in nine countries in the region.

Forensic nursing is a specialized field within nursing that combines medical and legal aspects to help victims of violence and trauma. Forensic nurses are valuable assets for analysis, determination, acquisition, and transmission of evidence in health issues that are the subject of forensic cases.[1] The concept of forensic nursing is widely recognized in many countries, but unfortunately not in the Balkan region. Therefore, there is no standardized educational curriculum for any of the didactic levels. Because nurses do not receive professional training on forensic cases, important deficiencies and problems occur during the detection of crime and the criminal.[2] The controversy is that although unrecognized and unspecialized, the forensic practice of nursing is applied in hospitals. It is relevant when nursing care is delivered to patients with trauma, potential liability-related injury, and death. To tackle this problem, BAFS brought together specialists from Turkey, Greece, Bulgaria Slovenia, Romania, Albania, Bosnia-Herzegovina, Kosovo, and Serbia to primarily establish "forensic nursing occupational standards and training." A recognized systematic training program is being developed by producing four-level curricula, certified training modules, and the training of trainers. The project includes activities for the establishment of an international medicolegal network and policy recommendations to ensure recognition and acceptance. Awareness-raising and dissemination activities are designed to maximize the impact. The project is also innovative in its management and evaluation method, which includes a six-stage data-driven approach that incorporates open communication and cooperation between project stakeholders.[3]

Crime has no borders but spreads from borders. It is essential to protect the evidence, which will in turn solve cases. Forensic nurses are an essential part of evidence collection and protection. This demands high-quality education and training.[4,5] This effort will contribute to the standardization and improvement of forensic nursing education across the Balkans, enhancing the health care and justice system. The project also establishes a collaborative platform for experts from other countries to share knowledge, expertise, and best practices in forensic nursing.

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Forensic Nursing; Education; Balkans

E12　Sexual Assault Has a Chokehold on Forensic Nursing

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Learning Overview: After attending this presentation, attendees will understand the components and benefits of forensic nursing examinations, the prevalence and risks associated with domestic violence and strangulation, and how forensic nurses are working toward preventing further victimizations.

Impact Statement: This presentation will impact the forensic community by expanding thinking toward the capability, capacity, and benefits of forensic nurses and the services they provide to various victims, resulting in an increase of an expansion of the type of victims served by forensic nurses and the care those victims are able to receive.

Forensic nursing is no longer limited to caring for only the sexual assault survivor and should not just be a two-hour, one-time visit. This presentation will propose the advantages of forensic nursing examinations for all victims of trauma, and the reasoning behind why forensic nurses are expanding into caring for victims of domestic violence, strangulation, human trafficking, elder abuse, child abuse, and much more. This expansion is important due to the fact that strangulation, in particular, may lead to death, and domestic violence usually involves more than one victim when children are in the home.[1] All types of assaults are occurring pandemically and need to be addressed.[2] Very limited resources, a lack of service availability, and minimal financial backing have led to forensic nursing focusing on sexual assault rather than expanding to caring for all assault patients. Follow-up visits have also been lost, either due to funding or the famous "patient not cooperating." As forensic nurses we have the duty to pursue follow-ups as a continuation of care and to perform the due diligence of ensuring we have provided the education to our patient leading to informed decisions regarding having follow-ups or not.[3] Every interaction with a patient is an opportunity for potentially life-saving education regarding the risks of victimizations and future recurrence, as well as a way to perform an assessment of their well being, and to plan further safety measures.[4]

Forensic nurses have the opportunity to provide holistic health care and evidence collection at the same time. Photographs can provide further details of injuries, including bite marks, corroborate the history of assault, and assist with educating the patient, peers, law enforcement, and others.[5] The camera used for photography can make a difference in the evidence collected, in the medical care a patient receives, and in the legal process. Invert filter photography can highlight what is already there and make it easier to see injuries.[6] More funding, stronger dedication, and greater attention is needed in order to succeed in the expansion in the field of forensic nursing. Victims who suffer from these different traumas need to know that they do not just have to survive what has happened to them, but that they are able to thrive. The focus of this presentation is to explain how an expansion of forensic nursing services to all victims of trauma improves patient outcomes, as well as to expound on the methodologies used during forensic nursing examinations.

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Forensic Nursing; Asphyxiation; Professional Responsibility

E13   The Implementation of a Risk Assessment Screening Tool to Detect Elder Abuse in the Emergency Department

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Learning Overview: After attending this presentation, attendees will be informed regarding: (1) elder abuse and its subtopics; (2) an elder abuse assessment tool (Elder Abuse Suspicion Index© [EASI]) designed for emergency department providers; and (3) United States and global statistics about elder abuse.

Impact Statement: This presentation will impact the forensic science community by providing information about the problem of elder abuse and it subtopics. Attendees will learn about the EASI tool and its importance in emergency departments.

The Emergency Department (ED) is often the only interface some elders who are victims of abuse have with formal health care services because they are less likely to have regular medical follow-up.[1] Physical and emotional abuse has been linked to long-term debilitating psychological effects, including anxiety, depression, and suicidal ideation and death.[2] Elder Abuse (EA) was originally categorized as a component under the family violence spectrum. That spectrum also includes domestic violence and child protection concerns.[2] While our elder population is increasing at a steady pace, there remains a deficiency of quality assessments, prevention programs, interventions, and the quality outcomes associated with their specific abuse.

Although violence has been perceived as a problem among those under the age of 30, EA is on the rise as this population is growing rapidly. The World Health Organization estimates the global population of people aged 60 years and older will more than double from 900 million in 2015 to about 2 billion in 2050.[3] The Centers for Disease Control and Prevention indicates 10% of older adults, those 60 years of age or older, are abused.[4] WHO's current evidence suggests that 1 in 6 older people experience elder abuse. Because EA takes on several forms, including physical, sexual, financial, neglect, and emotional, which are then fueled by the increasing number of persons over the age of 60 and the caregiving demands of the 21st-century family who are providing care to a family member, thus increasing the elder abuse crisis at hand.[5]

As our elder population numbers explode, the complexities of living longer are revealed. The world's awareness of EA is slowly creating those specialized assessments tools, elder protocols, and sustainable prevention strategies. Utilizing Dr. Yaffe's elder abuse suspicion index (EASI) tool will equip providers with a new tool to look at all the subtypes by initiating a six-question assessment during the triage segment of the elders ED visit.[6] Most EDs do not have any specialized policy or protocol to deal with EA. Child abuse and Interpersonal Violence (IPV) protocols took many years to develop into the standards we have today where every child gets an abuse evaluation with every visit.

One of the first child abuse cases was Mary Ellen McCormack. This 1874 case of child abuse was led by her champion Henry Bergh, who at the time was the director of the American Society for the Prevention of Cruelty to Animals. He brought this case up on her behalf and used statutes against cruelty to animals to ultimately remove her from the home.[7] Unfortunately, EA does not yet have a famous champion. Within the past 20 years, this EA crisis developed into a culmination between that of the health care system and the criminal justice system.[1] Elder abuse is currently and quickly becoming a human rights and a domestic violence concern.

The EASI tool is a six-question assessment tool that will raise an ED nurse's or provider's suspicion about EA to a level that may be reasonable for further investigations and or to propose evaluations by social services, adult protective services, or other victim service organizations. At this time, we must rely on the ED staff to implement their good clinical judgment when determining abuse by utilizing available health care bioethics and our states mandatory reporting laws to assess, treat, and report elder abuse cases. There are many screening tools used for elders with no cognitive impairment, but they are long and cumbersome in the ED environment.[1]

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Elder Abuse; Forensic Nursing; Age Assessment

E14   Forensic Nurse Focus: The Care of Persons With Disabilities

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Learning Overview: This presentation is designed to help participants to: (1) appreciate the factors which influence the incidence of violence and abuse within the community of individuals with disabilities; (2) adjust clinical forensic examinations to accommodate the special needs of individuals with disabilities; and (3) utilize information about the victim's social environment and caretaker interactions to identify triggers for abusive acts.

Impact Statement: This presentation will impact the forensic science community by bringing attention to the scope and underlying factors associated with abuse of individuals with physical and mental disabilities. The prevalence and severity of abuse and assault in persons with disabilities will be examined with a focus on the signs of abuse seen in this population. The social environment and behavioral interactions between victims and perpetrators will be discussed as they relate to incidents of intentional abuse. Understanding these factors is imperative for the establishment of plans to conduct a comprehensive forensic examination aimed at detecting evidence of intentional abuse and developing a strategy for preventing further abuse.

Persons with disabilities are a population that are more likely to be the victims of abuse than the overall public. Abuse against persons with disabilities is a crime that is significantly underreported by family and care providers. This unfortunate reality means that no one other than the medical personnel who encounter people with disabilities can detect and pursue justice for the victim. The forensic examiner must elicit specific information regarding the victim's social environment and behavioral interactions with those who commit acts of violence. In addition to understanding the individual's medical or mental health history, special physical and emotional issues must be carefully considered when conducting and documenting findings derived from a comprehensive forensic examination.

This presentation will outline physical and behavioral indicators of abuse that a forensic examiner should note when assessing a person with disabilities since many of these persons may not be able to effectively communicate information about abuse or assault. Statistics of abuse in this vulnerable population and their abusers will be presented.[1-3] Data indicates that an overwhelming number of disabled individuals will experience one or more abusive episodes during their lifetime

The forensic examiner should be an integral practitioner in our emergency departments and clinics where these individuals are brought for care. It is imperative that these nurses appreciate the environmental and behavioral dynamics associated with abuse and possess the knowledge and skills to illicit information and identify indicators of physical and emotional abuse. Comprehensive examinations require a plan that considers limitations inherent in the individual's disability since these factors influence how to successfully interact with them during forensic examination. Clinical environments can be quite overwhelming to this population, and forensic nurse examiners are well-equipped to provide safe, comfortable space for the disabled and to subsequently serve as a professional link between the medical and law enforcement teams.

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Forensic Nursing; Violence Against Women; Crime Prevention

E15 A Forensic Nursing Response to Workplace Violence Using the Worker Well-Being® Model

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Learning Overview: The goals of this presentation are to: (1) inform attendees about an occupational health conceptual model for Worker Wellbeing® when responding to workplace violence; (2) describe the potential relationship between adverse childhood experiences, workplace stressors, including workplace violence, the subsequent coping mechanisms, and worker well-being; (3) define the concept of Worker Wellbeing® in the application to the principles of forensic nursing; and (4) discuss the collaboration of forensic and occupational health nursing professionals to discover how trauma impacts worker well-being.

Impact Statement: The presentation impacts the forensic science community by introducing an innovative model to integrate the principles and theories in forensic nurse practices and occupational health nursing. This presentation demonstrates a model that conceptualizes relationships and frames collaborative activities for the forensic nurse community of practice wishing to incorporate occupational health nursing skills for the purpose of improved worker health and well-being.

Employees work one-third of their lifetimes, which totals approximately 90,000 hours.[1] Work is an active process, becoming a large part of and impacting a person's personal identity.[2,3] How a person perceives themselves and how the world perceives them is often tied to the work they do and how they do it.[2] In short, work impacts a person's body, mind, spirit, and overall well-being.[2,4,5]

The Pew Research Center reports that only half of Americans are happy or satisfied with their jobs.[3] Unemployment is detrimental to the well-being of workers and is often cited as a national benchmark for the wellness of society. Many households cannot survive without a worker in the home.[6,7]

The presentation introduces a concept analysis to establish the relationship between (1) the coping mechanisms of individuals with a history of adverse childhood experiences and workplace violence, and (2) the fundamental elements of the National Institute of Occupational Safety and Health Worker Wellbeing Model®[8]. The Worker Wellbeing Model® is largely based on the Social Ecological Model and acknowledges that worker trauma and coping mechanisms impact worker well-being at the home, community, and societal levels.[8,9] Roy's Adaptation Model nests in the model and demonstrates that individual trauma is influenced by adaptive behaviors, physiologic-physical, self-concept/group identity, role function, interdependence modes of coping function, and worker wellbeing bidirectionally. The model conceptualizes relationships and frames collaborative activities for the forensic nurse community of practice wishing to incorporate occupational health nursing skills for the purpose of improved worker health and well-being.

Worker Well-being® is defined as worker health status, evaluation and experience, policies and culture, and the physical environment and safety climate.[8] At the core is Roy's Adaptation Model, which includes the physiologic-physical, interdependence, role function, self-concept, group identity, and the impact on coping.[10] Together they promote an understanding of the inclusivity of a worker's health that extends from their lived journey through their current environment at home, in a community, and at work.[9]

The vast majority of people are workers at some point in their lives.[7] Many of those workers have experienced events in their lives, including events at work, that impact their well-being at work in profound ways.[11-13] The explanation of occupational health concepts in a forensic nurse practice influences the leadership necessary to improve the well-being and overall health of workers. The concept demonstrates connections between the forensic nurse and occupational health to officially establish a network of collaborators. The concept now has the potential to impact society on a grand scale.

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Workplace; Forensic Nursing; Occupational Incidents

E16   Forensic Nursing Science: Investigation as a Care Component in Trauma and Death

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Learning Overview: This presentation will justify why forensic nurse scientists are ideally prepared to conduct credible inquiries and investigations related to evidence encountered in a clinical environment. Furthermore, it confirms that these processes complement, but do not replace, the criminal investigative processes. Clinical forensic investigation is based on scientific principles and processes inherent to medicine and healthcare. Content of the presentation will confirm the obvious distinction between clinical investigations and criminal investigations.

Impact Statement: This presentation will impact the forensic science community by demonstrating that sharing the evidentiary findings and documentations of forensic nurses is vital in seeking justice for victims of trauma or the deceased with uncertainty of the cause and manner of death. Forensic nurses must strictly adhere to the scientific method to ensure credibility in a court of law during subsequent testimony and cross-examination procedures regarding evidence collection or forensic interpretations pertinent to the investigation. Finally, this presentation will demonstrate the value of investigation as a vital element to initiate plans for comprehensive forensic nursing care.

Forensic nursing introduces investigation as an instrument of the *scientific method* to allow for resolution of health and legal issues that offer the best possible care alternatives to meet the objectives of both. The function of the *scientific method* in forensic nursing practice provides the most accessible scientific results.[1] Investigation is the basis for all evidence-based nursing practice signifying a systematic search, assessment, and application of the scientific method pertaining to health and justice outcomes. In 1991, the American Academy of Forensic Sciences (AAFS) recognized forensic nursing as a scientific discipline eligible for membership defined as "The forensic aspects of health care applied to the scientific investigation and treatment of trauma and death…Pertains to the application of nursing science to legal proceedings which includes recovery of evidence from potential crime scenes and the body." In 1996, the United States Federal Bureau of Investigation (FBI) endorsed forensic nurse examiners and Sexual Assault Nurse Examiners (SANEs) as credible investigators.[2] Forensic pathologists are now employing SANEs to recover medical/forensic evidence and provide postmortem sexual assault examinations prior to autopsy.[3]

The AAFS elevated the integration of Forensic Nursing Science as a new section at the 74th Annual Meeting in Seattle, WA, on February 23, 2022. This advanced position will provide a forum for forensic nurse scientists and those seeking a forensic nursing role. Former President Obama and Congress signed into law that forensic SANEs will assist military physicians to help reduce and prevent sexual violence in the military.[4] This Academy presents an opportunity to develop and disseminate forensic knowledge for nurses as a member of a multidisciplinary team of health and justice professionals. The scientific method is defined as a method of investigation in which a problem is first identified; observations, experiments, or other relevant data are then used to construct or to evaluate hypotheses that support it. "The structure of the entire nursing process is predicated on maintaining a certain state of mind—an investigative, interpretive, dogmatic search for the facts and the truth. This concept is applied to the specific processes involved in the clinical investigation of trauma or death of the forensic patient."[5]

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Trauma; Death Investigation; Forensic Nursing

E17   The Forensic Nurse's Role in Investigating Trauma: Two Clinical Case Studies on Factitious Disorder

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Learning Overview: This is Part II of a two-part series exploring the forensic nurse's role as an investigator of trauma. In Part I, Virginia Lynch clarified the application of the term investigation as a process of the scientific method used as a component of care applied in forensic nursing science, demonstrating the need for clinical-forensic investigation and the purpose of maintaining a suspiciousness factor in the investigation of trauma or death. The intent of Part II is to demonstrate the need for clinical-forensic investigation and the purpose of maintaining a suspiciousness factor in the investigation of trauma or death by reviewing two clinical case studies on factitious disorder. This session will present two different patients with factitious disorder, also known as Munchhausen Syndrome, who presented to the emergency department for sexual assault exams; we will discuss how these cases interface with the current literature and implications for care of other patients with Factitious Disorder who present for sexual assault exams.

Impact Statement: This presentation will impact the forensic science community by informing attendees that forensic nurses may begin with believing, but they must maintain an index of suspicion and give themselves permission to question and investigate. The holistic investigation of fatal and non-fatal trauma presents unique challenges beyond the immediate treatment environs specific to the clinical and humanitarian resources in times of emergent need. Nurses are always patient advocates, but forensic nurses are also advocates for justice.

"Factitious disorder, or Munchhausen Syndrome, is a rare but serious disorder in which someone deceives others by appearing sick, by purposely getting sick, or by self-injury."[1] Factitious disorder is considered an inherently secretive disorder with a generally poor prognosis that often goes undiagnosed and untreated.[2] Patients diagnosed with factitious disorder pose a significant danger to themselves and have been known to cause significant and sometimes fatal self-injury. Alternative incentives such as desire for attention or affection may lead some patients with factitious disorder to seek victim status instead of patient status, thus causing these patients to present for sexual assault exams. This deception by patients poses a real challenge for health care providers in making diagnoses and providing appropriate health care for patients.

Forensic nurses should be prepared to identify patients who may have factitious disorder and be able to provide the services needed without supporting the victim role the patient seeks. Many cases lack definitive evidence. Forensic nurses must maintain a high index of suspicion with factitious disorder patients, pay attention to patient clues, and gather sufficient evidence to assist in the diagnosis and treatment of their patient. There is little by way of literature or training to specifically guide forensic nurses in the care of these diagnostically challenging patients, but investigation is the basis for all evidence-based nursing practice. Forensic nurses should rely on their scientific knowledge base and specialty skill set to provide the highest level of care when investigating factitious disorder and all trauma patients. Forensic nurses are educated in the sciences and have the critical thinking and investigative skills necessary to seek the truth and make sense of the clinical picture when evaluating clinical cases, ensuring the best possible outcomes for trauma patients and the prevention of similar future events that may result in death.

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Forensic Nursing; Trauma; Sexual Crimes

E18   Mapping Forensic Nursing Competencies for Disaster Situations

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Learning Overview: After attending this presentation, attendees will have a better understanding of the fundamental role of the forensic nurse in all aspects of disaster preparedness, prevention, response, and recovery.

Impact Statement: This presentation will impact the forensic science community by demonstrating how forensic nurses are central to all communities contributing to safety and health outcomes.

Introduction: The occurrence of natural and manmade disasters has been a growing global concern. Survivors' health and well-being are impacted due to the devastation from both public health emergencies and natural disasters. Faced with this challenging context, health professionals play a key role in achieving optimal outcomes. Forensic nurses are especially pivotal in safeguarding the public during and after these disasters. Forensic Nursing is a specialty that combines nursing knowledge with principles and practices of forensic science, being widely recognized for its contribution to the investigation of crimes, identification of victims, and assistance to justice. However, the role of forensic nurses in disaster situations has not been fully explored nor understood. The performance of forensic nurses in disasters is complex, involving specific and multidisciplinary skills to respond to the unique challenges presented in these scenarios. Although studies have explored the competencies of Forensic Nursing in other contexts, it was necessary to undertake a comprehensive review to map and synthesize the existing technical-scientific information sources on the specific competencies of Forensic Nursing in disaster situations. The aim is to provide a global view of their central role in response.

Objective: The present study aims to map sources of technical-scientific information related to the skills necessary for the performance of forensic nursing in disaster situations.

Method: A comprehensive scope review was conducted strictly following the methodology established by the Joanna Briggs Institute (JBI). The process was carried out in three distinct stages in which two independent reviewers completed detailed searches in selected databases, as well as in the Gray literature, using keywords and descriptors related to the theme through the mnemonic PCC (population = nursing professionals, concept = skills; and context = disasters). During the initial selection, titles, abstracts, and descriptors were carefully analyzed, with due observation of the eligibility criteria. A total of 28 sources were selected. Then, the selected publications were read in full, data were extracted, and their contents were analyzed.[1-10]

Results: Of the total number of sources selected, it was found that 24 were available in English, 20 of which were produced in the United States. From the mapping of sources, 24 competencies were identified, including: collection and preservation of traces; psycho-emotional care; direct care; link with the justice service; body management; resource management; maintenance of the chain of custody; registration and documentation with photography; training, and epidemiological surveillance.

Conclusion: The forensic nurse plays a fundamental role in all phases of disasters (prevention/mitigation, preparation, response and recovery), working in collaboration with people, families, and communities, through interdisciplinary actions. To achieve more efficient response standards, it is essential that the development of skills for the practice of forensic nursing in disaster situations is incorporated into the undergraduate and graduate training strategies of these professionals.

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Forensic Nursing; Disaster; Terrorism

E19 **The Role of the Forensic Nurse as a New Method of Managing the Quality and Timelines of Carrying out the Autopsy**

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Learning Overview: After attending this presentation, attendees will understand the role of the forensic nurse during the autopsy.

Impact Statement: This presentation will impact the forensic science community by demonstrating the need to integrate the figure of the forensic nurse in the autopsy field in order to improve the quality and timing of the assessment.

The professional figure of the forensic nurse was recognized for the first time in the United States in the 1990s in relation to a case of domestic violence and abuse. This figure has gradually been recognized in other countries such as Canada and also at a European level such as the United Kingdom, Holland, and Sweden.

In Italy, AILF was created in May 2009, later modified in 2016 into the Italian Legal and Forensic Health Professions Associations (APSILEF), which has among its many objectives that of recognizing the figure of the Legal and Forensic Nurse at the legislative and contractual level.

Forensic nursing is a young discipline in Italy, whose profile—unlike in other countries—is not yet well defined. During the completion of the autopsy, this figure, due to specific skills, performs the function of specialist of the discipline who gives full support to the coroner. The specialist activity of the forensic nurse finds a professional position in health care facilities such as penitentiary institutions, centers for minor violence or sexual violence, and also forensic medicine institutes.

We analyzed an autopsy case series in which the figure of the forensic nurse was used and the execution times of the activities carried out were evaluated through a questionnaire administered to the forensic operators regarding the analysis of the quality of the procedure. In the cases examined, all the activities that the forensic nurse performed before and during the autopsy examination were: verification of the functioning of the instrumentation, control of the cold room, documentation, preparation of the body, open-air preparation of the area concerning inspection/collection, instrumentation during the procedure, reporting through photos and videos, collection of biological fluids, weighing and positioning of tissues and organs in containers to be sent to the laboratory, recomposition, and hygiene of the body. The analysis of the questionnaires and the evaluation of the cases examined showed that the correct execution of the subsequent post-autopsy procedures, especially regarding the removal, storage, elimination, and cleaning of the instruments used, allow for the regularity of the examination and in some cases, ensure the safety of operators from probable contamination with any body fluids.[1,2] Furthermore, all these procedures, if carried out with the help of the various checklists, in addition to having a better coordination with the rest of the cooperative team, will also have benefits regarding the reduction of the error rate, complications, improving the safety of the procedure, and speeding up the success of the procedure to be carried out. It is very important that in drafting the checklist, there are all the indications regarding how, when, and how much to carry out an activity: for example, when it comes to biological sampling to be carried out on the body, it is very important to follow the various points step-by-step to best carry out the procedure, therefore what type of liquid to take, from which organ, in which container to keep it, etc.

From what has been said, the impact that the forensic nurse assumes on the timing and methods of autopsy is evident. However, these skills are not limited to strictly clinical and health ones but are extended, thanks to a specific path of specialized studies such as juridical and judicial ones. For this reason, this contribution is considered a complete, multidisciplinary, and essential assistance in the autopsy, forensic laboratory, and instrumental investigations.

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Forensic Nursing; Autopsy; Nursing

E20   Crowd Surge Triggers and Mitigating Factors

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Learning Overview: After attending this presentation, attendees will have a better understanding of how crowd surge events lead to injury and death. Mitigation strategies to prevent these devastating outcomes include human behavior response and engineering improvements

Impact Statement: This presentation will impact the forensic science community by demonstrating how identifying human factors coupled with improving engineering factors can decrease crowd surge morbidity and mortality.

Introduction: Crowd packing, crowd crush, crowd surge, or crowd stampede are all terms used for a group of people moving into an area beyond the capacity of that space. It may occur spontaneously, as a train during rush hour, or it may be planned such as at cultural or religious events, sporting events, political events, or an entertainment venue. One study found that between 1980 and 2012, there were 350 crowd surge incidents, which led to more than 10,000 deaths and 22,000 injuries and are the most common cause of mortality at a mass gathering after heat-related injuries.[1] They are often a response to real or perceived danger, a loss of physical space, or an attempt to receive personal gratification.

Morbidity and mortality are mostly related to two factors, human behavior and engineering deficiencies, but other factors may include direction of crowd flow and duration of high density.

The largest mass gatherings are the annual Hajj in Saudi Arabia and the Klumbh Mela in India, which occurs every three years. The majority of stampedes occur in developing countries from South Asia and Africa, and these have a 7.78 times higher fatality rate when compared to developed countries.

Methodology: A review of research was conducted using the terms: crowd packing, crowd crush, crowd surge, and crowd stampede using Google® Scholar. The search indicated the significant impact of human behavior and engineering deficiencies.

Human behavior that is dangerous beyond capacity of crowd, poor crowd control, and psychological unity or identification with the rest of the crowd may lead to the initiation of the crowd movement. Mass or crowd behaviors refer to those that occur at a group level and are referred to as mob psychology, swarm behavior, collective behavior, and herding. All capture the idea of looking at groups of people en masse and how crowds behave differently from individuals. Moving crowds become fluid with individuals who are no longer in control of themselves.

Engineering deficiencies usually involve a decrease in space for the flow of the crowd. These can be physical barriers and constrictions to movement, blocked or poorly identified exits, vehicles sharing space with pedestrians, and fire hazards from cooking or pyrotechnics. Because of the crowd's density and motion, the group becomes similar to a liquid, flowing as a whole; people in a crowd crush generally find themselves unable to control their movements as they are swept along by the many individuals around them. Children and lower-height people are more likely to be crushed in a crowd.

Results: This presentation will provide a more in-depth review of human factors as well as engineering factors that can make crowd surge dangerous or deadly. Among the mitigation efforts suggested are signposting—about entries, exits, et cetera—and making sure there's enough room for egress.

Technological advancements, notably Geographic Information Systems (GIS), Radio Frequency Identification (RFID), and digital footprints, may lead to early identification of potentially dangerous situations. Cellular telephone and social media are ubiquitous and, when tracked, can identify increases in localized crowd density and movement in real time. We will also propose avenues for future investigation regarding digital footprint and social media to track crowds and potentially provide predictive signs of crowd surge.

Summary and Significance: Understanding the unique and common drivers of mass behavioral responses to such events helps to better detect crowd behaviors, and in turn, develop strategies to mitigate responses to similar future events. Once crowd behaviors are unleashed, they are difficult to reign in suggesting that mitigation strategies should be proactive rather than reactive. Improvements in engineering design may mitigate physical limitations that improve crowd movement

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Crowd Surge; Forensic Engineering; Human Factors

E21   Interdisciplinary Research Related to Sexual Violence and Dating App Usage

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Learning Overview: This presentation will achieve two goals: (1) explain current interdisciplinary work that examines the relationship between dating app usage and online-to-offline Sexual Violence (SV), and (2) teach practices used by researchers to minimize retraumatization when working with victims of sexual assault.

Presentation attendees will understand the link between dating apps and SV and gain knowledge that may allow them to apply our participant safety measures to their own work with victims of SV.

Impact Statement: This presentation will impact the forensic science community by making the connection between dating apps and SV clearer, which may promote further research and enhance practice when providing care to patient-victims. By educating attendees on how to best conduct research with victims of sexual assault, this presentation has the potential to minimize retraumatization of victims of SV who are interested in helping to advance science surrounding sexual violence perpetration.

Sexual violence is a common experience; the Centers for Disease Control and Prevention (CDC) finds that one in four women and one in 26 men will become a victim of rape in their lifetime.[1] Additionally, dating app usage is very popular, as 30% of Americans have used a dating app.[2] A retrospective review of sexual assault medical examination charts found that 8% of 3,413 cases were incidents of technology-facilitated sexual assault.[3] Clearly, sexual violence is a common experience, and there is a connection between dating app usage and SV.[1,3]

In the current research, SV researchers and app developers will work together to create an app that will collect information about how consent is directly or indirectly communicated and/or perceived via partners who meet on dating apps (e.g., via uploaded text or image screenshots, or explanations of consent exchange from participants) and progress to face-to-face meetings. Once the app has been developed, an interdisciplinary team of psychology, human-computer interaction, social work, and forensic nursing experts will evaluate the app's content and capabilities. This process will encourage critique from multiple perspectives, which is intended to ensure that participants retain agency and enable the team to reduce the likelihood of retraumatization and labeling of victims and perpetrators. Following revision to the app based on the data collected from the qualitative analysis, dating app users will be iteratively recruited as participants to donate data via the app about their dating app usage in connection with their in-person sexual experiences. The app will ask questions from a trauma-informed perspective about participant consent practices and perceptions of consent as they relate to dating app usage. Feedback from participants will be used to modify the app to ensure participants feel comfortable. The combination of interdisciplinary expert-informed app development and iterative refinement of the app based on participant feedback will shed light on best practices for conducting research with victims of sexual assault and will help the forensic science community understand the issue of technology-facilitated SV.

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Rape; Interdisciplinary; Research

E22   Shaken Baby Syndrome—A Force to Be Reckoned With: A Case Review

Janean M. Fossum*, Forensic Nurse Consultants, Eugene, OR

Learning Overview: At the end of this presentation, participants will: (1) understand the procedures associated with a comprehensive clinical assessment of a potential victim of shaken baby syndrome (SBS); (2) explain the procedures for precise documentation of subjective and objective findings in the medical record that will subsequently contribute to forensic investigations designed to confirm or refute intentional acts of child abuse; and (3) appreciate the unique role of the clinical forensic nurse in advocating for justice for shaken baby syndrome victims who suffer long-term and permanent emotional and physical trauma.

Impact Statement: SBS not only results in fatalities for many infants, but those who survive often suffer long-term consequences. This makes it imperative to develop programs aimed at preventing this type of child abuse and to promptly identify potential victims who are brought to medical care facilities for assessment of head injuries. This presentation will impact the forensic science community by presenting a case study of a long-term survivor that illustrates key takeaways from the initial encounter and diagnosis, advancing to the present-day life of the victim.

SBS is a form of child abuse seen in children under the age of three with the incidence highest in boys under the age of one. Over the years there have been a multitude of names used to describe this syndrome such as abusive head trauma, shaken impact syndrome, inflicted head injury, or whiplash shaken infant syndrome. The survivors can develop severe cognitive impairment, seizure activity, blindness, and other neurological impairments. When potential victims are encountered in the clinical setting, the medical team must obtain a complete social history, including identification of risk factors. A comprehensive examination of the child and caretaker dynamics must be conducted by a Multidisciplinary Team (MDT), including a forensic nurse examiner.

A brief overview of the classic SBS type traumatic event and a review of the mechanism of action that produces the specific injuries seen will be presented. Most common are injuries such as subdural hematomas and retinal hemorrhage. It is found that the injuries seen can be caused from both the sudden rapid acceleration of the brain and the impact of the child's brain within the skull.

This case study presentation reviews a case from the time of the incident to current time to give a comprehensive picture of what it encompasses and a glimpse into the life of a survivor of SBS. The goal is to emphasize the critical importance of data collection of the MDT that will be important for confirmation of intentional abuse. The severity of injury to those that survive gives rise to the importance of stopping this crime through awareness and ongoing education. The attendee will gain a better understanding of this form of child abuse that results in up to a 40% fatality rate. A review of the socio-economic factors such as education and lifestyle choices seen as well as the mechanism in how these injuries are caused will provide a better understanding of SBS and the injuries seen in the case presented. I have been given permission to share his story.

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Forensic Nursing; Child Abuse; Violence Against Children

E23   "Compressed Baby Head": A New "Abusive Head Trauma" Entity?

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Learning Overview: The goal of this presentation is to inform attendees that the medical and forensic literature is of great help in identifying and recognizing even the most complex cases of child abuse, and the guidelines offer significant help and recommendations to handle the case and the abused child properly. To the best of our knowledge, no similar clinical cases have ever been described in the current literature.

Impact Statement: Medical personnel, pediatricians, and reanimators are the first individuals who may encounter complex clinical cases such as the one presented here. This presentation will impact the forensic science community by informing attendees that they need to know how to properly manage cases and ensure protection for abused infants and children.

Background: Child abuse represents an important issue in the medicolegal and social context. The term "child abuse" identifies multiple nosological entities that have been studied and categorized over the years. Indeed, "child abuse" encompasses abusive injuries of various types and entities, including acts of violence, sexual or non-sexual, as well as cases of neglect in the care, affection, and attention of a minor. In the past few decades, various aspects and mechanisms have been identified in child abuse case studies; however, constant research is needed in the field. After the case presentation, we will present a case of a new entity of abusive head trauma that has come to the attention of medicolegal experts. First, the American Academy of Pediatrics and later the Centers for Disease Control and Prevention (CDC) defined the term Abusive Head Trauma (AHT) to identify the set of clinical manifestations and injury mechanisms associated with abusive head injuries, judging the term to be more appropriate and precise.

Discussion: The trauma analysis performed on the cranio-encephalic district of the baby revealed quite peculiar lesions that led the authors to exclude that the injuries had been solely caused by violent shaking of the baby's head, as suggested by Shaken Baby Syndrome. Instead, we hypothesized that another lesion mechanism had been added to this one, namely latero-lateral cranial compression. The comprehensive and exhaustive analysis of the case led the coroners to present a new possible entity in child abuse trauma, namely "Compressed Baby Head." The presence, in fact, of two symmetrical fracture complexes on the parietal bones, one of which was larger (on the right) and one of which was smaller (on the left), led the authors to assume that this injury was produced by a double coeval impact, from both cranial surfaces, right and left, caused by a compressive action at the cranial level applied laterally.

The first lesion complex, at the level of the left parietal bone, presented a cranial fracture with a linear course, starting from the sagittal suture, with craniocaudal direction. There was an extensive subgaleal hematoma in the parieto-occipital area.

The second lesion complex, at the level of the right parietal bone, presented a fracture complex consisting of three radiating fractures branching off from the "point of impact" located at the level of the right parietal draft: inferior "branch" (A), with a linear course, extending cranio-caudally and involving the internal cranial plateau and the full-thickness diploe; upper "branch" (B), linear course, extending caudo-cranially, to the left, terminating at the posterior third of the sagittal suture; posterior "branch" (C), linear course, extending antero-posteriorly, ending at the lambdoid suture.

Taken together, the three fracture "branches" described above, together with the sagittal suture and the right branch of the lambdoid suture, identified the structure of a quadrangular baseball diamond-like dowel.

The diagnostic criteria proposed for the definition of "Compressed Baby Head" are: bilateral skull fractures or fracture complexes; the presence of bilateral perifractural cranial hematomas and/or hemorrhages, with similar characteristics in terms of extent and timing; cerebral oedema; retinal hemorrhage. The medical and forensic literature is of great help in identifying and recognizing even the most complex cases of child abuse, and the guidelines offer important help and recommendations to handle the case and the abused child properly. To the best of our knowledge, in the current literature, no similar clinical cases have ever been described. Thus, the case's uniqueness deserves to be brought to the attention of experts and the entire scientific community, as well as medical personnel, pediatricians, and reanimators.[1-5]

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Child Abuse; Forensic Analysis; Pediatric

F1   A Cholinesterase-Based Inhibition System for the Detection of Fentanyl and Fentanyl Analogs

Emilynn L. Banks*, Texas Tech University, Lubbock, TX; Giana Biddle, Texas Tech University, Lubbock, TX; Jan Halamek, The Institute for Forensic Science, Texas Tech University, Lubbock, TX

WITHDRAWN

F2 The Determination of Time Since Deposition of Blood Samples Via Alanine Quantification

Estreylla D. Sierra*, Texas Tech University, Del Valle, TX; Jan Halamek, The Institute for Forensic Science, Texas Tech University, Lubbock, TX

Learning Overview: The goal of this presentation is to provide a novel approach to determining the age of a bloodstain while looking and factoring in environmental conditions.

Impact Statement: Blood left behind at a crime scene can reveal valuable information previously unknown, but it is important to continue research and advancements within the forensic discipline to better assist criminal investigations. To this day, there is still a lot of uncharted knowledge about the age of a bloodstain, which can significantly impact an investigation. By providing additional methods of laboratory testing for bodily fluids, this presentation will impact the forensic science community by strengthening the forensic serology discipline within forensic science.

Forensic serology, also known as forensic bodily fluids, is one of the most important pieces of evidence that can be left at the crime scene due to their identifying values. Although bodily fluids like blood can provide an investigation with some answers, there is much more that can be accomplished with their presence at a crime scene. Apart from the detection and determination of bodily fluids and DNA at a crime scene, further research is needed to establish a chronological order of events, specifically the age of bloodstains. Previous approaches have been explored to determine the age of bloodstains but have been proven to be inapplicable for various reasons.[1-5] This research aims to focus on creating a new method to determine the time since deposition of bloodstains.

Utilizing a bio-affinity approach will allow us to achieve this due to blood's denaturing process and the biochemical mechanism of enzymes by quantifying a targeted substrate, alanine, to help evaluate the age of the stain. The analysis was performed and successfully tested in human serum solutions and aged in different environmental conditions for up to a month to mimic different crime scene situations. The samples were then collected and analyzed by applying the appropriate enzymatic cascade to quantify the alanine present, reflecting the amount of time passed since it was initially deposited. In addition, relative humidity and temperature were recorded to reflect and understand the effects of environmental conditions on the serum samples. This information was compared to that of stable conditions to comprehend the ability and effect of determining the age of a blood stain.

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Amino Acids; Bloodstains; Bio-Affinity

F3 **The Determination of Lead (Pb) in Cosmetic Samples by Graphite Furnace Atomic Absorption Spectrometer (GFAAS)**

Kapil Kumar*, All India Institute of Medical Sciences, Delhi, Delhi, India; Ashok Kumar Jaiswal, All India Institute of Medical Sciences, Delhi, Delhi, India; T. Millo, All India Institute of Medical Sciences, Delhi, Delhi, India

Learning Overview: After attending this presentation, attendees will have learned about the exposure and harmful effects of Pb on humans and the method for the determination of Pb in various cosmetic samples using GFAAS.

Impact Statement: This presentation will impact the forensic science community by providing a reliable method for the determination of heavy metal in cosmetic samples using GFAAS.

Background: Various cosmetic items such as talcum powder, lipstick, nail polish, toothpaste, face cream, etc. are used in our day-to-day life. The presence of high levels of Pb in these cosmetic samples is not good for humans as it affects numerous body systems like renal, cardiovascular, gastrointestinal, and hematological. A study was done by collecting random cosmetic samples to know how much Pb is present in them.[1-3]

Objective: Due to the lack of availability of quality control labs in developing countries, the determination of level of Pb or any other heavy metals is not possible for all cosmetic samples selling in local markets. So, the aim of this study is to determine the presence of Pb (as Pb is a very common heavy metal present in cosmetic samples) in various cosmetic samples by using GFAAS.

Methods: Different cosmetic samples were collected from the local market. These samples were digested by using a closed digestion system in a Microwave Digestion System (MDS) by using nitric acid and hydrogen peroxide and run on GFAAS.

Result: Cosmetic samples such as khol (kajal), face powder, lipstick, heena (mehndi), vermilion (sindoor), and nail polish, toothpaste, etc. are the most common samples used in daily life and were used for analysis. The normal limit of Pb in cosmetic samples must be between 10 to 20ppm, but a large amount of Pb (in ppm) was detected in the cosmetic samples.

Conclusion: The presence of a higher amount of Pb more than or equal to 50ppm was found in some samples. These cosmetic items can be very dangerous for us as Pb can easily accumulate in our body from them. The various cases come to us from pediatric emergency departments for suspected lead poising from accidently consuming cosmetic items as these are easily available to small children and are household items.

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Lead; Cosmetic Samples; Graphite Furnace Atomic Absorption Spectrometer (GFAAS)

F4   **Investigating Student Perceptions of Learning in the Flipped Classroom Setting Within a Forensic Science Course**

Lacey Victoria Sharman*, University of Central Florida, Kissimmee, FL; Amanda C. Chee-Awai, University of Central Florida, Oviedo, FL; Tamra Legron-Rodriguez, University of Central Florida, Orlando, FL

Learning Overview: After attending this presentation, attendees will have gained insight into forensic science students' perceptions of the flipped classroom. Mixed-methods research will be presented using survey results from a senior-level forensic science lecture-laboratory course. These results can assist educators in developing a greater understanding of student perceptions of learning regarding the flipped classroom setting.

Impact Statement: This presentation will impact the forensic science community by informing forensic science educators how mixed-methods surveys can provide a better understanding of student perceptions of their learning in the flipped classroom setting and how these perceptions can be used as an important piece of feedback in curricular changes.

A flipped classroom is an instructional method by which delivery of course content occurs online prior to class in the form of videos, readings, posters, etc. Students will then proceed to spend in-class time going over course concepts in more depth with a focus on problem solving, case studies, and group-based work. This instructional method is used to promote student engagement and peer-to-peer interactions, which have been shown to improve students' grasp of the material.[1]

Although a large body of work has been published related to the use of flipped classrooms and active learning in Science, Technology, Engineering, and Math (STEM), thus far, very little research has been completed on the use of flipped classroom learning in forensic science, nor on the impact it has on these students' perceptions of their own learning. There have been other studies surrounding the study of learning within flipped classrooms with subjects such as organic chemistry, but learning in forensic science education is a field that still needs to be explored.[2]

This study is set in a large, public, research institution in the Southeast and explores student perceptions of their learning within flipped classroom settings within a senior-level forensic science lecture-laboratory course. A mixed-methods survey with Likert scale questions and open-ended questions was given to students enrolled in a flipped forensic science course at the end of the semester. The responses to the open-ended questions were coded using thematic analysis by two separate coders. The coders then met to discuss student responses, came to an agreement on codes, and generated a complete codebook to categorize all survey responses. The responses from the survey will be presented, including the identified codes, general themes, and the frequency of the codes and themes.

A preliminary analysis of student responses to the survey shows three emerging themes. These themes include: (1) increased student accountability in the flipped classroom, (2) the ability to learn the material at one's own pace, and (3) the mention of working problems during class time. The Likert data and student demographics from the survey will also be presented. Preliminary analysis of student responses to the Likert scale questions shows that students generally agreed that the flipped classroom helped their learning. When asked if the flipped classroom improved their learning outcomes in the course, 50% of students surveyed agreed, 40% of students selected a neutral response (neither agree nor disagree), and 10% of students disagreed. The data and themes we have encountered throughout this research will not only help educators have a stronger understanding of student experiences in flipped classrooms but also how student perceptions of their learning can be used to enhance their course experience and student learning outcomes.

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Survey; Education; Forensic Science

F5 How Do Kinetic Energy and Firearm-to-Target Distance Influence Fracture Morphology?

Delaney Edwards*, Texas Tech University, Creedmoor, NC; Mariyam I. Isa, Lubbock, TX

Learning Overview: After attending this presentation, attendees will understand the relationships between kinetic energy, distance, and various fracture characteristics obtained in a series of gunshot experiments.

Impact Statement: This presentation will impact the forensic science community by providing data on the presence, quantity, and length of various fracture characteristics and their relationships with muzzle kinetic energy and firearm-to-target distance.

The proportion of homicides involving firearms has increased in the United States from 67.7% in 2016 to 79.2% in 2021.[1-2] Bullets frequently impact bone, with skeletal injuries in over 92% of gunshot homicides.[3] Forensic anthropologists would benefit from improved understanding of gunshot trauma, including how kinetic energy and firearm-to-target distance influence fracture patterns.

The goals of this study were: (1) to conduct gunshot experiments on flat bones using three firearms at two firearm-to-target distances, and (2) to investigate the relationships between kinetic energy, distance, and the resulting fractures.

Thirty bone-in pork shoulders consisting of the scapula and surrounding muscle tissue were acquired from a local butcher. Excess tissue was manually removed, leaving approximately 2.5cm of soft tissue around all bone surfaces. Gunshots were delivered by the first author and an expert shooter. All shots were aimed at the supraspinous fossa on the lateral aspect of the scapula. Full metal jacketed bullets were used in all experiments.

To investigate kinetic energy effects, three firearms generating different muzzle velocities were selected for this study: a .32-caliber pistol (230m/s), a 40-caliber pistol (290 m/s), and a .308 rifle (692 m/s). To investigate distance effects, two trials of 15 experiments were performed. In trial one, each of the three firearms were used to shoot five scapulae from 10cm. Experiments were repeated from 110cm in trial two. Several fracture characteristics were documented, including the presence of cone cracks; presence, and number of radiating fractures, crack branching points, circumferential, and concentric fractures; and Total Fracture Length (TFL).

Cone cracks were produced in 66.67% of experiments. Radiating fractures were present in all specimens and ranged in number from 2 to 15. Crack branching was observed in 33.33% of experiments, with the number of branch points ranging from 0 to 3. Circumferential fractures were obtained in 40% of specimens. No concentric fractures were recorded. TFL ranged from 13.29mm to 271.40mm (99.72 ± 66.90 mm) across the sample.

The results indicate relationships between kinetic energy and several fracture characteristics. The highest energy firearm (.308 rifle) produced significantly more radiating fractures than the lowest energy firearm (.32 pistol, p = 0.007). The .308 rifle also produced significantly more crack branching points than either of the lower energy firearms, including the .32 pistol (p = 0.024) and .40 pistol (p = 0.010). Significant differences in TFL were obtained between all firearms tested (p < 0.02). The .32 pistol produced the shortest TFLs (38.38 ± 21.14mm), the .40 pistol produced intermediate TFLs (100.67 ± 34.76mm), and the .308 rifle produced the longest TFLs (161.12 ± 65.25mm). No significant relationships were found between distance and any of the observed fracture characteristics.

The results of this study contribute baseline experimental data on the relationships between bullet kinetic energy, firearm-to-target distance, and fracture patterns. The number of radiating fractures, crack branching points, and total fracture lengths (TFLs) increased with increasing kinetic energy, while fracture characteristics were similar at the two distances tested. The results of this study can inform variables investigated in future experimental research and may justify further investigation of kinetic energy effects in human material.

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Anthropology; Trauma; Fracture

F6 Success Rates for Positive Identification Using Multiple Fingerprint Methods in a Mid-South Population

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Learning Overview: After attending this presentation, attendees will understand the quality differences in fingerprinting methods (ink, Mikrosil™, powder) to achieve positive identification, including the potential effect of age, sex, race, and manner of death based on a retrospective study conducted at a regional forensic center.

Impact Statement: This presentation will impact the forensic science community by examining the fingerprint method most likely to result in positive identification.

Positive identification is an important step in the medicolegal process. The primary methods of identification used are fingerprint, visual (photograph or in-person), dental, and DNA. Dental and DNA identification are only used when obtaining a fingerprint or locating next of kin for visual identification are unsuccessful. At the regional forensic center where this study was conducted, fingerprinting is often the first identification method and is typically done using ink. When ink is not successful, Mikrosil™ and powder are used. Positive identification may be delayed without fingerprints of sufficient quality. This study examines which fingerprint method results in the most positive identifications, whether ink should be continued to be used as the primary approach, and if there is a difference in success rate between fingerprint methods based on age, sex, race, or manner of death.

A retrospective review was performed for cases between 2017–2022 where ink was the primary fingerprinting method. Of the 7,024 cases brought to the regional forensic center, 6,043 were fingerprinted and of these, 5,384 received a fingerprint "hit" resulting in a positive identification. When examining a fingerprint for quality, the examiner looks at the clarity of features, quantity of features, and specificity of features.[1] The 659 remaining cases did not have fingerprints on file (n=601), or the print was not of a high enough quality (n=58).

In 250 cases, a single finger (right thumb) was fingerprinted using ink, powder, and Mikrosil™. Individuals over the age of 65, under the age of 18, and any case that did not have clear ridges (e.g., decomposed or extensive trauma to the hands) were excluded. An expert latent fingerprint examiner with over 50 years of experience analyzed the quality of the three prints for each case and determined which print would be best to submit for identification. More than one print, for each case, could be selected if they were of equal quality. Chi-square tests were performed to assess whether there was a relationship between fingerprint method and sex, age, race, or manner of death.

In 47 cases, more than one printing method was determined to be of equal quality to another: Mikrosil™ and ink (n=25), Mikrosil™ and powder (n=8), and ink and powder (n=14). All three methods were determined to be of equal value in only two cases (n=2). However, low sample sizes of combined fingerprinting methods prevented a robust statistical assessment. Of the 201 remaining cases, ink was identified as the best quality print (42%, 84/201), followed by Mikrosil™ (39%, 78/201), and powder was last (19%, 38/201). These results were then broken down and compared to the four variables of sex, age, race, and manner of death. There was no statistically significant difference between sex (p=0.786), age (0.796), race (0.387), or manner of death (p=0.250) and fingerprinting method.

These results indicate that the ink method is most successful in producing a quality print for positive identification, suggesting that it should be continued to be used as the primary fingerprinting method. There is no difference in quality of fingerprints based on sex, age, race, or manner of death. Based on this study, ink is recommended to be used as a first approach over Mikrosil™ and powder for all cases.

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Positive Identification; Fingerprint; Ink

F7 Gunshot Defense Wounds: The Need for a Specific Classification

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Learning Overview: After attending this presentation, attendees will understand a case of murder by gunshot with the victim's injuries localized on the forearms and hands attributable to an attitude of self-defense.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of categorizing defensive gunshot injuries as well as in the case of stabbing (defense) injuries.

Defense wounds are injuries that occur when a victim protects themselves during aggression. They generally are localized on the forearms or hands, rarely on the feet or legs, because of the attempt to protect the eyes, face, chest, and brain.[0] The above-mentioned finding is explained by the fact that the head and neck are the targets of choice in the majority of assault cases, and it is the instinct of the victim to raise his/her arms to ward off the attack to protect the vital organs. These wounds could have a considerable medicolegal significance because they indicate that the victim was conscious, at least partly mobile, and not taken completely by surprise.[2]

Defense wounds in firearm injuries are infrequent and hard to classify, except the stab wound, which has an exhaustive description in literature. For this reason, gunshot defense wounds lack an exhaustive taxonomy. In some shooting deaths, there is an entrance and an exit wound in the forearm or hand, then the projectile repenetrates the trunk or the head.

The topic of this work is to present two cases of murder with multiple firearm injuries, referring to the defense attitude of the victim during the homicide aggression. In the first case, a 50-year-old man was shot by a smooth-bore shotgun and two shotgun shells were found next to the dead body. The second case involved a 33-year-old man who was shot by a gun and two 38mm pistol bullets were found during the autopsy.

This presentation focuses on an analysis of the gunshot wounds and stab wounds in reference to the defensive attitude of the victims.

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Ballistics; Murder; Wound

F8 **A Raman Spectroscopic Analysis of Drugs in Contaminated Fingermarks: The Effect of Temperature and Time Since Deposition**

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WITHDRAWN

F9   Dating Traumatic Brain Injury With a Dura Mater Immunohistochemical Assay: An Ongoing Study

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Learning Overview: The goal of this presentation is to introduce an innovative technique for studying the post-traumatic interval in traumatic brain injury.

Impact Statement: This presentation will impact the forensic science community by describing an innovative immunohistochemical approach to traumatic brain injury. We will show how immunohistochemistry can have a wider field of application in forensics.

After attending this presentation, attendees will be aware of an innovative method to date traumatic brain injury by applying immunohistochemistry to the dura mater. This presentation will impact the forensic science community by showing a reliable method to estimate the survival time interval after a traumatic brain injury (post-traumatic interval). The attendees will discover new applications of immunohistochemistry in forensics, which may have a relevant impact in the field.

Traumatic brain injury is a common cause of violent death and a key issue for forensic pathologists. The primary damage is represented by cell necrosis/apoptosis, while the secondary damage results from the local inflammatory response. Several authors used immunohistochemical assays to date the traumatic brain injury by using several markers of the local inflammatory response of the central nervous system. The main limitation of immunohistochemistry in forensics is the protein alteration due to cadaveric autolysis and putrefaction.[1,2]

The present study aims to use immunohistochemical stains on samples of human dura mater that is made by connective fibrous tissue and shows a certain degree of structural preservation after death. The authors tested three markers (CD15, CD38, and CD3) expected to be expressed by the central nervous system tissue respectively 10–20 minutes, 3 hours, and 2 days after a traumatic brain injury. The markers identify three cells involved in the inflammatory response: granulocytes, macrophages, and T-lymphocytes. The ongoing study is including autoptic samples collected from subjects with traumatic brain injury (cases) and without it (controls). This report will present the promising preliminary results and the future perspective of the research.

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Dura Mater; Traumatic Brain Injury; Immunohistochemistry

F10   An Evaluation of Explosive Odor Signature Persistence on Training Aid Substrates

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Learning Overview: After attending this presentation, attendees will gain an understanding of the chemical odor profile persistence of explosives, namely Composition 4 (C4) bulk material, using commonly utilized substrates such as Getxent tubes for impregnation purposes. Odor profiles after impregnation times with the explosive C4 will be monitored using headspace chromatographic methods to monitor key odor volatiles over a period of up to eight weeks of shelf life at standard laboratory conditions to understand odor fate for optimized canine detection performance and training purposes.

Impact Statement: This presentation will impact the forensic science community by providing a scientific foundation in the understanding of odor persistence utilizing recent developments in impregnation substrates such as polymer tube technology. Understanding odor profile stability on these impregnated materials over time provides forensic personnel within law enforcement, military, search and rescue, and other first responding agencies who utilize these training aids for canine detection application with a better understanding of odor fate and dissipation concepts in order to develop optimal training regimens to better prepare canine teams for operational deployments.

Explosives threaten the well-being and lives of civilians, fire service, law enforcement, and military personnel daily, with injury and fatality reports from the United States Bomb Data Center (USBDC) totaling 127 in the year 2021.[1] The need for detection of these explosives is imperative, with canines being most utilized as bio-detectors for their ability to locate and signal target explosive odors.[2] However, certain explosives are not available to canine handlers for training due to safety regulations, transportation protocols, and other restrictions related to budget or resources, so other training methods are required.[3] One such option is a non-pseudo alternative.[3] Non-pseudo alternatives include encapsulating the true (pure) material, diluting the true material, or impregnating a material by adsorbing the true material's Volatile Organic Compounds (VOCs) from its headspace.[3] The advantage of impregnating a material via headspace of a true material is ease in transportation and handling of the adsorbent, therefore bypassing any risk of detonation or safety restrictions.[3] Getxent polymer tubes is a recently developed impregnation alternative available to handlers, as the tubes are an impregnable material, previously cited in peer-reviewed research, including odor from Triacetone Triperoxide (TATP)[4], smokeless powder, and biomedical matrices.[5,6] However, to date, no research has been openly disclosed studying their ability to retain primary VOCs of C4 bulk material and more so, the persistence of key odor volatiles retention across time on this targeted substrate. The goal of this research is to evaluate different impregnation times of the Getxent technology to monitor C4 VOC retention and subsequent persistence across an eight-week time period under standard laboratory conditions.

Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) proved to be a reliable instrumental approach to monitor key odor volatiles emitting from the impregnated polymer tube devices after exposure to C4 bulk material at different collection times. The study provides a first approach to instrumentally validating impregnation-derived training aid devices for C4 and provides a qualitative and semi-quantitative platform to monitoring odor markers from explosive materials of interest to canine and sensor detection of energetic material.

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Absorption; Canines; Solid Phase Microextraction

F11   The Opioid Revival: A Case Report Where Toxicology Meets Music

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Learning Overview: The goal of this presentation, starting from an unusual case in toxicological matters, is to stimulate some reflections on the close connection between the restrictive measures of the pandemic, the social and psychological unease of young people also conveyed through music, and the dynamics regarding the supply of substance abuse.

Impact Statement: This presentation will impact the forensic science community with the present case, which started from an interesting toxicological analysis of the combined use of psychotropic drugs and synthetic opioids, and intersects with new social and public health problems: the pandemic, isolation, the increase in psychiatric pathologies, and the increase in the abuse of prescription substances. Numerous cases of deaths resulting from overdoses from new substances, if deepened, could bring out new social dynamics related to drug use and new emerging problems.

In September 2021, a 19-year-old boy was found dead in his bedroom. During the inspection of the scene, the judicial staff found cartons of drugs with empty blisters of alprazolam, oxycodone + paracetamol, quetiapine, sertraline, and two bottles of codeine syrup. The judicial authority ordered an autopsy to detect the cause of death. The clinical history examination showed a story of borderline personality disorder and a suicide attempt by stab wounds one year earlier. The boy had embarked on a path of psychotherapy, then interrupted and occasionally resumed electronically during the third wave of COVID-19. The presence of codeine bottles, which the boy's mother reported were not part of his standard therapy, and carbonated drink, raised the suspicion of lethal intoxication with "Purple Drank," a new recreational drug composed of codeine, promethazine, and soda.

During the autopsy, a urine sample and a blood sample from the inferior vena cava were taken for the execution of appropriate toxicological tests. The search for substances was performed by qualitative analysis with Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Mass Spectrometry (LC/MS). Quantitative analysis was performed by LC/MS. Investigations did not find codeine but rather a lethal cocktail of multiple drugs: alprazolam [344ng/ml], quetiapine [47ng/ml], sertraline [290ng/ml], and oxycodone [154ng/ml]. The results of the toxicological investigation showed that the blood concentrations of alprazolam (0.344mg/L), sertraline (0.290mg/L), and oxycodone (0.154mg/L) exceeded the therapeutic range. The simultaneous intake of alprazolam, sertraline, and oxycodone is suitable to determine an inhibitory effect on the Central Nervous System (CNS), with associated severe respiratory depression and consequent death. The doses found in this case were compared with those found in other studies in the literature. As the "Purple Drank" that was suspected at first, oxycodone and other opioids have been the protagonist of a growing interest among young people in recent years, becoming the new "Saturday night high."

Oxycodone; Opioids; Drugs

F12   Establishing the Manner of Death: A 3D Reconstruction of a Case of Hanging

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Learning Overview: The goal of this presentation is to underline the importance of the 3D reconstruction of a crime scene for establishing the manner of death.

Impact Statement: This presentation will impact the forensic science community by encouraging attendees to perform further in-depth studies concerning 3D reconstruction techniques.

Establishing the manner of death is one of the most challenging tasks for forensic pathologists. This is the case of a 24-year-old woman who was found dead in the early morning on a flyover. The body was sitting on the ground with the back leaning against the wall. The neck was encircled by a white phone charger cable knotted to the staircase's handrail.

According to witnesses, the victim argued with her boyfriend and tried to jump out of his car while coming home from a wedding party the night before. At approximately 12:30 a.m., she left home alone with her phone charger in her hand.

Due to such self-harm behaviors, the first hypothesis was suicide by hanging. However, the ligature mark encircled the neck twice and crossed horizontally above the thyroid cartilage. The ligature crossed immediately beneath the thyroid cartilage and encircled the neck horizontally, and the two ends of the cable overlapped forming a cross-over point in the front-right of the neck. Then the ligature passed obliquely through the nape where it gradually disappeared, forming a gap in the mark. The mark was sharply defined, stiff, yellow, and parchment-like.

The hanging mark is usually a single mark slanting downward from the knot to the noose and becomes shallowest near the knot, especially if the noose is knotted with a hold knot.[1] On the other hand, a typical strangulation mark completely encircles the neck many times without gaps following a horizontal plane overlying the larynx or upper trachea.[2,3] So, the geometry of the ligature mark in the present case raised some doubts about the manner of death. The 3D reconstruction of the occurrence confirmed that the hanging was feasible without any external intervention.

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Forensic Science; 3D Technology; Hanging

F13   Electric Scooter-Related Accidents: A Possible Protective Effect of Helmet Use on Head Injury Severity

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Learning Overview: After attending this presentation, attendees will understand the injury characteristics in accidents involving electric scooter use, the importance of dedicated lanes for these means of transport, and the role of helmet use in reducing the fatality rate.

Impact Statement: This presentation will impact the forensic science community by helping to assess new risks for the community. In particular, in the case at hand, it emphasizes the need to extend the helmet obligation to all ages in order to reduce the number of e-scooter-related injuries.

Background: At the end of 2017, rental electric scooters (e-scooters) were first introduced in the United States as a new means of transport.[1] Despite electric scooters use proliferating in Italy since 2019, actionable data regarding injury incidence and patterns associated with electric scooter accidents are limited.[2] This study aims to analyze the rate and clinical and demographic features of electric scooter accidents accessed in the Emergency Department (ED) of Fondazione Policlinico Universitario A. Gemelli IRCSS in Rome, Italy.

Methods: This retrospective study included all patients older than 18 years riding an electric scooter admitted to the ED from June 2019 to April 2022. Personal data, injury circumstances, helmet use, and health data were collected. Abbreviated Injury Scale (AIS) codes of all diagnoses were recorded, and the Injury Severity Score (ISS) was calculated for each patient.

Results: The analysis includes 92 patients admitted to the ED due to an e-scooter accident during the study period, with an increase in years. The median age of the patients was 28 (Interquartile Range [IQR], 19.5–39), with 16 (17.4%) female patients. Thirty-two patients presented bone fractures, especially concerning the extremities and the face districts. The medical history shows that only one patient wore a helmet. In most cases (94.6%), the injury was caused by either a fall or loss of balance. In addition, the median ISS in the study cohort was 3, with the highest AIS represented by AIS Pelvic-Extremity and AIS External. Finally, statistical significance was found between AIS Head-Neck and severity of trauma.

Discussion: E-scooters have become a familiar sight in cities worldwide recently, with many new companies renting them for use. But their arrival has also brought new safety concerns. The most frequently affected region was the extremities, especially the upper limbs like those observed in specific non-motorized mobility devices, which present a delicate balance.[3] In addition, among the upper limb fractures, the most commonly affected region was the hand: this could be mainly accounted for by the fact that these vehicles are also used on urban roads that may have a road surface that has potholes that can easily alter the static of the car and cause the fall. For these reasons, these modes of transportation should only run on dedicated streets such as bike lanes and not on urban roads. Although most injuries reported are minor, the meager rate of helmet use is critical. In fact, the correlation between AIS head-neck and trauma severity was statistically significant, so helmet use could reduce trauma severity. However, despite increased restrictive measures against these vehicles in Italy, helmets are still not compulsory for individuals over 18 years of age.[3] Implementing compulsory helmet use for electric scooters for all ages could be a protective factor for a patient incurring head trauma on urban streets.

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Electric Scooter Accidents; Injury Patterns; Helmet

F14 Contention and Restraint: Medicolegal Aspects of a Controversial Practice

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Learning Overview: The purpose of this presentation is to highlight the lack of clear legislative references in Italy about the issue of patient restraint. Such gaps lead to an overly variable interpretation about liability profiles of health care professionals. This condition places Italy in contrast to the United States context, where such an aspect is highly regulated by local, state, and federal laws and other health care accreditation organizations.

Impact Statement: This presentation will impact the forensic science community as it is intended as a call for health care personnel to use restraint in a reasoned and transparent manner, with the documentation in the medical records of all procedures adopted. This would allow for an objective assessment of liability profiles in litigation cases. However, the lack of clear directives in Italian law about this topic makes such assessment anything but straightforward.

"Is it lawful to restrain a patient?" The term "restraint," long debated, refers to any means of physical constriction of a person's freedom of movement or normal access to his or her body.[1] Despite the paucity of knowledge about the topic, it is well known that the use of restraint is far from sporadic in Italian social-health facilities, especially in psychiatric and geriatric settings.[2-4]

Effectively, restraint in the medical-health care setting is neither provided for nor governed by any rules in the current Italian legal system. The normative references today are based on the articles of the Constitution such as Article 2, Article 13, and Article 32. In ordinary legislation, other normative references come from the second subparagraph of Article 40 of the Criminal Code. In addition, ethical codes of some health professions have indicated some principles about restraint, even with different accents and tones.[5-7] In this normative silence, in 2018 the Court of Cassation expressed itself in its sentence 50497/2018 inherent to the Mastrogiovanni's court case, in which the Supreme Court enunciated some pivotal principles. Consequently, in such a setting, different profiles of liability can be configured for the health care professional, both in civil and criminal law.

Therefore, two illustrative cases of medical-legal litigation are presented, showing two opposite eventualities. The first is a case of an incorrect way of application of restraint: an elderly subject, suffering from chronic encephalopathy with moderate cognitive impairment, was admitted for widespread pain. During hospitalization, he developed episodes of psychomotor agitation, during which he removed his bladder catheter, causing himself hematuria. In the medical records there were no annotations regarding any restraint, but the nursing diary reported: "calmer patient, means of restraint removed after doctor evaluation." Thus, the relatives decided to sue the hospital because the restrain was applied without the consent of the patient himself and in absence of any kind of information to the family.

The second concerns a case of erroneous application of the means of restraint. A 54-year-old patient was hospitalized for cerebral ischemia with agitation and left hemiplegia. He was admitted with a Fall Risk Scale indicative of "high risk." After an overnight episode of psychomotor agitation, treated pharmacologically without success, the doctors decided to use mechanical restraint. The following day, the doctors reported that the patient was confused but calm, so they decided to remove the restraining devices (straps, rails, etc.). However, the patient, during the usual dialysis session, fell out of bed, resulting in head trauma with a fatal outcome.

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Restraint; Professional Liability; Forensic Medicine

F15   **"One Ring to Find Them All": The Potential of Scanning Electron Microscopy (SEM) With Microanalysis/Energy-Dispersive X-Ray Spectroscopy (EDS) in a Murder Suspect Case**

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Learning Overview: Cases of falling from a height are well known in the forensic world. This presentation illustrates a case of a fatal fall from a height under suspicious circumstances in which the main query was: homicide or suicide? After attending this presentation, attendees will understand the importance of exhaustive forensic investigation and comprehensive radiological, genetic, and electron microscopic analysis.

Impact Statement: This presentation will impact the forensic science community by showing the usefulness of innovative instrumental investigations such as SEM with microanalysis/EDS and its ability to enhance the work of forensic pathologists and direct investigations.

A 32-year-old man fell out of a window on the fourth floor of a building. Medical staff responded to the scene and found the man in a comatose state (GCS 3). He was then taken to the emergency department where he underwent therapeutic treatment and diagnostic imaging exams that revealed multiple fractures and internal injuries. A few hours later, the man died.

An investigation by law enforcement revealed that the house was being illegally occupied by a couple and used for drug dealing. The people who were present agreed on the same version of events: the man had taken a lot of drugs, was agitated, and had thrown himself out of the window.

Prior to the autopsy, a Computed Tomography (CT) scan of the body revealed multiple fractures of the skull, ribs, pelvis, humerus, and femur. The autopsy confirmed extensive polytrauma. On the face, in addition to seven other blunt injuries, there was a sharp injury under the lower lip that could not be attributed to the fall from height.[2] The lesion was removed (skin patch) and analyzed with different devices and analysis. Toxicological analysis was positive for cocaine and morphine, but the blood concentration was not fatal. The cause of death was determined to be contusive polytraumatism after precipitation. This type of injury can be the consequence of the secondary impact from a great height (at least 12m) onto an asphalt surface.[1-3]

Further investigation confirmed the clear edges of the lip injury (stereomicroscope and digital microscope) and identified the presence of 1,291 particles of inorganic material inside the wound (tomographic analysis and SEM with microanalysis/EDS). Among these, in addition to substances considered common or ubiquitous, residues of platinum and silver, materials used in jewelry, were found, as well as particles of tin, commonly used in the protective surface tinplating of ferrous alloys (tinplate) and other metals and, in some particular products, also for coating cutting blades.

Analysis carried out by SEM on some rings belonging to the subjects who were present in the apartment on the evening of the man's death revealed the presence of the substances found inside the above-mentioned lesion. The subungual sampling also showed a mixed trace with the presence of DNA from one of the subjects present in the house.

These findings introduced doubt about the accidental/suicidal/homicidal nature of the man's fall and led to further investigation of the subjects present in the house.

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Scanning Electron Microscopy; Fall From Height; Sharp Injury

F16   **"All Women Are Equal, but Some Women Are More Equal Than Others": The Italian View of Cesarean Section on Maternal Request Between Women's Self-Determination and Malpractice**

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Learning Overview: This presentation shows the Italian situation regarding the choice of childbirth, particularly on the Cesarean Section on Maternal Request (CSMR). After attending this presentation, attendees will understand that women do not have the same choice in childbirth due to the lack of specific guidelines. An increase in medical-legal litigation often follows this problem.

Impact Statement: This presentation will impact the forensic science community by demonstrating the need to develop guidelines for a shared and informed choice of mode of delivery to ensure equality and dignity in the treatment of women and to reduce litigation related to this issue.

Today, the rate of cesarean sections is increasing worldwide. An underlying important phenomenon is CSMR. CSMR is an elective cesarean section without medical and/or obstetric indications.[1] The CSMR rate is often unknown but increasing. It ranges from 1 to 48% in public hospitals and more than 60% in private hospitals.[2] Women claim their right to choose how to give birth. Forcing a woman to undergo a vaginal delivery can affect not only her physical well-being (e.g., major vaginal lacerations) but also her psychological well-being (e.g., tocophobia) and, therefore, her health. This falls under the concept of "obstetrical violence."[3] This term includes all abusive and/or disrespectful acts by health care professionals (doctors, midwives, nurses, and health care personnel) toward women throughout the reproductive sphere.

In general, obstetric violence occurs when a woman's physical integrity and, more generally, her health—understood as psycho-physical, mental, and social well-being (as defined by the Constitution of World Health Organization [WHO], 1946)—is violated. Over the past 20 years, various countries have developed national and international guidelines for recognizing a woman's right to choose childbirth, although clear guidelines in this direction have not been established. In Italy, the rate of CSMR is about 9% of all cesarean sections, but it is underestimated.[4] According to the Italian guidelines of the Superior Institute of Health (SNLG-ISS) of 2012 (updated in 2016), a maternal request without clinical reasons does not represent an indication for a cesarean section.[5]

The fear of medical-legal repercussions influences the decision-making process on the mode of delivery, especially when the mother requests a cesarean section. According to Law 219/2017, the doctor and the patient must establish a "therapeutic alliance" based on informed consent, in which the woman's autonomy meets the doctor's competence, autonomy, and professional responsibility.

In Italy, there is no informed consent to natural childbirth.[6] CSMR is currently contrary to good care practice, so the doctor has no professional obligations and is entitled to refuse the request for a cesarean section. However, the legislation does not explicitly state that the doctor must refuse such a request. As a result, choice is not available to all: some women receive CSMR, while others are denied it. In addition, doctors often work in fear of litigation due to the lack of guidelines. In a perspective where women claim their right to health, not only physical but also psychological, the need to develop informed consent for childbirth in general and a guideline on CSMR is unavoidable. This is important both to ensure equal opportunities for all women and to clarify the medical responsibilities and the legal consequences of this choice.

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Cesarean Section; Obstetrical Violence; Psychological Health

F17   **The Postmortem Identification of Decomposed Bodies Using Mikrosil™ Fingerprint Impressions**

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Learning Overview: After attending this presentation, attendees will be familiar with Mikrosil™ Casting Putty. It is used to create a cast of a finger that renders enough fingerprint ridge detail so that a fingerprint or latent print examiner can make a positive forensic identification from deceased individuals in an active state of decomposition. Fingers are as subject to decay and putrefaction as is the rest of the body, dependent on external conditions and time since death. Fingerprint powder and ink are not the most suitable method for fingerprint collection under these conditions. This study indicated that Mikrosil™ Casting Putty is the most reliable and suitable method for fingerprint collection on those cases in an active-to-advanced stage of decay.

Impact Statement: This presentation will impact the forensic science community by demonstrating the effective use of Mikrosil™ Casting Putty when it comes to forensic identification on deceased individuals in an active stage of decomposition. Currently, the means of identification for these deceased individuals can be invasive and time consuming. Mikrosil™ Casting Putty is not only a reliable and efficient process, but the cast can be used for establishing a positive identification.

Introduction: This research quantitatively compared Mikrosil™ to the most common fingerprinting methods for deceased individuals, those being fingerprint powder, fingerprint ink, and after finger rehydration in their ability to render a positive forensic identification from deceased individuals who were in an active stage of decomposition. This study establishes the reliability and benefits of Mikrosil™ Casting Putty.

Methodology: The phrase active stages of decomposition are divided into three categories: mild, moderate, and advanced. A list was generated of those who were in an active stage of decomposition who needed to be forensically identified between October 2022 and December 2022. The fingerprinting method that can render the best ridge detail based on an individual basis will be utilized to collect the fingerprint impression. All fingerprint impressions collected will be sent to a latent print examiner, who will examine the impression to generate a positive (or negative) forensic identification. All subjects that have been identified will be documented and attributed to the fingerprinting method utilized. All fingerprint impressions were then sent to California Identification (CAL-ID) and examined by a latent print examiner to generate an individual identification. If the result from CAL-ID is "negative–not suitable for comparison," then a different fingerprinting method from the three categories was utilized. This second impression sent to CAL-ID will be documented and attributed to the fingerprinting method utilized and how many were positively identified by fingerprint ink, fingerprint powder, and Mikrosil™ for quantitative and qualitative analysis.

Results: Mikrosil™ Casting Putty can create a fingerprint impression of a deceased individual in an active stage of decomposition that will lead to a positive forensic identification. A total of 685 cases were created between the months of October to December of 2022. There was a total of 63 people who were in a stage of active decomposition. Of those 63 decedents, 38 were identified through Mikrosil™ casting putty. One decedent was identified through fingerprint powder, 20 decedents were identified through fingerprint ink, and 4 decedents required advanced methods for identification.

Conclusion: Mikrosil™ Casting Putty is a more reliable fingerprinting method that can be used on those who are deceased and in an active stage of decomposition when compared to the common fingerprinting methods, and it rendered the most positive forensic identification. It also avoided the need for more invasive and time-consuming fingerprinting methods that would have had to be utilized otherwise, such as the finger rehydration process.

Stages of Decomposition; Fingerprint Identification; Mikrosil™ Casting Putty

F18 Fungal Interaction in Mammalian Decomposition in the New Jersey Pine Barrens

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Learning Overview: This presentation will demonstrate the importance of understanding fungal interaction in the decomposition of mammalians within the New Jersey Pine Barrens for the use of death investigators. After attending this presentation, attendees will better understand another factor that can aid in accelerating or decelerating decomposition.

Impact Statement: The aim of this research is to study the mammalian decomposition rates in the presence of fungi native to the New Jersey Pine Barrens. This presentation will impact the forensic science community by providing information regarding fungal interactions within decomposition in areas of acidic soil. Because biological processes are different depending on location and environmental factors, this provides a basis for New Jersey and the eastern side of the United States regarding fungal interaction and how it can affect Total Body Score (TBS) interpretation, which affects Postmortem Interval (PMI) calculations. The specific research questions of this study are: How do fungal species interact in mammalian decomposition? Does the presence of these fungi accelerate the decomposition process? How does decomposition compare between burial in a soil matrix and surface deposition? Does a soil matrix accelerate or inhibit decomposition? It is hypothesized that in the presence of fungi, the mammalian specimens will decompose at an accelerated rate compared to a sterile soil matrix and compared to surface deposition.

Soil derived from the New Jersey Pine Barrens was used for this study as was fungal species specific to this environment. As cited in various scientific articles, the main area of fungal focus during decomposition is ammonia metabolizing fungi and/or keratinase metabolizing fungi.[1,2] For this study, keratinase metabolizing fungi that can be found in the New Jersey Pine Barrens was chosen, in particular: *Fusarium oxysporum, Trichoderma viride,* and *Chrysosporium keratinophilum.*[2-4] The soil used for the study was dug up from a wooded area in Winslow Township, NJ. The soil consisted of soil horizons O, A, and B mixed together to simulate the burying of a body. The mammalian specimens chosen for the study were mice. In particular, 30 mice were chosen and split into five categories and given a subject number: negative control one, negative control two, positive control, specimen species one, specimen species two, and specimen species three. Negative control one were mice left unburied to simulate surface deposition. Negative control two were mice in sterilized soil. Positive control were mice in unsterilized soil. Specimen species one were mice in *Fusarium oxysporum*-inoculated soil. Specimen species two were mice in *Trichoderma viride*-inoculated soil. Specimen species three were mice in *Chrysosporium Keratinophilum*-inoculated soil. Decomposition of the mice was documented on days 0, 3, 6, 9, 13, 20, and 30. Documentation includes the weight of each subject number to track loss of mass, the soil pH, and TBS. Results of the study showed that the fungi did have an impact on the decomposition process.

This research will greatly benefit the forensic community by allowing crime scene investigators to have more available methods for detecting bodies and pathologists to determine time of death. Research such as this has been sought out throughout the forensic and mycology fields. Authors such as M. Tibbett and D.O. Carter have stated in their book *Soil Analysis in Forensic Taphonomy*: "We hope mycologists and forensic scientists will collaborate to take the concept of forensic mycology forward into an accomplished and effective forensic science tool." [1]

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Decomposition; Soil; Fungus

F19   Deadly Fettuccine With Bolognese Sauce: A Case of Sudden Death in a Woman Suffering From Achalasia

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Learning Overview: After attending this presentation, attendees will be aware of a particular type of death caused by compression of the mediastinal structures due to a food-filled megaesophagus in a patient affected by achalasia.

Impact Statement: This presentation will impact the forensic science community by reporting a multidisciplinary approach allowing a thorough postmortem diagnosis of a rare disease such as achalasia.

Achalasia is a rare disease whose incidence ranges from 0.3 to 1 case per 100 000 per year. The most frequent cause of death is malnutrition, followed by aspiration pneumonia, hemorrhage, and tumor.[1] The average person usually begins to suffer from typical symptoms such as regurgitation, chest pain, and dysphagia that worsen with time; because of the slow onset of this illness, it usually takes many years to establish the diagnosis and to initiate the proper treatment. Most of the subjects affected by achalasia must undergo repeated invasive and risky procedures to improve their symptoms.[2]

In a small town in northern Italy, a 61-year-old woman was found unresponsive on the floor of her bedroom. Given the presence of post-mortal phenomena, the emergency team could only ascertain her death. In order to clarify the cause of death, the local sanitary authority contacted the woman's general practitioner, who stated that his patient only suffered from gastroesophageal reflux disease and stomachache (pyrosis). A forensic autopsy was performed, followed by histological and immunohistochemical investigation. At the external examination, some undigested food was noticed in the oral cavity in addition to congestion and cyanosis of the head, neck, and upper torso. During the autopsy, an enlarged spindle-shaped esophagus with a circumference of 19cm and considerable wall thickening was observed: at its opening, a bolus of undigested food material of roughly 500g was found, whose composition was quickly identified as flat spaghetti (fettuccine). The trachea was slightly compressed by the esophagus' increased bulk. Furthermore, the same food fragments were also present in both the upper and lower airways.[3]

At the histological examination, the most relevant findings consisted of pulmonary edema and diffuse visceral congestion, together with consistent hypertrophy of the esophageal muscle tunic, with areas of fibrotic substitution and extensive leukocyte infiltration. According to the macro and microscopical esophageal aspect, an immunohistochemical analysis was performed to characterize the leukocyte infiltration (CD3 and CD20).[4] These results, in addition to the subject's medical history and the autoptic findings, led to the postmortem diagnosis of achalasia. On this basis, it was possible to ascertain the cause of death as acute cardio-respiratory failure following a severe mediastinal compression of lower airways, large veins such as vena cava and nerves in a woman suffering from achalasia with megaesophagus.[5]

This presentation will impact the forensic science community by reporting a case of an esophageal achalasia presenting as sudden death in an apparently young woman.

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Sudden Death; Autopsy; Respiratory Failure

F20   The Climate's Influence on Fingerprint Quality

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Learning Overview: After attending this presentation, attendees will understand more about the climate's impact on the quality and comparison of fingerprints found indoors and outdoors in Southwest Wisconsin.

Impact Statement: This presentation will impact the forensic science community by providing information relating to the quality of fingerprints and the comparison of those fingerprints found on a specific substrate. In addition, important data will be provided based on the fingerprints developed from aluminum soda cans and glass bottles exposed to the local climate conditions and indoor climate-controlled conditions between Spring 2022 and Spring 2023 at the field study station Forensic Investigation Crime Scene House (FICSH) in Platteville, WI.

This study hypothesized that climate will significantly impact the quality of a fingerprint found on a non-porous surface. It was also hypothesized that the quality would negatively impact the comparison of a fingerprint. A key objective of this research was to identify how long fingerprints could last on objects placed indoors and outdoors. Observations were made and recorded after fingerprints were developed, photographed, and assessed for their overall quality.

For this research, fingerprints were placed on numbered aluminum soda cans or glass bottles using human perspiration, chip grease, and lotion. The aluminum cans and glass bottles were separated into two groups. The first group were placed outside on the ground in a grass field isolated by a chain link kennel fence. This simulated an item left outside on the ground but protected from scavenging animals and the wind. The second group was placed in a climate-controlled basement. Fingerprint quality data was collected based on observations made after fingerprints were developed using magnetic and traditional fingerprint powders. A scale was developed to determine the quality of the fingerprints. Analysis of Variance (ANOVA) test and t-tests were used to analyze the data. Significant differences were indicated by p values <0.05.

Results of the study indicated that climate in southwest Wisconsin impacted the quality of fingerprints found on non-porous surfaces. On average, fingerprints placed on outdoor cans and bottles were of a worse quality than fingerprints on indoor cans and bottles. The data supported the observations that fingerprint comparisons had a direct relationship to the quality of the developed latent fingerprint. The data indicated that a comparison between fingerprints was significantly influenced by the duration of exposure to their respective climates.

Information on the quality of fingerprints left on non-porous surfaces found in southwest Wisconsin was reported for the first time by these findings. This information is critical for determining whether a developed/processed fingerprint will have a higher probability to improve the outcome of a comparison.

Fingerprint; Climate; Quality

F21 Investigating the Effect Flipped Classroom Structure Has on Perceived Student Engagement in a Post-Secondary Forensic Science Course

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Learning Overview: After attending this presentation, attendees will understand the importance of student engagement in educational environments and the impact flipped classroom structure has on student engagement in forensic science. Results from a mixed-methods survey will be presented, including student perceptions of their experience in a flipped, senior-level forensic science course.

Impact Statement: This presentation will impact the forensic science community by underscoring the importance of student engagement in learning environments and the impact of flipped classroom structure on student engagement. Understanding student perceptions of their learning environments can help educators in designing these environments in order to better improve the quality of education.

This study focuses on a senior-level, flipped classroom, forensic science course and perceived student engagement regarding the flipped classroom structure. A flipped classroom can be structured in many ways, but the general idea is to open class time for activities involving higher-order thinking and leave direct instruction and delivery of basic information for outside of class time.[1] It has been well documented in the Science, Technology, Engineering, and Math (STEM) education research literature that the flipped classroom structure has a positive effect on student learning outcomes and course engagement. Engagement in learning is defined as the quality of a student's investment in learning and can be used to guide effective learning.[2] This means that if a student is engaged in the class, they will be more active in their learning, resulting in better learning outcomes. Engagement has also been shown to be linked to student motivation and, therefore, course success.[3] Despite the numerous studies conducted on student engagement in the flipped classroom in various STEM disciplines, little research has been done regarding how this course structure affects student engagement in forensic science courses. This is critical for this field of forensic science education because engagement has been shown to affect learning and motivation, which then affects student outcomes. The objective of this study is to investigate perceived student engagement within a flipped classroom forensic science course.

The study was conducted at a large public research institution in the southeast in a senior-level forensic science lecture-laboratory course. Data was collected via a survey, which included 14 Likert scale questions, 2 of which had a free-response section. The free-response questions were individually coded by two coders, who then came together to discuss the results and agreeability of the codes. A preliminary analysis of student responses to the survey shows three emerging themes. These themes include more opportunities to practice, the ability to ask questions, and increased engagement with other students in the course. The Likert data and student demographics from the survey will also be presented. Preliminary analysis of student responses to the Likert scale questions show that students generally agreed that the flipped classroom is more engaging. When asked if the flipped classroom is more engaging than a traditional classroom, 67% of students surveyed agreed, 25% of students selected a neutral response (neither agree nor disagree), and 8% of students disagreed. Engagement is very important for student outcomes because of its close relationship with effective learning and motivation.[1,3] To better understand student engagement, this study investigates perceived student engagement within a flipped classroom structure, specifically for a senior-level forensic science course. Results from this study can be used to inform educators on perceived student engagement in active learning courses as well as possible improvements that can be made to maximize student engagement and learning outcomes.

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Education; Forensic Science; Survey

F22   Characterizing Flow Cytometry Analysis of Pollen Mixtures

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Learning Overview: After attending this presentation, attendees will have a better understanding of how flow cytometry data of pollen mixtures can be clustered by multivariate mixture model using log-normal and normal distributions.

Impact Statement: This presentation will impact the forensic science community by introducing an automated method to analyze pollen mixtures by flow cytometry.

Forensic palynology is the application of pollen and spore analysis to civil and criminal legal cases.[1,2] As a forensic tool, pollen is used as a proxy to connect individuals or items to a crime scene. While its use in the United States has been limited, it has seen great success in Europe, the United Kingdom, and New Zealand where documented cases involving rape, homicide, robbery, terrorism, genocide, and illegal pollution have all utilized forensic palynology.[1,3]

Currently accepted methods use Transmission Light Microscopy (TLM) or Scanning Electron Microscopy (SEM) to identify pollen grains based on morphological features; however, these techniques are laborious and often time-consuming.[1,4,5] In addition, the limited available information about the technique, a severe lack of specialized palynologists, and diminished funding for forensic pollen studies have all contributed to its almost complete absence in United States crime labs.[1] To alleviate these issues, we have explored the use of flow cytometry to sort individual pollen grains based on the physical characteristics of individual grains. This method relies on the same fundamental basis of traditional pollen morphological analysis, while also providing high-throughput screening capabilities needed by modern crime labs.

As a test sample, three mixtures of willow (*Salix*) and mustard (*Brassica*) were prepared in different mass ratio in Phosphate-Buffered Saline (PBS). The mixtures and blank samples were analyzed using flow cytometry. The variables acquired for each event were forward and backward scattering, as well as fluorescence. Many clustering techniques such as K-means and finite mixture models have been used to analyze flow cytometry. However, finite mixture models using normal distributions depend only on the distribution that is not realistic.[6] A multivariate mixture model using a log-normal distribution and normal distributions is proposed and implemented in the mixture of pollen flow cytometry between willow and mustard. Based on the properties of a log-normal distribution, it could deal with data that have right skew distribution.[7] The signal from pollen mixture contains willow, mustard, and background clusters. Then, the data in willow and mustard clusters have multivariate normal distributions. Because a histogram of the data in background cluster tends to be right skew, a multivariate log-normal distribution is fitted in the background data. Therefore, our proposed multivariate mixture model could deal with the mixture of pollen.

Before we applied the proposed method on the data set, we simulated a data set that has three clusters. Data in the first cluster has a multivariate log-normal distribution and data in other clusters have different multivariate normal distributions. By comparison, our proposed model has more proficiency in terms of accuracy, bias, and mean square error than a multivariate mixture model using normal distributions. Our approach is applied to the analysis of a mixture of willow and mustard pollen. The approach separates the background from the species. The species are also modeled with their own distributions.

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Pollen Grains; Flow Cytometry; Multivariate Mixture Model

F23 Determining Possible Causality of Inherent Fluorescence in Fingerprint Residue

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Learning Overview: After attending this presentation, attendees will have a better understanding of inherent fluorescence in fingerprint residue through a comprehensive literature review and meticulous data analysis. The study highlights the need to consider additional factors, such as diet and product-related influences, and the need for future research to explore these variables. This study contributes valuable insights into the complexities and limitations surrounding inherent fluorescence and provides a foundation for further forensic investigations.

Impact Statement: This presentation will impact the forensic community, particularly in the area of fingerprint analysis, by uncovering the presence of inherent fluorescence in fingerprint residue and highlighting its complexity, with support for further research. The absence of significant correlations underscores the need for ongoing investigation into the underlying factors influencing inherent fluorescence. These insights enrich the knowledge base and stimulate advancements in forensic science, improving our understanding of this phenomenon and its impact on forensic examinations.

The introduction outlines the research objectives, including a literature review on fluorescence and the formulation of hypotheses related to geographical, genetic, and environmental factors. The study contributes to expanding knowledge in the field and unraveling the underlying causes of inherent fluorescence in fingerprint analysis.

Methodology: This study encompasses a thorough literature review on fluorescence, establishing the research framework. The data was obtained on fingerprint residue samples from 304 subjects, including individuals with military experience in different regions.

Each individual deposited a latent print on a template that was mailed back to the investigators. The samples were analyzed using a TracER Laser (Coherent, Inc.) for inherent fluorescence and validated using indanedione staining. This procedure ensures reliable data acquisition and enables meaningful comparisons across the samples, supporting the research objectives.

Results: The statistical analysis (chi-square) indicated there were no significant differences between inherent fluorescence in fingerprint residue and the variables investigated (p-values >0.05). Geographical factors such as residence and regions deployed for military service also did not show a significant change with inherent fluorescence. A higher proportion of participants from the United States displayed inherent fluorescence that was not statistically significant (p-value >0.10). Similarly, the test relating inherent fluorescence and ethnicity indicated no discernable genetic differences (p-value >0.18). Age and sex assigned at birth were also examined, indicating no significant associations with inherent fluorescence (p-values >0.18 and 0.36), respectively.

Conclusion: The study demonstrated there is no significant correlation between inherent fluorescence in fingerprint residue and geographical regions, genetic connections to specific racial identities/ethnicities, or exposure to similar environments (p >0.05). These findings indicated that other factors, such as diet or product-related influences, may play a role in inherent fluorescence. More research is needed to explore these factors and improve our understanding. Latent print examiners should exercise caution when interpreting fluorescence. Developing standardized protocols and procedures can enhance fingerprint analysis techniques. More research is needed to explore these factors and improve our understanding of inherent fluorescence in order to advance the field and improve interpretation.

Inherent Fluorescence; Fingerprint Residue; Latent Prints

F24　The Influence of Handwashing and Handedness on the Fingermark Microbiome Over Time

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Learning Overview: This presentation will demonstrate the effects of handedness and handwashing on the microbiome population of latent fingermarks over time. After attending this presentation, the attendees will have a better understanding of the dynamics of the microbial signature of individuals and how several environmental factors can influence the microbial population on fingermarks deposited over 192 hours.

Impact Statement: This presentation will impact the forensic science community by showing how environmental conditions can influence the microbial signature on an individual's latent fingermark and how that signature may change over time. During a crime scene investigation, the microbiome composition could reveal characteristics about a potential suspect's lifestyle or habits.

The skin microbiome varies between individuals and based on body location, environmental conditions, and several other factors regarding their geography and lifestyle.[1] The diversity and relative abundances of the microbiome may decrease over time when a fingermark is deposited on a surface, which may reduce the individuality of the microbial signature.[2] Several core taxa have been identified to exist on fingermarks of multiple people, as well as unique taxa that can individualize a donor.[1,3] The microbiome composition of an individual's fingermark may change over the course of a few months; however, a study has yet to evaluate the modifications that occur within the first week after deposition.[4] Handedness and handwashing demonstrated to have a significant influence on the composition of bacterial communities after one month on palm-marks, but no data demonstrated the succession of microbes from the time since deposition.[3] Understanding the gradual succession of microbes on fingermarks is important at crime scenes as it may aid in establishing time-since-deposition.

To fill the gap of knowledge on how the fingermark microbiome is modified in a short period, fingermarks from both hands from four donors, including negative controls and skin swabs, were aged on glass microscope slides over a period of 192 hours at room temperature in complete darkness. A group of slides was transferred to a -80°C freezer every 48 hours before DNA was extracted from all samples and sequenced for bacterial 16S rRNA gene. Preliminary analyses revealed similarities in the macro-population (top 5 most abundant Families) among donors but differences in the micro-population (the remaining 5% of the population abundance). Intrapersonal variation between the dominant and non-dominant hands of the same donor were detected as well as between washed and unwashed conditions. A change of the microbiome was expected as the microbial population adjusts and adapts to existing on a new substrate. Understanding how the microbiome signature changes over time is of paramount importance if this type of DNA analysis is to be routinely conducted for personal identifications and time-since-deposition determinations in the future.

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Fingerprint; Microbial; Environment

F25   Deaths of Irregular Migrants in California

Marni LaFleur*, University of San Diego, San Diego, CA

WITHDRAWN

F26 Death Due to Miracle Mineral Solution (MMS)

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Learning Overview: After attending this presentation, attendees will learn about MMS toxicity, symptoms, mechanism of action, what to look and test for in the decedent, and its contribution to death.

Impact Statement: This presentation will impact the forensic science community by increasing attendees' understanding of the effect of MMS toxicity and its mechanism, which will enhance medical examiners' ability to determine an accurate cause and manner of death.

MMS is an aqueous solution of chlorine dioxide, a powerful industrial bleaching agent, made by mixing sodium chlorite with an acid. MMS has been falsely promoted as a panacea for various conditions; however, its ingestion can lead to severe health consequences. Adverse effects include nausea, vomiting, diarrhea, dyspnea, minimal urine output, diffuse pallor, and cyanosis. Chlorite's molecular structure is analogous to nitrite and shares a similar effect on the cellular level. The action of chlorites leads to the production of methemoglobin and causes methemoglobinemia, renal failure necessitating renal replacement therapy, and hemolysis requiring blood transfusion among other potential complications.[1,2] Patients with MMS toxicity often exhibit elevated levels of methemoglobin, usual exceeding 50% saturation. Moreover, acute hemolysis can lead to Pulmonary Embolism (PE).[3] Furthermore, elevated methemoglobin levels have been associated with the severity of acute PE.[4] It should be noted that methemoglobin saturation can range between 0—3% in a normal healthy induvial; however, an increase in saturation corresponds to progressively worsening symptoms (Table 1).[5]

Postmortem detection of MMS (sodium chlorite) is challenging; however, methemoglobin levels can be tested and are typically very elevated in cases of MMS toxicity. It is usually extremely elevated; in our case, it was above 80% saturation. It is important to consider that postmortem levels of methemoglobin tend to rise, especially in aged and decomposed specimens, necessitating careful interpretation. Therefore, when MMS toxicity is suspected, the timely collection and appropriate storage of blood samples becomes imperative. Proper handling and preservation of samples can significantly contribute to accurate and informative analysis.[6]

Despite our decedent having multiple risk factors for pulmonary embolism, the temporal relationship between the use of MMS, the high level of methemoglobin, and associated hemolysis strongly indicate that MMS played a pivotal role in her demise. Consequently, the manner of death was changed from natural to accident.

This case highlights the mechanism of MMS toxicity and its contribution the cause of death by producing methemoglobin in extremely high levels. It also emphasizes that MMS can induce hemolysis and pulmonary embolism especially in susceptible patients with risk factors such as obesity, cardiovascular disease, and a history of Deep Vein Thrombosis (DVT) and PE. Methemoglobin should always be tested in patients with MMS toxicity and should be done promptly in the postmortem state.

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Miracle Mineral Solution; Methemoglobin; Sodium Chlorite

F27   Missing and Murdered Indigenous People: The American Southwest

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Learning Overview: After attending this presentation, attendees will have a better understanding of the status of missing and murdered indigenous people in the United States, with a special focus on the American Southwest.

Impact Statement: This presentation will impact the forensic community by demonstrating and promoting awareness about how profound the problem of unsolved cases of missing and murdered indigenous people are in the United States and how forensic evidence is often lacking due to the circumstances. While the whole of the United States will be included in the presentation, there will be a special focus on the American Southwest where the majority of Native American disappearances and deaths have been recorded. Canada has also been grappling with the problem of First Nations indigenous missing and murdered, and information as to how our Canadian neighbors have expended efforts toward addressing the issue will be incorporated as a comparative template.[1] In that context, the United States' Not Invisible Act of 2019, which was enacted in 2020, and its new committees will be highlighted to show the ways in which law enforcement is trying to incorporate input from affected communities.[2]

Statistical information from the United States for Missing and Murdered Indigenous People (MMIP) is derived from a nearly 20-year collection of reports on Native American homicide reports spanning 1999–2017.[3] This range encompasses the pre-COVID-19 pandemic era. However, the latest information about the 2023 task forces and consultation groups in New Mexico and Arizona and their strategic plans to contribute to law enforcement and family support will also be included for the most recent information.

Finally, the disparity in focus and application of resources is a controversial and ongoing issue with regard to the ethnicity of the missing and murdered will be discussed. This presentation will address this component and will include comparatives of the MMIP to the general population as well as other people of color in the United States. Further, issues specific to MMIP that distinguish the impact on its population will be outlined with regard to jurisdictional, environmental, and social problems particular to those communities that further complicate matters.

General Statement of Conclusion: This presentation will demonstrate the challenges and objectives designed to overcome the obstacles and disparities for solving cases of MMIP in the United States. While the focus is on the American Southwest, the overall problem facing Native American communities across the United States will be highlighted. Attendees will learn the unique circumstances that law enforcement and affected communities face that handicap successful resolution. Attendees will gain an appreciation and awareness of how challenges are currently being addressed to overcome the obstacles to solving cases of MMIP.

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Indigenous; Missing Persons; Homicide

F28   Is the Famous California Palm Tree a Serial Killer? A Unique Mechanism of Death in Tree Trimming Accidents

Sami Souccar*, Los Angeles County Department of Medical Examiner, Tustin, CA; Brice Hunt, Los Angeles County Department of Medical Examiner, Los Angeles, CA

Learning Overview: After attending this presentation, attendees will have learned that the majority of tree trimming accidents involve the iconic California palm tree, scientifically known as *Washingtonia robusta*, commonly referred to as the Mexican fan palm tree. This study aims to shed light on the mechanism of death, report its frequency, understand why it occurs specifically with this palm tree species, and present the findings from autopsies.

Impact Statement: This presentation will impact the forensic science community by increasing understanding of the types of asphyxia associated with the *Washingtonia robusta* palm tree and its findings on autopsies.

Tree trimming worker accidents in Southern California have become all too common. California, according to the Occupational Safety and Health Administration (OSHA), reported one of the highest number of injuries and fatalities with an average of 5.25 deaths per year from tree care incidents. Asphyxia accidents all involve the iconic California palm tree. The Mexican fan palm tree is a single-trunked palm tree that can grow up 100ft in height. They are considered invasive to Californian and were imported from Mexico and planted in late 1800s and early 1900s. Despite the allure of these majestic trees, maintaining them has proven to be both expensive and perilous.

In our case series, we examined 96 cases of tree trimming accidents over the past 20 years at the Los Angeles Medical Examiner's office, autopsy findings, and the cause of death of these accidents. Most accidents involved falls from great heights, entanglement with equipment gears, or traumatic injury from the heavy fronds of the palm trees. Blunt trauma emerged as the primary cause of death in these cases. Other causes of death in tree trimming are electrocution and self-inflicted wounds such as chain saw injuries. However, autopsy findings revealed a different mechanism of cause of death. Traumatic asphyxia was determined as the cause of death in 12.5% of the total fatalities from 2003 to 2023.

Traumatic asphyxia is interchangeable with mechanical asphyxia or suffocation. How does this palm tree entrap the worker? In our cases, workers scaled the palm trees and positioned themselves under the dead fronds, beginning trimming operations. Tragically, the weight of the severed fronds caused a deadly cascade, pinning the workers against the tree trunk and severely impeding their ability to breathe and they became entrapped. Given the workers' often elevated positions, swift assistance by first responders proved challenging and complex. In entrapment, individuals find themselves trapped in an airtight or relatively airtight enclosure. Initially, there is sufficient oxygen to breathe. However, as respiration continues, they exhaust the oxygen and asphyxiate.

The classical signs of asphyxia are congestion of the neck and upper body with petechiae. Petechiae can also be found in eyes on the sclerae, conjunctivae, and eye lids. Retinal hemorrhage and cyanosis can also be seen. However, all of the above-mentioned signs are non-specific and can occur in deaths from other causes. In our cases, the most common external finding on autopsy were scleral congestion in 58% of the cases, followed by petechial hemorrhage of conjunctivae and scleral seen in 50% of the cases. Other external findings included facial plethora, head and neck congestion, and minor non-fatal trauma such as abrasions and lacerations. The most common internal finding is pulmonary edema and congestion; however, this finding is highly unspecific and can be seen in many autopsies due to different causes. Internal findings were none-specific such as pulmonary edema and congestion. An interesting finding in one case, which had no external finding other than minor non-fatal abrasions and lacerations, was small bowl wall petechial hemorrhage, congestion of the mesentery, and generalized congestion of the peritoneum.

Traumatic asphyxia due to palm tree trimming, specifically *Washingtonia Robusta*, is more prevalent than initially believed. The increased awareness of this type of death in the occupational health sector led to the development of training and certification requirements for workers performing tree trimming duties. Autopsy findings were consistent with those of asphyxia due to mechanical causes. While some external and internal findings can aid in diagnosing asphyxia, specific signs are not always present, making it essential for medical examiners to consider the broader context and circumstances surrounding each case.

Accident; Tree Trimming; Palm Tree

F29   The Effects of Overlapped Latent Fingermarks on Skin Microbiomes Deposited Over Time

Lauren Welch*, University of New Haven, Monroe, CT; Josep De Alcaraz-Fossoul, University of New Haven, West Haven, CT

Learning Overview: This presentation utilizes 16S rRNA microbiome sequencing data from latent fingermarks that were deposited on a glass surface and aged in darkness for up to 96 hours. This experiment demonstrates the significance of microbial succession and the relative abundance of populations from a single donor when compared to the results of overlapping microbiomes from two different donors.

After attending this presentation, attendees will have a better understanding of the dynamics of microbes as signatures that could: (1) help aid in identifying suspects; (2) identify the changes in microbial populations over time and how this may hinder identifications; (3) investigate how overlapping microbiome populations from two different donors can influence each other over time; and (4) narrow down the timeframe of the crime.

Impact Statement: This presentation will impact the forensic science community by showing how microbiomes from fingermarks could be employed to indicate time-since-deposition and how to observe the importance of how "aged" microbiomes may affect the dynamics of two overlapped and different microbiomes deposited at different times. During a crime scene investigation, overlapping fingermarks of the suspect and victim may occur. However, these convoluted microbial signatures may behave differently from their singled counterparts. The microbes present could reveal possible lifestyle characteristics of a suspect and/or a victim that could not be revealed by conventional human DNA analyses.[1,2]

The human skin microbiome has shown to be unique to the host and may reveal distinctive signatures among individuals and different areas of the body.[1,2] It may also reflect on many aspects of someone's life such as the surrounding environment, diet, use of medications, and exposure to organisms, among others.[3] The uniqueness of microbiomes could be a valuable tool for the forensic community because it could potentially help build a profile of a suspect and possibly identify an individual.[4] However, microbial signatures recovered from surfaces may be prone to modifications over time that could hinder the potential use for human identifications.[5]

It has been shown that "touch microbiomes," which are transferred from human skin, can persist and thrive on surfaces for a certain time that cause microbial signatures to age.[6] Foreign microbes from a skin-to-skin or object-to-skin contact would not survive too long on the receiving person's skin since they may not be fit for that environment. However, there is limited information on how two different overlapping populations that were deposited on an object at two different times can affect the presence of a mixed donor population.

In the current research, touch microbiomes from aged overlapping latent fingermarks are examined. Also, the research investigates the visual quality of latent fingermarks using 2D imaging and 3D examination concurrently with the quantity and quality of DNA recovered. Latent fingermarks were aged for 96 hours on glass slides and stored in complete darkness. A group of specimens were frozen at -80°C at time 0 and again at 48 hours and 96 hours. DNA was extracted, sequenced (16S rRNA), and alpha and beta diversity analyzed. It is expected that the results will close the gap of knowledge on how microbiomes evolve over time, especially in cases of overlapping populations from two different donors on the same surface and how useful they may be at identifying their donors.

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DNA; Microbiology; Fingerprint

F30   **The Use of GIS Mapping to Assist With Forensic Recoveries of Historic Aircraft Crash Sites**

Audrey M. Lauerhass*, Middle Tennessee State University, Columbus, OH; Tiffany B. Saul, Middle Tennessee State University, Murfreesboro, TN; Adam Fracchia, Middle Tennessee State University, Nashville, TN

Learning Overview: After attending this presentation, attendees will understand how Geographical Information Systems (GIS) can be utilized to create a predictive model that can be used to direct forensic and archaeological efforts at large-scale scenes.

Impact Statement: This presentation will impact the forensic science community by demonstrating how the use of predictive modeling can assist in the efficient use of resources and increase the likelihood of locating items of interest at large-scale sites. This point will be demonstrated through a case study involving missing personnel from a World War II crash site.

Large aircraft crash sites present complex recovery scenes. Recovery efforts are further complicated when significant time has passed between the event and the recovery process, such as on-going efforts to recover unaccounted for service personnel from World War II. In instances where large surface scatter is a consideration, determining the boundaries of the debris field and location of positional pieces of Aircraft Wreckage (ACW) can assist with the development of forensic recovery and excavation strategies. The accurate documentation and analysis of spatial relationships is crucial for a more effective and efficient recovery methodology. The use of GIS mapping technologies not only allows for a greater spatial understanding of the debris field, but can also be used to create predictive models to inform forensic experts and concentrate efforts and resources in areas more likely to contain items of interest such as personal effects or human remains.

This presentation discusses the archaeological efforts to recover missing service personnel at a World War II crash site and the integration of GIS mapping of recovered aircraft wreckage. Spatial control was generated with a metric grid established across the site via total station and a GPS network. Over two years, portions of the roughly 450-meter by 350-meter site were accessible in both forest and agricultural fields. When accessible, these areas were systematically scanned via metal detection in 10-meter by 10-meter squares. Metal detection hits were sampled and documented. All ACW was researched and analyzed based on its original location on the aircraft. The coordinates of positional ACW were entered into GIS mapping software along with other spatial data, including property boundaries, terrain, Light Detection And Ranging (LiDAR) imagery, and the location of excavation units. Additionally, the number of metal detection hits per 10-meter squares were recorded in GIS mapping software and were used to create color-coded heat maps. These density maps of wreckage allowed for the creation of a detailed distribution map of the aircraft debris field. The combined mapping overlays showing ACW density and positional ACW guided the excavation strategy in the third year of the recovery project. Supplemental metal detection in the third year further expanded the testing grid and allowed for a more complete description of the aircraft's position on the current landscape.

The formulation and adaptation of this methodology in the field allowed for a more precise search for human remains. The constraints of weather, terrain, personnel, equipment, potential unexploded ordnance, and land access limited a large-scale survey and were further complicated by 80 years of taphonomic processes. The ability to collect data systematically over multiple years and analyze it in GIS software allowed for an effective integration and use of resources and time in the field. Ultimately, the success of the field survey and the use of mapping technologies demonstrates the utility of predictive models generated using GIS software at large-scale scenes to develop more focused archaeological recovery strategies.

Forensic Archaeology; GIS; Spatial Modeling

F31   The Application of Forensic Genetic Genealogy in Criminal Investigation

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Learning Overview: After attending this presentation, attendees will be more aware of the application of forensic genetic genealogy in criminal investigation and how criminal DNA is combined with forensic genetic genealogy.

Impact Statement: This presentation will impact the forensic science community by empowering criminal investigators in how to utilize forensic DNA technology in conjunction with forensic genetic genealogy to uncover new investigative leads, aid in solving cases, and prevent wrongful convictions.

The dilemma in DNA forensics arises when biological evidence of a suspect is discovered at a crime scene, but there is no DNA database available for comparison, rendering it ineffective.[1,2] This limitation primarily stems from the absence of a national DNA database. In the current scenario, if the true perpetrator remains at large or their DNA information is not on record, the absence of DNA comparison becomes a predicament, resulting in case delays, investigative impasses, and eventually turning the case into a cold one. Consequently, the lack of comprehensive national DNA databases also restricts the application scope of forensic DNA analysis. Confronting the challenges posed by DNA trace evidence, advancements in forensic genetics and genomics, alongside the integration of genetic and gene technologies, have paved the way for two novel approaches.

Forensic Investigative Genetic Genealogy (FIGG) and Forensic DNA Phenotyping (FDP) have revolutionized the field of forensic science.[3-6] These techniques offer immense potential for narrowing investigation scopes and successfully identifying perpetrators. FIGG enables the tracing of individual genealogy, including ancestral composition, ethnic migration, and genetic heritage across continents. By leveraging ancestry markers, investigators can eliminate potential suspects, narrow down investigations, and uncover how biological evidence was utilized at crime scenes. Past studies have utilized numerous neutral polymorphisms to differentiate genealogies, offering insights into the historical evolution of family trees and genetic disease variations. These advancements hold promise for advancing criminal investigations and promoting justice.

FDP is a distinct branch of forensic science that differs from offender profiling. It utilizes specific gene expressions to predict Externally Visible Characteristics (EVCs) of individuals and create lifelike portraits.[7,8] FDP also integrates information such as criminal modus operandi, biogeographical data, medical records, and family traits to aid criminal investigations. This article aims to inform law enforcement agencies about the application scope and legal foundation of FDP genetic technology and databases, drawing on a comprehensive literature review. FDP has demonstrated the capacity to accurately determine physical attributes like pupil color, hair color, hair density, and skin tone. It can also identify Minor Physical Anomalies (MPAs) such as eye distance, nose distance, earlobe shape, single and double eyelids, and thumb curvature.[9-12] These advancements significantly enhance the accuracy of physiological descriptions in forensic investigations.

Ultimately, the integration of FDP information with medical databases has the potential to significantly narrow the investigative scope and clarify case details. This advancement would represent a groundbreaking development in achieving the objectives of judicial science, namely the identification and apprehension of criminal suspects or the rectification of wrongful convictions.

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Crime Scene Investigation; Forensic DNA; Genetics

F32   Multidisciplinary Teamwork: The Excavation of a Grave Enshrouded by a Banyan Tree

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Learning Overview: The goal of this presentation is to illustrate to the larger forensic community an example of how a typically simple forensic activity can instead present with extreme challenges. Photographic documentation and first-hand accounts of how those challenges were actioned will be used to demonstrate these challenges and subsequent success.

Impact Statement: This presentation will impact the forensic science community by reminding practitioners not to underestimate how complex even simple activities can be, as well as to share the unconventional methodology that led to success. This multidisciplinary teamwork reinforces collaboration, and the tenacity of success here underscores the Academy's theme of "Justice for All."

The United States Department of Defense POW/MIA Accounting Agency (DPAA) conducts disinterments of remains originally interred as *Unknown* from cemeteries across the world for the purposes of identification and has faced a wide range of challenges in the process. Disinterments from the National Memorial Cemetery of the Pacific (NMCP) in Honolulu, HI, have been a particular focus of efforts with 1,654 occurring since 2001. The recent disinterment of a casket containing the remains of an unknown WWII serviceman from grave number N 271 at NMCP presented unique challenges, and its execution and subsequent success warrant sharing with the AAFS forensic community. The remains of this individual (designated as *Unknown X-536 Barrackpore*) were interred at the NMCP in 1949 and believed to be from the Battle of Myitkyina, a 1944 loss of approximately 272 United States servicemembers in Burma. A total of 56 caskets containing unidentified human remains believed to represent at least 104 individuals from the Battle of Myitkyina were disinterred from the NMCP between 2018 and 2022 with minimal complications. The final casket, X-536 in grave N 271, was fraught with unusual challenges to an extent that it may have remained interred in perpetuity, but in keeping with the Academy's theme of "Justice For All," the DPAA-Laboratory declined to leave the remains of *Unknown X-536* behind.

A robust and symbolic banyan tree (*Ficus microcarpa*) had been planted directly atop the N 271 grave site; part of a row of carefully tended trees along the central pavilion of the NMCP. As a prominent feature of the cemetery, officials made preservation of the tree's vitality a priority and forbade disruption to the tree or its massive root system. Cemetery staff had previously been combatting invasive species and tending to other health issues of the many banyan trees present, already at tremendous expense. Banyan trees span up to 30 meters in canopy with a main trunk up to 2 meters in width. They are known to sprout aerial roots that can turn into supplementary trunks and their root system is massive, containing multiple tap roots. The challenge was to disinter the remains inside the casket *without disinterring* the casket itself to minimize disruption to the tree immediately above and around it. The forensic archaeology team of the DPAA-Laboratory along with a contracted team of certified arborists, worked in tandem to excavate the sediment around the root network for partial access to the casket. A variety of tools atypical to forensic archaeology was used, including an air spade and venturi vacuum for excavation. A minimal portion of the casket was exposed, and an even smaller aperture was cut in the casket exterior to access the remains inside. The multidisciplinary teamwork, acrobatic maneuvers, and non-standard methodology required to excavate this grave—while ensuring the vitality of the tree—enabled the successful extraction of an undamaged bundle of remains from a uniquely precarious place of interment.

Archaeology; Anthropology; Exhumation

F33   Driving While Intoxicated: Why Are DWI Cases Being Dismissed in New Jersey?

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Learning Overview: After attending this presentation, attendees will be aware of the growing interest in New Jersey (and in other states) about Driving While Intoxicated (DWI) cases and their dismissals; they will understand the importance of investigating the reasons why these cases are dismissed and the geographical distribution of DWI dismissals across New Jersey counties. Attendees will learn what the major substances detected in DWI cases are and will identify potential trends in terms of dispositions and location. Attendees will also realize how important it can be to monitor these data to design and offer appropriate interventions to help the courts and to sensitize the public about the seriousness of DWIs.

Impact Statement: Cases of DWI-related fatalities have thankfully been reduced in the past years by several campaigns and programs designed to make people more aware of the issue; however, DWIs continue to be recorded in all states and, sadly, some of those accidents have serious consequences. This presentation will impact the forensic science community by informing attendees about the efforts that the New Jersey Statewide Traffic Safety Project and Kean University have made to gain a better understanding of the handling and final dispositions of DWI cases in New Jersey. The community will also realize the importance of identifying trends in these data and will appreciate the impact that these analyses can have on the general public.

According to the National Highway Traffic Safety Administration (NHTSA), every day in the United States one person dies every 39 minutes in drunk-driving crashes. In 2021, of the 42,939 traffic fatalities, 31% of the people lost their life due to alcohol-impaired driving. Thanks to various campaigns put in place to make citizens more aware of the importance of DWI, the rate of fatalities has decreased by more than 30% in the past 30 years; however, the numbers continue to remain high. In 2021, in the State of New Jersey, there were 869 deaths due to alcohol impairment. Even though there are significant penalties associated, DWIs are handled as traffic offenses in NJ, as they are not considered criminal offenses. It has been noticed that in the past years, there has been a significant number of DWI dismissals following an arrest, without evident reasons. New Jersey is not an isolated state in this matter, and this is why several Departments of Transportation and Traffic Safety have started to investigate this trend. The New Jersey Statewide Traffic Safety Project, in collaboration with Kean University, has begun to gather DWI data from all counties to gain a better understanding of the nature of the offenses and the reasons behind their dismissals. Through the analysis of these data across different years, it will be possible to identify potential trends in specific counties and to produce a report that will allow the development and implementations of interventions accordingly. Here we present the analysis of more than 120,000 cases of DWI in the State of New Jersey for the years 2018–2022; our report highlights the courts, the dates of the DWI, the substance, the disposition, and the reason for such disposition. No identifying information will be included in our analyses nor in the final report.

Traffic Safety; DWI; New Jersey

F34   **Approaches to Investigating Textile Damage in Stabbing Cases: A Forensic Analysis and Reconstruction**

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Learning Overview: After attending this presentation, attendees will have gained insights into the diverse methodologies and techniques used in the investigation of stabbing assaults. Attendees will understand how textile damage analysis and stabbing simulations may reveal characteristics about the weapon and the assailant and aid in reconstructing fatal stabbing events. Additionally, attendees will learn about advancements in interpretation and trends in this field over the past two decades.

Impact Statement: This presentation will impact the forensic science community by offering valuable insights to attendees by exploring methodologies used in textile damage reconstruction and stabbing simulations. A comparative analysis of manual and mechanically assisted approaches will be presented, highlighting their advantages and limitations for potential implementation in forensic investigations.

Fatal stabbing incidents are a significant contributor to homicides, especially in regions with limited access to firearms. The analysis of the wounds (when present) and damage inflicted on clothing can offer valuable insights into the weapon used and may aid in the reconstruction of events that unfolded during the incident. Over time, numerous methods and techniques have been employed to analyze and reconstruct stabbing incidents, contributing to the substantiation of investigative theories. It is essential to note that each method has its unique starting point and aim, leading to specific advantages and disadvantages. By understanding these distinctions, investigators can choose the most appropriate approach to unravel the complexities of fatal stabbing cases, enhancing the accuracy of their findings and supporting the pursuit of justice.

An extensive review has been conducted with the aim of examining the diverse methodologies and techniques available for forensic textile damage analysis and stabbing simulations. The review also focuses on the current trends in this field. Its ultimate goal is to contribute significantly to the development of a comprehensive and standardized approach in forensic science for analyzing and reconstructing stabbing assaults, with a focus on textile damage.

The review conducted in scholarly databases utilized targeted keywords ("forensic textile damage analysis," "textile damage reconstruction," and "stabbing simulation"), resulting in the synopsis of more than 100 articles. Each article's specific attributes, such as the utilized methodology for damage reconstruction (manual, mechanical, or mechanically supported), number of samples or participants, subject's age and gender, prior training, handedness, applied stabbing technique, force, substrate, and knife characteristics (including blade and handle type) were systematically recorded.

This presentation will discuss the advantages and limitations of the three validated approaches that have dominated the studies: trials involving one or more participants, trials using instrumented knives, and studies utilizing stabbing machines/apparatuses. Furthermore, data will be provided on how the textile damage resulting from the use of different methodologies is influenced by several key parameters related to the individual committing the stabbing. These factors include the assailant's prior experience, the specific stabbing technique employed (e.g., overarm, underarm, thrust, and slash), the type of knife used, the physical characteristics of both assailant and victim, and the dynamics of their interaction.

Textiles; Stabbing; Reconstruction

F35   Unraveling an Unusual Homicide: Asphyxia Due to Obstruction of Respiratory Orifices in a Subject With Acute Benzodiazepine and Tricyclic Antidepressant Intoxication

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Learning Overview: This presentation delves into a crime scene that initially appeared to be a suicide but was later determined to be a homicide through a meticulous and multidisciplinary investigation, combining detailed crime scene examination, autopsy, histological, and toxicological analysis. A global multidisciplinary approach was essential to clarify the true dynamics of the event and eliminate the initial hypothesis of suicide. The goal of this presentation is to highlight the critical need for thorough investigations to distinguish between homicide, simulated suicide, or accidental acts.

Impact Statement: Understanding the complexity of such cases can greatly impact the forensic science community, emphasizing the necessity of meticulous examinations and collaborations to achieve accurate reconstructions.

Suffocation occurs when the external respiratory orifices (nose and/or mouth) are obstructed, preventing the passage of air. The method of occlusion can vary, involving direct means such as hands or indirect means using objects like pillows, towels, or clothing pressed to the victim's face.[1] Detecting subtle signs of asphyxia can be challenging, as the presence of ecchymotic areas mimicking the shape of fingertips may be observed in cases involving hands, while compressive effects on the labial mucosae often provide key diagnostic evidence in other cases.[2]

Suffocation typically involves an imbalance of force between the victim and the aggressor, making it more prevalent among vulnerable individuals such as infants, children, the elderly, women, or individuals in an unconscious state due to the influence of alcohol or narcotics.[3]

We present an unusual case of homicide involving a 56-year-old woman with acute benzodiazepine and tricyclic antidepressant intoxication. A comprehensive multidisciplinary approach helped unravel the true dynamics of the event and underscored the significance of accurate investigations to differentiate between homicide and simulated suicide or accidental acts.

The investigation centered around the discovery of a deceased 56-year-old woman in her bedroom. The presence of various medications, including antidepressants, benzodiazepines, and anticonvulsants, along with a two-bladed sharp object on the bedside table led the initial inquiry toward a potential case of suicide due to drug intoxication.

The medical examiner observed distinct signs such as intense cervico-cephalic congestion and cyanosis of the labial mucosa. Notably, contusion-escoriative areas were found on the upper and lower lips, as well as linear, obliquely oriented continuity solutions on the left forearm, indicating an aggressive encounter. Further indications of subungual cyanosis and a body temperature of 28.8°C provided vital clues.

The autopsy and toxicological analysis confirmed elevated levels of lorazepam and amitriptyline in the blood and gastric contents, suggesting acute respiratory failure due to unconscious consumption of high doses of these substances. The contusive areas on the labial mucosa were consistent with occlusion in the buccal cavity, realized by the soft tissues. The victim was in no position to resist due to the pharmacological effects of the drugs. The absence of defensive injuries on the corpse further supported this interpretation. The inflicted cutting injuries on the left upper limb were indicative of a sharp object and were likely caused during the victim's vulnerable state.

In conclusion, a comprehensive multidisciplinary approach was essential in elucidating the true dynamics of the event and eliminating the initial hypothesis of suicide. This case highlights the critical need for accurate investigations to differentiate between homicide, simulated suicide, or accidental acts.

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Asphyxiation; Medicolegal Death Investigation; Drug Analysis

F36   ***Staphylococcus Aureus* Strikes Again! Never Underestimate a Furunculous Infection: An Autopsy Case Report of Acute Bacterial Spinal Meningitis in a Young Female**

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Learning Overview: This presentation shows an autopsy case of spinal cord meningitis in a young female and its controversial clinical care. The clinical signs and symptoms of spinal meningitis are not very clear or suggestive. In this case, the diagnosis was made postmortem. Here, it will be presented how the coroner achieved the diagnosis by the autoptic technique of all brain and vertebral specum. After attending this presentation, attendees will understand the method of approaching an uncommon case of suspected spinal meningitis and how this team evaluated the clinical care and its responsibility in the young female dead.

Impact Statement: This presentation will impact the forensic science community by presenting a way to achieve the diagnosis of spinal cord meningitis. After a critical analysis of clinical presentation and an evaluation of medical care, here are answered the questions of some hypotheses: first, if any other causes of death can explain clinical presentation; then, if alternative medical management should avoid death.

Reported here is a case of *Staphylococcus aureus* septicemia complicated by meningitis and extensive spinal cord injury. The peculiarity of this case report is the foci of septicemia, represented by an axillary abscess, drained a few days before death. *Staphylococcus aureus* was found in both samples of carbuncle and liquor.

Infection complications involving the spinal cord due to skin infection are very rare and their occurrence in the literature is based only on case reports.[1] So, due to the lack of epidemiological and clinical literature, clinical manifestation could be linked to another disease. The spinal cord may be injured by compression due to a spinal abscess, by ischemia due to vasculitis, shock, herniation, arachnoiditis, or by myelitis, so signs are related to meningeal irritation and fever.[2,3]

In this case, the patient was agitated, confused with fever, and had a very positive Lasègue test (straight leg raise test), that was misread as a sign of L5-S1 discal herniation. Brain complications have been described after a few accesses in the emergency department and it was diagnosed as bacterial meningitis: it was too late. Despite emergency care, intensive care unit admission was useless.

During the autopsy, skull removal and total vertebral laminectomy were performed. After, the spinal dura mater incision, there was a clear pus retention, and the diagnosis of death was accomplished.

The brain was enclosed in very tense leptomeninges; once removed, the increase in brain volume was observed, the convolutions were hardly recognizable. The venous sinuses were pervious, the blood inside them was fluid. The spinal cord was covered with a greenish, corpuscular fluid. Histological samples were obtained after formalin fixation of the brain and spinal cord (eviscerated *en bloc*). The leptomeninges and spinal subarachnoid space was completely covered with inflammatory cells, then identified by immunohistochemistry. The results will be better presented at the conference.

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Neuropathology; Meningitis; Medical Liability

F37   Closing Cases and Reducing Risk: A New Training Curriculum for Law Enforcement on Conducting Investigations With Transgender Crime Victims

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Learning Overview: Using perspectives from public health, prevention science, and forensic anthropology, attendees will develop an understanding of why transgender people are at an increased risk for crime victimization, the scientific and investigative challenges in resolving such cases, and the best practices for forensic scientists and law enforcement when engaging with the transgender community. In connection to this, after attending this presentation, attendees will be aware of a new, evidence-based, training curriculum that has been created for law enforcement about understanding transgenderism and the related implications for preventing and investigating crimes with gender-variant victims.

Impact Statement: This presentation will impact the forensic science community by proposing that current approaches to investigating cases of transgender decedents do not often take systemic and structural factors contributing to victimization into account, thus complicating the identification process and impeding case resolution. An evidence-based training curriculum impacts the scientific and legal community by establishing strategies to follow in cases involving transgender victims of violent crime.

Transgender people are at an especially high risk for violent crime victimization, with The American Medical Association and the Department of Justice declaring homicides against transgender people an epidemic and crisis, respectively.[1] Research suggests that, in addition to homicide, gender variant people are disproportionately represented in sexual and domestic violence victimization prevalence data.[2] Furthermore, recent studies have highlighted the challenges associated with properly identifying gender variant decedents.[3]

Despite this, there is very little evidence-based training available for law enforcement on understanding transgenderism, working within such communities, investigating crimes with gender variant victims, and identifying transgender decedents. In response to this, a new in-person, interactive, mixed-pedogeological training curriculum has been developed for law enforcement that integrates research and applied case work from the fields of public health, prevention science, and forensic anthropology. This new training program has been designed using existing research from a variety of scientific disciplines, best practices from the field of violence prevention, and new research conducted by the presenters on transgender homicide victimization.

More specifically, this training curriculum aims to teach law enforcement participants about the macro- and micro-level risk factors that contribute to violence against the transgender community, the reasons why transgender individuals are less likely to access assistance from law enforcement, and how presenting themselves as informed allies can aid in reducing transgender crime victimization while assisting in successfully investigating incidents once they have occurred. Last, this program will focus on homicides and missing persons cases involving gender variant victims. Drawing on methods used by forensic anthropologists in unidentified remains cases, strategies will be taught for reconciling (likely) lived gender identity with chosen names/family, understanding clues from clothing and gender affirming practices during life, and contextual interpretation of material culture and the skeleton.

To this end, the proposed presentation is designed to: (1) highlight the need for such a training for law enforcement personnel; (2) identify the key components and learning goals of the training program; (3) explain the potential benefits of improving law enforcement competency in this area; (4) provide the results of a formative evaluation, conducted with law enforcement, that demonstrates the effectiveness of this new curriculum; and (5) offer recommendations for its implementation.

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Law Enforcement Training; Forensic Anthropology; Transgender

F38   Emerging Paradigms in Forensic Facial Comparison Through Artificial Intelligence

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Learning Overview: After attending this presentation, attendees will be aware of recent efforts to understand and represent different sources of uncertainty when considering facial images for their comparison in facial identification scenarios. Such efforts aim to reduce bias in Forensic Facial Comparison (FFC) techniques that are currently not recommended (i.e., photo-anthropometry and facial superimposition). Attendees will also learn about novel methodologies based on Artificial Intelligence (AI) and 3D images, the purpose of which is to improve the reliability of the procedures.

Impact Statement: This research is focused in two aspects. The first is gathering as much *a priori* information as possible from a facial image to improve the reliability of analysis techniques. The second is to propose innovative methods based on AI to reduce bias and to speed up and ease the process of facial identification. We sincerely believe this topic will be of great interest to attendees and impact the forensic science community.
FFC has become increasingly significant as an identification technique in recent years, thanks to advancements in technology such as the proliferation of mobile devices, surveillance systems, and the emergence of AI. However, certain forensic methodologies have not fully adapted to these recent technological developments, and the reliability of different approaches within FFC remains a matter of ongoing scrutiny.

For instance, international organizations, such as the Facial Identification Scientific Working Group (FISWG), discourage the use of photo-anthropometry or facial superimposition. This recommendation is based on the presence of various factors that restrict their applicability, including image quality and the acquisition conditions required for making accurate comparisons. These conditions involve a known focal length and camera-subject distance, as well as similar scenarios where the subject is in the same pose and facial expression.

This work is devoted to showcase advancements in AI-based methodologies aimed at addressing the complexity of the problem and overcoming the identified limitations. First, we will explore automatic techniques for estimating the camera-subject distance in facial photographs, along with the automated marking of landmarks in both 2D and 3D images.[1-3] These approaches aim to minimize biases and subjectivity in the process. Furthermore, we will introduce the application of automated facial superimposition methods using an innovative 3D-2D paradigm, leveraging the advancements in 3D scanning technologies. Last, we will present a dataset of images in realistic scenarios and 3D facial models specifically designed to drive progress in FFC methodology.

Additionally, we will present a comprehensive study on the influence of facial expressions on FFC. High-quality facial scans for 32 individuals have been obtained by using a DI4D photogrammetry-based scanner. Different expressions were captured for each individual, totaling a sample of 160 facial scans. A set of 52 facial landmarks was annotated on each scan, and a displacement assessment was performed based on the Procrustes analysis. The study allowed us to determine facial regions with soft tissue stability and regions with a different variability pattern related to each expression.

In conclusion, modeling uncertainty in the form of anatomical variations related to different facial expressions is a crucial step to understand the limitations of the technique and design novel and reliable AI-based FFC methodologies that minimize biases from multiple sources.

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Facial Identification; Artificial Intelligence; 3D Technology

F39   The Reliability of the Viola-Jones Algorithm for Detecting Pose Variant Facial Features

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Learning Overview: After attending this presentation, attendees will understand how to detect human facial features in photos captured in varied poses. The Viola-Jones algorithm can help forensic experts working in the field of facial recognition to extract facial features in a convenient manner. Attendees will also learn how well this Cascade Object Detector-based method performs in detecting facial features in facial images captured in varied poses.

Impact Statement: This presentation will impact the forensic science community by elaborating on the ease with which human facial features can be detected from an individual's side profile, which will be helpful for forensic experts who are still relying on antiquated and time-consuming methods to detect the facial features.

Globally, criminal activities are surging inexorably due to various reasons, such as technological advancements, economic deprivation, moral value deterioration, unfair laws and regulations, and others. Parallel to this, crime recognition has also increased as a positive outcome of technological developments. Uncountable criminal acts are being recorded by surveillance systems, which are analyzed in order to identify the perpetrators involved. The face, being the prime source of identification, is generally analyzed by adopting numerous approaches. Face detection is the primary step to determine faces or non-faces in the questioned images. For this purpose, Viola-Jones algorithm is used often as it can identify human faces despite its dimensions and background.[1] This algorithm can reduce detection errors, and detection accuracy of 92.24% can be achieved.[2] Keeping these points in mind, in this present study, an attempt has been made to study the accuracy of the Viola-Jones algorithm in detecting human facial features from the side profiles of human faces. Also, the limitations of this algorithm have been studied.

In the present study, a total of 2,000 human facial images in five different poses were collected. All the images were captured using the Canon® EOS-1300D in broad daylight. The collected samples were then analyzed using MATLAB® software. The analysis was carried out by implementing the Cascade Object Detector-based Viola-Jones algorithm, and facial features, namely, the full face, nose, mouth, and eyes, were successfully extracted.

The results of this study vividly prove that facial fractures can be successfully detected from the side profiles of human faces using the the Viola-Jones algorithm. Full face came out to be the most highly detected facial feature (88%), followed by the eyes (69%), the mouth (42%), and the nose (33.5%). However, certain limitations of this algorithm, such as no detections, false detections, and multiple detections, were also observed.

To conclude briefly, the ever-increasing rate of crime necessitates an improvement in the methods of investigations, especially those dealing with the identification of individuals. Hence, the Viola-Jones algorithm can be successfully implemented in the forensic analysis of questioned images involving face recognition.

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Forensic Science; Feature Detection; Viola-Jones Algorithm

F40   The Marion Figure-Ground Hypothesis: A Perspective on the Art-Science Relationship in a Facial Reconstruction (A-SR) in a (FR)

Daniel Marion, Jr.*, Colorado Coroners Association, Denver, CO

Learning Overview: This presentation will seek to conceptualize what the blend of art and science in manual forensic facial reconstruction might look like. In the 77-year United States history of literature on facial reconstruction, including the most recent (Chapter 2—History and Research of Facial Identification), there is no mention of how the art and science in the facial reconstruction process interface.[1]

Impact Statement: This presentation will impact the forensic community by establishing that Manual Forensic Facial Reconstruction (MFFR) is an Ill-Structured Problem (ISP).[2] Knowing the type of problem will help the forensic artist as well as the end-users of this forensic service.

Using a two overlapping circle Venn Diagram (VD), Necker Cube (NC), and the Heisenberg Certainty Principle, the presenter will seek to give a visual conceptualization to the blend of the Art and Science in the Manual Forensic Facial Reconstruction process.

Knowing the type of problem will help not only the forensic artist but the end-user of this forensic service. This is important for several reasons, First, no two skulls are morphologically the same, not even identical twins.[3,4] Second, if the forensic artist follows an explicit algorithm, it will not necessarily result in the optimal reasonable likeness of that Jane/John Doe in that human skulls are bilateral and not mathematically symmetrical. Third, MFFR requires a practitioner/artist to have, at a minimum, an Associate of Arts in Art with a sculpting skill level and a basic anatomical knowledge of the bony architecture of the human skull.[5]

A VD is two circles that overlap at their nearest circumferences with each circle representing one of two different things like ideas, states of being, and/or conditions. An NC is a line drawing of a cube, in which the front and back faces of that cube appear to visually shift their initially observed positions back and forth continually. The Heisenberg Uncertainty Principle (HUP) states that the exact velocity and location of an electron cannot be determined at the same time.

The above (VD, NC, and HUP) all appear to have somewhat difficult conceptual characteristics to explicitly explain. That is to say, at what point in time precisely did the overlap of the two circles with their separate states of being become comingled in the VD? At what exact moment does the front face of the NC become back face and to the front again. What prevents the point pin timing of being able to observe the velocity and location of an electron in Heisenberg Certainty Principle? The inexplicability of the characteristics of the VD, NC, and the Heisenberg Certainty appears to be the same in kind with the Art and Science amalgamation in for Manual Forensic Facial Reconstruction. This would make any explanation of the Art/Science relationship implicit at best, tending toward experientially tacit.

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Forensic Art; Facial Reconstruction; Art/Science

F41   An Update on the Status of Facial Identification

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Learning Overview: This presentation will provide attendees with the latest events, standards, legislation, proficiency result, and status of an emerging certification program for the growing facial examination field.

Impact Statement: This presentation will impact the forensic science community by informing attendees that as the criminal justice community and other stakeholders continue to rely on surveillance system and closed-circuit images as evidence obtained during criminal activity, understanding the increased need for (and dependence on) skilled forensic facial analysts is a key piece to proper applications of the discipline.

Although dependence on "eyewitness" testimony as an element of criminal investigation goes back millennia, the use of facial recognition technology to support the justice system has a relatively recent history. Since practical applications of the Facial Recognition (FR) system came into play in the early 1990s, concerns over accuracy, bias, and potential misuse (among others) have prevented the optimal implementation of the enterprise. Studies show that highly accurate FR algorithms combined with skilled Facial Identification (FI) analysts can mitigate many of these concerns. Despite this, FR and FI continue to make headlines in the United States and around the world. Initial reluctance to deploy FR systems (and support with qualified FI analysts) has been tempered with the demonstrable benefits from proper applications, methodologies, training, and adherence to sound standards and best practices. This presentation is intended to further inform the forensic science and other stakeholder communities as to how the facial analysis and examination discipline continues to expand and gain acceptance in the criminal justice system and the court of public opinion. Recent and current events wherein facial recognition technology and subsequent facial analysis conducted by skilled practitioners, have demonstrated the benefits of FI. Other elements in the presentation include: (1) some of the published standards or those in development to regulate the application of FI; (2) status of the proposed certification programs for the Facial Examiner and Facial Reviewer; (3) additional customers for FI practices (historical photo confirmation, missing person/fugitive updates); and (4) the future of the Facial Identification Discipline.

The presentation will include summaries of standards that have been approved and published as well as standards in development. Additionally, attendees will be provided with insight into emerging technologies, such as improved algorithms, Artificial Intelligence, etc. that will have a direct impact on the accuracy and effectiveness of FR systems and the support offered through FI. It is hoped that participants will see the value in the continued utilization of facial examination in conjunction with the deployment of an effective FR system. Additionally, the proposed development and deployment of an accredited certification program will establish defendable scientific rigor for the discipline.

Standards; Accuracy; Facial Identification

F42 Forensic Podiatry: An Important Discipline in the Analysis of Feet, Footprints, Gait, and Trackways

Kewal Krishan*, Panjab University, Chandigarh, Chandigarh, India

Learning Overview: After attending this presentation, attendees will understand the significance of an important and emerging subdiscipline of forensic science, forensic podiatry, which examines, interprets, and evaluates pedal evidence, in particular, footprints, footwear, and gait. This may motivate young forensic scientists to take up this discipline for research and practice.

Impact Statement: This presentation will impact the forensic science community by presenting a greater understanding and insight into methodologies and tools available to assist in the analysis of footprints, footwear, gait, and human remains, and, importantly, an understanding of how these principles may be applied to other scientific and forensic specialties.

Forensic podiatry is comparatively a new scientific subdiscipline of forensic science that deals with the examination of pedal evidence generally encountered at crime scenes. According to DiMaggio and Vernon, it is defined as the application of sound and researched podiatric knowledge and experience in forensic investigations to show the association of an individual with a scene of crime or to answer any other legal question concerned with the foot or footwear that requires knowledge of the functioning foot.[1] Forensic podiatrists contribute to personal identification in crime scene investigations whenever foot or footprint-related evidence is recovered from the crime scene. The need to establish the identity of dismembered remains may arise in cases of mass fatality incidents such as terrorist attacks, mass murders, transport accidents, tsunamis, floods, and earthquakes. Dismembered and mutilated remains are usually encountered in these mass fatality incidents. There is an increased likelihood of the recovery of feet (often enclosed in shoes) separated from the body in mass disasters such as high-powered explosions and bomb blasts, airplane crashes, and other high-impact transportation accidents. In this regard, forensic podiatrists can collect evidence related to the foot and help in the identification of the individuals from the foot and its parts. Furthermore, forensic podiatrists conduct the examination of footprints generally recovered at the crime scene. Footprints are commonly encountered at almost every crime scene in the form of bare footprints, socked footprints, or shoeprints.

There are many ways in which footprints can be used in establishing personal identification in forensic podiatry. The analysis of bare footprints involves the identification based on class, intermediate class, and individual characteristics of the footprints.[2] For example, features such as corns, pits, ridges, humps, creases, hammertoe deformity, an extra toe or missing toe in the foot impression, and flat-footedness are characteristics of the footprints that may be utilized as forensic evidence in establishing personal identification. This kind of physical evidence can positively link a suspect to a crime, or it can prove one's innocence. By using different anthropometric methods, the stature, sex, and body weight can also be estimated from the footprints recovered at the crime scene. As stature and body weight can provide an idea about the size of the individual, they can give useful clues to a forensic scientist in a criminal investigation. Apart from these shreds of evidence in forensic podiatry, gait analysis, step/stride length analysis, and identification and documentation of the trackways can also furnish some indication about the criminals involved in a particular case.

The presentation will discuss various methods of personal identification related to pedal evidence that is usually recovered from the crime scene or scene of occurrence in the form of human remains, footprints, or questioned footwear.

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Forensic Podiatry; Pedal Evidence; Personal Identification

F43 Further Observations Into the Linking of Footprints With Footwear—Forensic Implications and Interpretations

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Learning Overview: After attending this presentation, attendees will comprehend the utility and methodology of linking footwear with the footprints of the perpetrators in forensic examinations and crime science investigations. This knowledge will help attendees in forensic practice and in conducting further research into this area.

Impact Statement: This presentation will impact the forensic science community by teaching new information for associating footwear with footprints by studying the distinctive features and characteristic marks created by the sole on the insole of the footwear.

The footprints are usually recovered at the scene of crime, which may help in identifying the perpetrator in many situations. In the past, many studies and forensic casework have revealed that a foot impression has certain features that may help in linking the crime with the criminal.[1] Comparison of the shape, size, and morphological features of the footprints has been successfully used in criminal investigations. Due to these distinguishing morphological features of the footprints of a person, the marks left by the foot on the insole of the footwear may also be distinct. The impressions on the insoles of the footwear may depend upon the pressure exerted by the sole of the foot at different points.[2]

The main objective of the present study was to identify the weight-bearing marks and the characteristic impressions of the soles on the insoles of the footwear in North Indian participants. In other words, the study will ascertain the weight-bearing areas/pattern of the sole created and darkened by pressure on the insoles of the footwear, which may be helpful in matching/non-matching the footwear recovered at the crime scene. The study also compares the various characteristics of the footprints with the insole pattern on the footwear of a person for identification purposes.

The data for the present investigation were collected from 200 participants (100 males and 100 females) from Chandigarh, a city of North India. A pair of footwear (rubber slippers) was given to each participant for about five months. The participants were instructed about the use of rubber slippers with certain precautions. Side by side, the foot impressions were also taken from the subjects with the standard technique using an inking method. The slippers were collected from them after five months and were studied and analyzed for the insole impressions. Upon examination, in most cases, the ball and the heel regions show maximum weight-bearing areas of the foot and consequently a clear marking has been left on the insole of the slipper. The marks are also different in different slippers, clearly indicating the weight-bearing variations in the individuals. In many cases, the big toe and the instep region also show some weight-bearing marks, which may be well correlated with the foot impression of the person. Generally, the obese participants show a rich impression of the instep region, indicating the association of the body weight with the insole impression of the footwear. Overall, the study concludes that the weight-bearing marks on the insole of the footwear may be helpful to a great extent in associating the footwear found at the crime scene with the perpetrator. The study will discuss some of the limitations of the work and further recommendations for future researchers.

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Forensic Podiatry; Crime Scene Investigation; Footprints and Footwear

F44 **The Casting of Shallow Footwear Impressions: A Field Test of Comparisons Between 3D Photographing and 3D Laser Printing Methods**

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Learning Overview: After attending this presentation, attendees will be aware of two new innovative methods of casting shallow footwear impressions. While the 3D photographing can provide a three-dimensional image of a shallow impression (<2mm in height) and compare with the suspect's shoe (2D vs. 3D comparison), the 3D laser printing is able to not only take a 3D photograph but also produce a 3D cast for a 3D-to-3D and a positive mold-to-a-positive original comparison. In the field, the two portable devices were able to successfully capture sufficient details of the impressions in an *in situ* (non-intrusive) manner and with much less time than a dental cast.

Impact Statement: This presentation will impact the forensic science community by providing two cutting-edge methods of casting shallow footwear impressions in the field. Attendees will learn the equipment, the principles, and the procedures, as well as examine the actual 3D photos and the 3D laser printing cast at the presentation.

Footwear evidence is one of the most abundant forms of evidence but is also the most overlooked evidence at crime scenes, as well as the most easily disturbed or damaged by outsiders. In reality, footwear evidence should be the first consideration as potential evidence since the perpetrator has to enter and walk around the scene. It should be the first processing work before any other types of evidence processing procedure because it can be easily ignored and missed. According to the reports of police agencies, footwear evidence is being collected in over 70% of their cases, but most of them become unusable due to various reasons. One of the challenges lies in the fact that any shallow footwear impressions (<2mm) cannot be casted by regular dental stone, dental Polyvinylsiloxane (PVS), or wax-dental mixer methods because the height of tread ridges is less than 2mm and thus cannot separate the details by the liquid for a cast, making these impressions abandoned as valuable probative evidence.

The two devices were used to cast the same footwear impression in the field for a comparison purpose as two quasi-experimental tests. The following brief results are reported. The new 3D photographing device is able to take a photo of the impression and detect the class and the subclass levels of characteristics. Then the 2D image is converted into a 3D image in eight minutes and used to compare with the suspect's shoe (or the shoe's 3D image). At the class level, the 3D virtual image clearly displays the direction, the size, the curve, the logo, the mid portion, and the edge. At the subclass level, the 3D virtual image depicts relatively visible tread features, such as main ridges, main grooves, block shapes, ridge widths, groove widths, and thickness. Yet, the comparison is a 3D virtual image with 3D positive original ridges (the shoe). At the individual level, some of the features are not so visible due to its reversed virtual image details. On the other hand, the cast by the 3D laser printing is not only better on the class and the subclass details, but also on the individual details (e.g., cuts, nicks, scratches, imbedded materials, heal wears, and even gait estimates). As a better probative value evidence, it is a true 3D positive cast to a 3D real positive ridges (the suspect's shoe) with a same siding and greatly improves the evidence's accuracy, reliability, competency.

Based on the field results from the ground truth tests, the author argues the following advantages from the combination of the 3D photographing and the 3D laser printing casts: (1) both methods are able to detect footwear impressions with less than 2mm ridge height; (2) the 3D casting process (one virtual and one real) is an *in situ* (non-destructive) method as a first option; (3) the 3D laser printing cast can provide a positive cast to a positive original comparison with a same siding; (4) as a non-destructive method, possible trace evidence, such as hair, fiber, glass, paint and biological or botanical evidence (e.g., blood, pollen, or leaves) still remain intact and untouched on mud, dirt, soil, or snow. Finally, both of the casting processes take much less time than that required for a regular dental stone method. The next move is to apply these two methods to other impression evidence such as tire, tool, and even palm impressions for International Organization for Standardization (ISO) validity tests.

3D Technology; Admissibility; Imaging

F45   Selected Trends From COVID-19 Prospective Data Collection at the Tarrant County Medical Examiner's Office

Lilly G. Crowns*, Southlake, TX; Stacey Murthy, Tarrant County Medical Examiner's Office, Fort Worth, TX; John Briggs, Tarrant County Medical Examiner's Office, Fort Worth, TX

Learning Overview: This presentation utilizes COVID-19 data collected in real time to illustrate trends at the Tarrant County Medical Examiner's Office (TCMEO). The emphasis of this presentation is not only having a better understanding of trends during a pandemic, but also the strong collaborative efforts of multiple departments with the fostering of autonomy in data collection of potential trends.

Impact Statement: This presentation will impact the forensic pathology community by refining points of data collection when faced with emerging pandemics and trends and fully utilizing available departments to obtain thorough data collection in a timely manner. It will also emphasize the need for cross-departmental collaboration.

The TCMEO encompasses three additional counties (Denton, Parker, and Johnson) and covers cases from Lubbock County.[1] Tarrant County is a sizeable metropolitan County in Texas, and its Medical Examiner Office (MEO) also receives cases from surrounding rural counties. Prospective COVID-19 data was collected from 2020 to 2022. The total population of the counties covered by TCMEO is 3,726,142 (as of 2021).[2]

Selected trends being evaluated include demographics (numbers of COVID-19-positive decedents based on age/race/location), positivity rate in decomposition cases, causes of death in COVID-19-positive cases, and suicides related to COVID-19.

Evaluation of the data showed multiple trends. There were 447 COVID-19 deaths during this period. The other manners of death were 14.2% natural, 10.1% accident, 1.9% homicide, 3.0% suicide, and 0.8% undetermined for Tarrant County in 2021.[1] An unanticipated outcome of the data collection was the effect COVID-19 had on mental health and insight into the decedent's reasoning for suicide. Most of the suicides were in those over 50, and most of them had no underlying health issue besides COVID-19 and possibly mental health. Also, from the trends, we saw the most positive COVID-19 cases were in the over-50 population and the Caucasian population. Our four other counties (Denton, Parker, Johnson, and Lubbock) represent more rural populations. From the data, these areas showed a lower percentage of COVID-19-positive cases compared to the urban Tarrant County. The demographic data allow us to evaluate trends throughout the pandemic and potential future correlation and collaboration with public health. These findings can help allocate resources to at-risk areas.

This data was collected prospectively by our Chief Forensic Death Investigator (FDI) and entered into spreadsheets. The Chief FDI recognized the gravity of COVID-19 early in the pandemic and had the foresight to start collecting the data. This is an example of autonomy given to staff in the MEO and the resulting collaborative efforts benefiting the office and potentially national public health. Having engaged staff who can identify potential trends and understand the future implications lends to a stronger research program. MEO must encourage staff to monitor for trends because MEOs are often the first area in which outbreaks are noticed. This is not just for infectious diseases but also drugs, gun violence, etc.

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COVID-19; Cause of Death; Suicide

F46   Predictive Models for the Diagnosis and Prognosis of COVID-19: Tools for Assessing the Evaluation of Health Responsibility in a Case of a "Culpable Epidemic"

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Learning Overview: After attending this presentation, attendees will be aware of the advantages and limitations of the use, according to medicolegal methodology, of the predictive diagnostic-prognostic models of COVID-19 for the evaluation of health responsibility in the case of a "culpable epidemic."

Impact Statement: This presentation will impact the forensic science community as it represents an example of definition and subsequent application of a method of assessing health responsibility in a case of suspected "culpable epidemic" from COVID-19, an event that occurred within a residential facility for the elderly during the first pandemic period.

More than three years have passed since the outbreak of COVID-19, the pathology caused by SARS-CoV-2 infection and responsible for interstitial pneumonia, Severe Acute Respiratory Syndrome (SARS), multi-organ dysfunction, and death. As of July 20, 2023, COVID-19 has affected more than 760 million people, leading to a progressive increase in hospitalizations worldwide. An important question of debate is how the management took place in specific health care contexts, such as residential facilities for the elderly, where people who are particularly susceptible to the more serious consequences of SARS-CoV-2 infection are housed, and who have therefore suffered with particular severity the first wave of the pandemic.

The epidemic spread of SARS-CoV-2 infection in facilities for the elderly has led to an increase in disputes between health professionals and citizens on the verification of the existence of related professional liability. Factors related to these structures such as the number of guests, their condition of fragility as well as structural and organizational deficiencies have made them an ideal place for the development of SARS-CoV-2 epidemic outbreaks, especially during the first pandemic wave, characterized by management difficulties, including the lack of diagnostic swabs.[1] In Italy, some structures have been accused of "culpable epidemic," giving rise to the need to establish a method, respectful of the medicolegal criteriology, through which to assess the causal link between a possible omissive behavior by the health workers involved and the spread of SARS-CoV-2 infection and consequent damage.[2]

In this study we analyzed the health documentation of 740 patients in the period between February 1 and December 31, 2020, guests of an Italian residential facility called into question for suspicion of "culpable epidemic" for the death of 410 guests.

The evaluation considered both organizational and management issues and the overall clinical situation of the patients, which was investigated using diagnostic and prognostic predictive models related to COVID-19. The predictive models applied were selected through a literature search, favoring those that demonstrated higher quality to the verifications by subsequent meta-analyses and systematic reviews.[3]

Therefore, based on the chosen models, for each patient the possibility of a link between SARS-CoV-2 infection and the outcomes was evaluated, privileging in the evaluation the role of comorbidities and the efficiency of the diagnostic-therapeutic procedures implemented.

The work carried out made it possible to recognize a "threshold value" useful for identifying responsibility, as well as its extent, in the context of a complex case such as the culpable epidemic, and provided a useful example of comparison in similar cases relating to outbreaks of SARS-CoV-2 and other diffusive infectious diseases.

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COVID-19 Infection; Culpable Epidemic; Prediction Models

F47 Humanitarian Forensics: A Call to the Scientific Community From the International Committee of the Red Cross

Pierre Guyomarc'h*, International Committee of the Red Cross, Geneva, Switzerland

Learning Overview: After attending this presentation, attendees will be aware of the recent evolutions of humanitarian forensics as applied by the International Committee of the Red Cross (ICRC) in multiple contexts, underlining the gaps that limit the impact of its action.

Impact Statement: This presentation will impact the forensic community by offering a series of observations based on humanitarian crises and mass fatalities having occurred during the past three years and proposing avenues to overcome the related challenges by increasing targeted research and collaborations.

The Forensic Unit of the ICRC celebrates its 20th anniversary in 2024, and its members have been participating in the AAFS (among other fora) regularly, sharing their experiences through workshops and presentations, and ensuring a link between the implementation of humanitarian forensics and the scientific community. From the promotion of the proper management of the deceased in emergencies to the strengthening of national medicolegal systems and the support to parties to conflict in complying with their legal obligations, a wide range of services is provided by the ICRC.

With the recent large-scale events (e.g., the conflicts in Ukraine, Ethiopia, Sudan, the COVID-19 pandemic, earthquakes in populated areas, the continuous migration crises), ICRC forensic staff have witnessed both successes and failures of local systems and organizations to address the consequences of mass fatalities. Humanitarian forensics covers the whole chain of operations from the search for missing persons, the recovery of human remains, their transport, storage, analysis, and identification to their final disposal. Failing to ensure minimum standards at any step of an operation will have a negative effect on the dignity of the deceased, the rights of their relatives, and the state of their community. With the increase of conflicts, disasters, migration, and other situations of violence, the areas that would benefit from additional efforts include:

- Updating and creating new guidelines to implement International Humanitarian Law obligations for armed forces and parties to a conflict; proposing better systems to ease the search, recovery, identification, and accounting of deceased combatant.
- Improving the coordination among States and international organizations when responding to emergencies; promoting the creation and revision of national mass fatality planning (i.e., inclusion of the management of the deceased in mass casualty plans).
- Leading research on the perception of death and the needs of communities form various cultures and religions, adapting consequently the procedures of managing the deceased in the relevant areas.
- Reforming policies and regulations in medicolegal systems to ensure proper civil registration (at both birth and death), and the management of unclaimed bodies.
- When death occurs in a different country than the one of origin (i.e., in migration cases), the data management (collection and exchange) still requires improvement, including the specific challenges of managing the deceased at sea.
- Innovating on the production of equipment for the death care industry, toward more sustainable material and protecting the environment.
- Refining protocols to handle fatalities from various types of infectious diseases, considering the needs of the communities and the protection of the body handlers (from COVID-19 to Ebola outbreaks).
- Building a defined career path for humanitarian forensics professionals through adapted curricula that will better prepare for field work.

The ICRC will focus its efforts toward these goals during the following years and will need the support of other international organizations, state institutions and universities to reach them.

Emergency; Mass Fatality; International

F48 **Asylum Seekers and Victims of Torture in Sicily, Italy: The Role of Forensic Medicine in the Mediterranean Migratory Crisis**

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Learning Overview: The goal of this presentation is to analyze evidence of torture found during forensic inspections and present our methodology, aiming to provide new methodological proposals for improving the accuracy and reliability of forensic assessments in immigrant cases. This presentation aims to significantly impact the forensic community by sharing the comprehensive findings of our five-year forensic inspections focused on detecting evidence of torture among migrants and asylum seekers.

Impact Statement: This presentation will impact the forensic community by showing the successful implementation of the Istanbul Protocol, a crucial tool in our assessments, and proposing new methodological approaches to enhance the accuracy and efficiency of forensic investigations. Additionally, we will emphasize the importance of educating health care workers and local associations about recognizing evidence of maltreatment, enabling early detection and intervention in cases of potential torture.

Immigrants seeking international protection status in Italy often claim to be victims of persecution in their home countries, necessitating forensic assessments to support their asylum applications.[1] Forensic examinations are usually required by the local committees in charge of granting or refusing the international protection status, which implies political asylum, or, if the requirements for the "refugee status" are not fulfilled, the subsidiary protection and special temporary permits.[2]

The Institute of Forensic Medicine at the University of Palermo is involved in two crucial aspects of illegal immigration cases: age estimation in unaccompanied minors and forensic assessment of alleged torture, following the Istanbul Protocol guidelines.[3] In this study, we analyze evidence of torture found during forensic inspections and present our methodology, aiming to provide new methodological proposals for improving the accuracy and reliability of forensic assessments in immigrant cases.

Two hundred thirty-six forensic inspections were performed during five years (2018–2022) according to the Istanbul Protocol. Each examination was always performed in the presence of a cultural mediator, a forensic specialist, and a psychologist. Informed consent was always requested for taking pictures during the inspection. Most of the subjects were men aged 20–30; in the case of women, the examination was performed by a forensic specialist. The inspection was committed long after the torture, with a mean time of six months. All types of lesions were observed (blunt force trauma, thermal injuries, asphyxiation, ligature marks, stress position, falanga, cutting, and electric injuries). The most frequent injuries were blunt force injuries and trauma (82%). Falanga was observed in 18 cases. Genital mutilation was detected in 20 patients. Most of the migrants who suffered from torture passed through Libyan prisons. All our data (sociodemographic data, information about the journey, the torture method, inspection findings, and physical and psychological sequelae) will be presented.

Our inspection aims to provide evidence of torture to the local commissions, according to the Istanbul Protocol. Moreover, a multidisciplinary approach is needed in some cases to strengthen evidence of torture and for age estimation: after the inspection, the subject is addressed to the odontologist and radiologist. In many instances, especially for musculoskeletal injuries, the patient underwent Magnetic Resonance Imaging (MRI) and/or X-ray examination. The findings will be shown during the presentation. Our inspection is frequently performed a long time after the episodes of torture. Signs of torture could disappear, thereby leading to the need for a comprehensive and complete approach in all branches of forensic disciplines. Moreover, our visit has a medical and humanitarian purpose. In our report, we focus on all the health care assistance needs and the humanitarian aspects of the migrant to address such issues to the local commissions, health care facilities, politicians, and local volunteer associations.

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Torture; Migrants; Forensic Analysis

F49   **Multiband Imaging Methods in Humanitarian Forensic Medicine to Enhance the Readability of Documents From Mediterranean Shipwreck Victims**

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Learning Overview: The goal of this presentation is to provide attendees with an understanding of multiband imaging techniques and their application in the analysis of severely deteriorated documents related to the victims of the Mediterranean Sea migration crisis. Attendees will learn about the use of Reflected Infrared photography (IRR), Visible light photography (Vis), Reflected Ultraviolet photography (UVR), and Visible Fluorescence induced by Ultraviolet light (UVF), and how the interactions between various materials and these different forms of radiation can yield diverse results. This presentation will illustrate how such techniques are employed to enhance the legibility of illegible, water-damaged documents like identity documents, handwritten letters, parts of diaries, and photographic identifications. Attendees will also learn about the specific post-processing methods employed, such as perceptual local contrast enhancement algorithms and open-source applications designed for written documents and how they can dramatically increase readability.

Impact Statement: This presentation will have a significant impact on the forensic science community by demonstrating the potential of multiband imaging and computational post-processing techniques to restore and enhance the legibility of severely deteriorated documents. These methodologies represent a crucial tool for forensic experts working in humanitarian forensic medicine, helping them to identify victims of calamities like shipwrecks. By learning about these techniques, forensic scientists will be better equipped to handle similarly challenging scenarios in their own work, whether related to migration crises, natural disasters, or other events leading to document degradation. The potential expansion of these methods beyond the present case could foster innovation and increase efficiency in forensic examinations, thereby significantly advancing the field of forensic science.

Multiband imaging generally involves taking multiple images of the document under examination using different wavelengths of the electromagnetic spectrum, such as X-rays, Ultraviolet (UV), Visible (Vis), and Infrared (IR). These non-invasive techniques are commonly used for the analysis of cultural objects, which are often subject to conservation constraints. In this work, we used IRR, Vis, UVR, and UVF. These techniques can provide different results of the object, depending on the specific interaction between the materials and the radiation used to maximize information.

These analyses were applied to a sample of documents curated by the Laboratory of Anthropology and Forensic Odontology (LABANOF) of the University of Milan, which is carrying out a project to identify migrants who died in the Mediterranean Sea. Twenty-five identity documents, handwritten letters, parts of diaries, and photographic identifications that have become illegible were selected. These items may provide forensic experts with insight for the identification of the victims. So far, in the field of humanitarian forensic medicine, no case studies about the restoration of documents of migrants through photographic and computational post-processing techniques have been produced.

These documents deteriorated following various factors. The prolonged exposure to water of such a fragile material caused severe wash-out of text and images. Moreover, intense and sudden variation of temperature and humidity at the time of recovery accelerated the oxidation process of paper and ink, and exposure to light caused subsequent ink fading. All these factors have made the visibility of text and images extremely difficult. Photographs of the documents were taken in the laboratory with different instrumentation and conditions based on the specific technique. Once the images were acquired according to the different bands, they were further processed to increase the low visible contrast between the text and the background. In all cases, this approach started from unreadable visible light images and resulted in processed images with a significant increased legibility.

When multiband photographic techniques were applied, only visible photography was able to provide a satisfactory outcome in terms of legibility, which was subsequently used in the post-processing step, together with the individual R, G, or B channels selected. As for computational techniques, perceptual local contrast enhancement algorithms significantly improved the original image. By varying the different parameters in the algorithms, the process was adjusted according to the type of material (i.e., text or images). In addition, open-source applications designed to enhance the legibility of common written documents were tested on the ability to increase the readability of the documents.

Preliminary results showed that the presented approach was efficient in making legible most of the documents. By combining the consolidated multiband approach with an innovative and specific post-processing method, these techniques were able to make highly deteriorated documents visible again.

Humanitarian Forensic Medicine; Multiband Imaging Methods; Identification

F50   Breaking Barriers: Empowering Forensic Dermatologists in Asylum Applications Through Identifying Evidence of Torture in Ethnic Skin Variations

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Learning Overview: After attending this presentation, attendees will better understand the forensic dermatologists' roles and significance in providing evidence for asylum applications. Attendees will also learn of the biological differences between Caucasian skin and People of Color (POC) to understand the variations in torture injury presentation between these individuals. A detailed evaluation of the methods and presentation of physical and psychological torture that asylum seekers face will help attendees gain a better insight and appreciation of how forensic dermatologists assess these victims.

Impact Statement: This presentation will impact the forensic science community by highlighting the gap in current research into the presentation of torture injuries in POC. The community will be exposed to torture wounds on Caucasian skin and can compare these to colored skin. In addition, this presentation will also raise awareness of the shortcomings of forensic dermatology training and aid in shaping guidelines for future practice.

Asylum applications are increasing worldwide. However, the number of trained forensic dermatologists cannot match this growth. Forensic dermatologists are the professionals responsible for assessing the injuries and scars of torture that asylum seekers present to provide compelling evidence for their applications. However, there is a lack of research into the differences between injury presentation in colored and Caucasian skin. This information is especially crucial since most asylum-seeking individuals are of Iranian, Albanian, Afghan, Indian, Iraqi, Bangladeshi, Syrian, Sudanese, Eritrean, and Pakistani origin. This caveat has created a gap for this study to investigate.[1]

This study aims to review the published literature, via peer-reviewed databases, on the various mechanisms of torture and critically evaluate how forensic dermatologists assess relevant injuries to support asylum applications. Furthermore, it explores the appearance of torture in colored skin compared to Caucasian skin.

Forensic dermatologists assess victims of torture as per the Istanbul Protocol. However, international guidelines and current research fail to consider that colored skin has varying skin pigment/melanin content, lipid content and skin layer thickness, which differ from Caucasian skin. These differences cause altered injury presentation in colored skin. For these reasons, this study's results specify that people of color are more likely to present with post-inflammatory hyperpigmentation, reduced erythema, varied bruise color, and keloid scars. This work offers an understanding of what forensic dermatologists must be aware of and trained further in assessing these dissimilarities.

The results of this study indicate that the most common methods of torture suffered by asylum seekers are beatings, suspension, asphyxiation, burns, and electrical and sexual torture. These victims experience psychological torture yet go under-reported due to a lack of physical evidence and stigma. Various dermatological conditions such as Steven-Johnson syndrome, Toxic Epidermal Necrolysis, and Facial Erysipelas and cultural practices such as cupping, coining, and moxibustion mimic the presentation of torture injuries. Forensic dermatologists must be skilled in combining all this knowledge and being able to differentiate between naturally occurring phenomena and genuine cases of torture.

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Forensic Dermatology; Torture; People of Color

F51   The Contribution of Immunohistochemistry in Assessing Cruelty as an Aggravating Factor of Homicide

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Learning Overview: The goal of this presentation is to utilize a case file to demonstrate how immunohistochemical analysis can be used to date wounds inflicted a short time before death. In this case, this allowed understanding of the duration of the aggression, which was crucial to assess cruelty as an aggravating factor.

Impact Statement: This presentation will impact the forensic science community by underlining the role of immunohistochemistry in the reconstruction of the dynamics of a crime. Widespread application of this analysis could improve the amount of information provided to the court to assess future cases.

In the Italian penal code, cruelty as an aggravating factor of a homicide occurs when the methods of executing the offense demonstrate the intent to inflict excessive suffering on the victim and show a *quid pluris* compared to the activity required for the consummation of the crime itself. Because of the intrinsically subjective nature of this definition, proving cruelty during the trial is often a difficult process that can spark intense discussion and fierce opposition. Here we present a case where the contribution of the forensic pathologist was crucial in addressing this matter.

The body of a 33-year-old man was found in his apartment after a violent fight, with multiple abrasions, contusions, and lacerated wounds involving the head, face, trunk, and limbs, amputation of two fingers, and avulsion of the mandibular central incisors. Subsequent investigations identified six individuals potentially involved in the crime.

Forensic investigations were conducted, including imaging, autopsy, histological and immunohistochemical examinations, and toxicological analysis.

The Computed Tomography (CT) scan and autopsy of the body identified multiple fractures of the ribs, face, fingers, and spine, pulmonary and cerebral edema, laceration of the spleen, hemorrhagic infiltration of the soft tissues surrounding the lesions, and the presence of digested food in the airways. A toxicological screening by immunochemistry on a urine sample revealed only Tetrahydrocannabinol (THC) positivity.

Skin samples were collected from various wounds in different anatomical regions (head, fingers, elbow, knee) and subjected to immunohistochemical analysis to search for CD15, CD45, LCA, P-selectin, and E-selectin, a series of markers of the wound healing process. Each of these markers appears with a specific timing, making them a useful tool to date the lesions. The results showed a timing ranging from wounds inflicted one hour before death to injuries inflicted in *liminae vitae*.

The cause of death was determined as a synergism between hypovolemic shock and asphyxia due to inhalation of regurgitated food. None of the injuries was fatal by itself. The type of injuries observed, such as amputation, lacerations, and the immunohistochemical demonstration of an aggression lasting about one hour, allowed the aggravating factor of cruelty to be proven during the criminal proceedings. At all levels of judgement, the judges upheld the view expressed by the forensic pathologists.

Homicide; Torture; Immunohistochemical

F52   **A Probabilistic Genotyping Analysis of Touch DNA and Transfer Evidence**

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Learning Overview: The goal of this presentation is to describe the performance of touch DNA in a defined transfer setting and analysis with probabilistic genotyping software. While allele counting is a common process among laboratories for determining the usability of low-level samples, probabilistic genotyping analysis can offer a more thorough understanding of the usefulness of low-level data.[1]

Impact Statement: This presentation will impact the forensic science community by informing forensic laboratories and other forensic personnel of the statistical power of low-level data.

The amount of touch DNA deposited on a surface is typically dependent on the individual's shedder status and the amount of pressure applied to the object.[2,3] This project was completed in two phases. The first phase of this project narrowed down deposition procedures, best shedders, and if a profile could be generated. The second phase focused on the transfer of touch DNA with two contributors.

Probabilistic Genotyping (PG) software, STRmix™, was utilized to examine the statistical confidence of potential contributor genotypes to better assess the usefulness of the data. For Phase II sample analysis, the numerator for all ratios included both contributors, while the denominators were designed to assess each contributor as a Person Of Interest (POI). Each POI deconvolution was run in triplicate.

The PG analysis was evaluated for accuracy in the POI genotypes. Only likelihoods that met the 99% 1-sided Highest Posterior Density (HPD) were reported to communicate profile statistical strength, rather than only an allele count.[4]

As much of the data in this study was below potential stop at quants and calculated stochastic thresholds, laboratories without probabilistic genotyping would not define this data as usable. Additionally, when only focusing on allele counts, the same count does not always mean the presence of the same alleles, which adds to the complexity of evaluating the usefulness of touch/trace DNA evidence.

Touch DNA, while seemingly prominent, is not a good source of DNA to confidently decide on the presence of an individual. As seen through this experimentation and probabilistic genotyping analysis, it is unlikely a useful DNA profile is obtained only through transfer. This study also supports the need for validation and understanding of the performance of low-level data and transfers, which encourages the need for further research with current science and as science improves in the future with low-level data.

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Touch DNA; Transfer Evidence; Probabilistic Genotyping

F53 How to Talk to Your Genetic Genealogist: The Language of Kinship

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Learning Overview: After attending this presentation, attendees will better understand the terminology and methodology of genetic genealogy and, specifically, forensic genetic genealogy.

Impact Statement: This presentation will impact attendees by "translating" the language of genealogical research—as it relates to kinship determination—for scientists and law enforcement with the goal of enhancing communication, eliminating misperceptions, and countering false information.

While genetic genealogy has the power to help solve previously unsolvable cases as well as uncover new types of crimes, its relative newness means that there may not always be commonly understood terms to describe the work being performed. In addition, many of the terms used in the field—especially in communication between scientists, law enforcement, and genealogists—are inaccurate, misleading, or confusing. Just as "touch DNA" has become a misnomer since it is now known that DNA can be transferred without "touching," describing what it is that genetic genealogists do and understanding the terminology they use will be useful in creating a multicultural, multilingual, inclusive environment where we can communicate effectively and not proliferate false information.

In nearly every newspaper article reporting on a cold case that has been solved with the use of genetic genealogy, the way in which it is described is misleading. Labs are often described as performing genetic genealogy when, in reality, the labs are processing DNA to generate a DNA profile so that it can be uploaded and used by genetic genealogists to perform their research. Genetic genealogy involves identifying the DNA contributor's matches, building family trees, and finding connections between the trees. Genetic genealogy is not "new technology;" the DNA profile is not "uploaded to family tree databases over months;" neither law enforcement nor the genealogists have "access to troves of genetic information" that they "can then link to the DNA of unknown suspects;" and they do not interview hundreds of people in a family tree.

Additionally, terminology is important. Knowing how to talk to your genealogists using genealogical terminology will lead to better communication overall. While scientists and others sometimes describe the familial relationship between two people in terms of steps, degrees, Short Tandem Repeats (STRs)**,** Single Nucleotide Polymorphisms (SNPs), or ranges, genetic genealogists use centimorgans (cMs) and familial relationship terms, including grandparents, aunts, uncles, and cousins. A "second degree relationship," for example, means one thing to a scientist and another to a genealogist. Accuracy and consistency in terminology is key to having productive discussions about a case.

The genetic genealogist starts with the DNA results provided via the lab's work and then performs the genealogical research necessary to push the case forward. It is important that the community and the public better understand what genetic genealogy actually is, what information is accessed, and how the work is performed. Common definitions and terminology are key to making this a success.

Genetics; Genealogy; DNA

F54   Forensic Genetic Genealogy: Not Just for Solving Homicides

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Learning Overview: This presentation will highlight the importance and responsibilities of medical examiner/coroner offices to identify all unidentified remains utilizing updated technology and databases, including Forensic Investigative Genetic Genealogy (FIGG) on all cases, regardless of their suspected or known cause and manner of death. After attending this presentation, attendees will understand the importance of identifying remains not only for law enforcement to determine what happened to the decedent, but also to give the family closure. Attendees will learn about the different databases, technologies, and collaborations with law enforcement agencies, as well as ways to obtain funding for FIGG testing.

Impact Statement: This presentation will impact the forensic science community by demonstrating the importance of identifying unidentified decedents using FIGG to decrease the number of Unidentified Human Remains (UHR) at Medical Examiner/Coroner (ME/C) facilities, close/solve older cold cases, and help families put their loved ones to rest. This will promote solving of cold cases as, by identifying the UHR, law enforcement can then work their way backward to further investigate potential causes and manners of death, as well as give a grieving family closure.

According to the National Crime Information Center (NCIC), there were 521,705 people reported missing in 2021, with 93,718 of these still missing in 2022.[1] There have been 545 cases solved using FIGG, with 280 of those being unidentified cases.[2] National Missing and Unidentified Persons System (NamUs) has 14,463 unidentified cases in their system to date.[3] FIGG companies have worked with law enforcement agencies to collect DNA samples from violent crimes in order to help them identify a suspect through whole genome sequencing that is then uploaded into GEDMatch Pro and Family Tree DNA databases. This allows genealogists to work on the family trees of the people with the highest shared centimorgans in order to find a relative of interest.

Although this is important for solving violent cases, ME/C agencies can utilize this same technology simply to identify unidentified remains. There are several FIGG companies that collaborate with ME/C offices and can assist in crowdfunding for unidentified decedents if funding is an issue. FIGG gives ME/C offices the technology to identify people, which can then give investigators insight into further personal circumstances and may lead to the cause and manner of death. If no autopsy samples for SNP testing are available, exhumation of remains may be necessary, and law enforcement may assist. Our agency has identified 3 unidentified decedents using FIGG, with another 14 currently undergoing testing, and 5 that will need to be exhumed. All of the above decedents had previously been entered into Combined DNA Index System (CODIS) as well as NamUs (some for over 20 years), without resulting in an identification. It is important to recognize that FIGG is not only useful in solving crimes but is also a powerful tool from a purely humanitarian perspective, making identifications to provide families with the final answers of what happened to their loved one.

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Unidentified Persons; DNA; Medical Examiner

F55   **Is There a Lesser-Value Type of Violence? Older People Abuse: "The Silence of the Lambs"**

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Learning Overview: After attending this presentation, attendees will be aware of the results of an electronic literature research showing a low number of results and little attention paid to the topic of elder abuse.

Impact Statement: This presentation will impact the forensic science community by highlighting the scarce attention that has been given through the years to the topic of abuse of older people, with the hope of giving a new impulse to begin more research focusing on this topic.

The World Health Organization (WHO) describes gender violence as a real global health problem with a major impact not only on the victims' physical and mental health, but also on the economics of the National Health System. Gender-based violence has been also extended to all types of subjects defined as fragile: children, older people, women, men, and disabled people. Elder abuse, still more frequent in women, is a far less socially debated issue, with many possible forms: physical, sexual, psychological, abandonment, neglect, economic-financial, pharmaceutical, discriminatory, and institutional.

Today, the most common types of violence are neglect and psychological or emotional abuse, which creates a picture that is clearly different from the past, dominated by physical and sexual violence.

An electronic literature research was carried out using the keywords "elderly abuse" on various online sources such as PubMed®, Google® Scholar, Web of Science®, and Science Direct®. The keywords "elderly abuse" has led to a low number of results: PubMed®: 78; Google Scholar®: 5,750; Web of Science®: 85; Science Direct®: 197.

The research showed a low number of results and little attention to this topic in the scientific literature. The presentation highlights how the theme of older people abuse is unfortunately still underreported and not adequately addressed in scientific literature, as well as in real-life discussion or politics, being a form of violence certainly underestimated by the public.

Studies often fail to understand the data regarding the most susceptible elderly population (i.e., subjects with dementia or living in nursing homes), thus showing an incomplete evaluation of the phenomenon. We are certain that it is necessary to broaden the theme by also considering other aspects, such as inadequate care and the safeguarding of the elderly. Moreover, the lengthening of life prospects cause the need for special attention to this category and a better protection for their relative vulnerability.

Elder Abuse; Violence Against Women; Sex Offender

F56   A Differential Diagnosis Between Elder Neglect and the Pathophysiology of Aging: Medicolegal Aspects From a Case of Death by Hypothermia

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Learning Overview: Attendees of this presentation will gain knowledge of the complexities and considerations involved in identifying and investigating elder abuse cases. This case study discusses the challenges in differentiating between age-related pathologies and potential elder abuse, given the pre-existing health conditions typical of advanced age. Attendees will learn about the multifaceted process of evaluation, including assessing signs of malnutrition, inadequate hydration, skin lesions, and other indications of possible neglect. This presentation underscores the importance of comprehensive data collection and analysis, such as medical reports, testimonies, and material evidence. Attendees will also be exposed to the medicolegal tools and guidelines for investigating suspected cases of fatal elder abuse, highlighting the necessity of accurate interpretation of physical and laboratory findings for proper classification.

Impact Statement: This presentation will impact the forensic science community by fostering a deeper understanding of elder abuse, a rising public health problem expected to escalate due to increasing elderly populations. It emphasizes the medicolegal approach's pivotal role in ensuring a fair assessment of the facts, thereby safeguarding the rights of vulnerable elderly individuals and promoting justice. Moreover, it encourages forensic professionals to adopt proactive strategies and preventive measures to minimize future incidents of elder maltreatment. Overall, this presentation will contribute to the forensic community's capacity to better identify, investigate, and prevent elder abuse, enhancing their ability to help provide justice for this vulnerable demographic.

Elder abuse is a growing public health problem, and in almost 60% of cases, the perpetrator is a family member. Early recognition and prevention of abuse by focusing on both patients and caregivers are indispensable. This study presents a case of death by hypothermia in an elderly man, followed by an accusation of neglect against the son. The aim is to analyze the challenges posed by the differential diagnosis between age-related pathologies and elder abuse, with a particular focus on the medicolegal aspects of the case.

A 77-year-old man was found deceased in his apartment in winter. The autopsy highlighted "classical" findings of hypothermia, which was confirmed as the cause of death. However, attention was drawn to the potential negligence by the son, who was responsible for the care of the elderly father.

The differential diagnosis proved particularly complex, as the elderly individual also had chronic conditions and frailties typical of advanced age. It was necessary to carefully evaluate signs of malnutrition, inadequate hydration, skin lesions, and other indications of possible neglect. Simultaneously, it was essential to exclude the hypothesis of a role played by pre-existing health conditions. All relevant data, including medical reports, testimonies, and material evidence, were collected and analyzed. Accurate documentation played a fundamental role in determining whether there were sufficient grounds to support the accusation of neglect.

The discussion will focus on the complexity of the differential diagnosis in similar cases, highlighting those medicolegal aspects to be explored, including tools and guidelines for investigating suspected fatal elder abuse. The medicolegal approach plays a key role in ensuring a fair assessment of the facts in order to protect the rights of vulnerable elderly individuals and help pursue justice.

With the increasing number and proportion of elderly individuals in our population, cases of elder maltreatment, a prevalent form of domestic violence, are expected to rise. Accurate interpretation of physical and laboratory medicolegal findings is crucial for proper classification of these cases, certifying the cause and manner of death, and implementing preventive measures to avoid future incidents.

Elder Abuse; Neglect; Hypothermia

F57   Caregiver Burden and Elder Abuse: Medicolegal Perspectives from Two Fatal Cases

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Learning Overview: After attending this presentation, attendees will have a better understanding of how the caregiver profile influences the dynamics and injury pattern in fatal cases of elder abuse.

Impact Statement: This presentation will impact the forensic community by highlighting the importance of focusing on caregiver-related risk factors to prevent the phenomenon of elder abuse in the home environment.

Elder abuse is a common form of domestic violence, consisting of a failure to act or an intentional act by a caregiver that causes or creates a risk of harm to an elderly person. Victims are mostly elderly people with cognitive and physical impairments, making them incapable of self-care and over-reliant on caregiver support. The authors present two cases of fatal elder abuse by different caregivers, each presenting an unusual profile of physical abuse.

The victims were an 82-year-old woman with cognitive impairment who died while being assisted by a privately hired caregiver and a 101-year-old woman who was beaten by her daughter suffering from a psychiatric illness. In the first case, the autopsy showed a massive subdural hemorrhage with multiple contusions of different colors, consistent with repeated maltreatment. The elderly woman of the second case was the victim of an escalation of violence that caused extensive external injuries, leading to death by subdural and epidural bleeding. While the shared living situation and the high level of caregiver burden may have been major risk factors in both cases, the inability to cope with the stress was exacerbated by the coexisting psychiatric disorder of the daughter's victim.

Physical abuse may occur differently depending on many factors, including socioeconomic background, physical and mental health of both victims and perpetrators, and the relationship between the caregiver and the elderly. Postmortem examination findings play a crucial role in profiling the abuser and clarifying the setting in which the maltreatment occurred. Studies in various countries have investigated abuse-related risk factors, showing in about half of the cases a perception of stress or excessive burden in caregivers.[1,2] Moreover, most abusive behaviors have been shown to be perpetrated or worsened despite contact with specialist service, suggesting that more targeted and enhanced interventions are needed to identify the situations at risk for caregiver stress and take more effective support measures.[2,3]

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Elder Abuse; Domestic Violence; Blunt Force

F58   **Estimating the Biogeographical Origin of the Unidentified Bodies of the Shipwreck of April 18, 2015, in the Mediterranean: A Comparison of Genetic and Anthropological Assessments**

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Learning Overview: After attending this presentation, attendees will have gained a comprehensive understanding of the methodologies employed in geographic origin estimation in the context of forensic genetics and anthropology, particularly regarding victims of Mediterranean shipwrecks. This presentation will unpack how genetic investigation is applied to victim identification, exploring correlations between the quality of analytical results and taphonomic condition of cadaveric remains. Attendees will also learn about the significance of biogeographical ancestry estimation through various techniques, the implications of the timing of victim recovery, and the importance of early identification procedures. In addition, this presentation will delve into the complexities and discrepancies between different ancestry estimation methods and the influence of reference population choice.

Impact Statement: This presentation is poised to significantly impact the forensic science community by offering valuable insights into the potential challenges and solutions in victim identification and geographic origin estimation, particularly in the context of migratory crises. It highlights the urgent need for more refined methods, underlining the importance of a multidisciplinary approach that integrates forensic genetics, anthropology, and odontology. By sharing these research findings, the forensic community will be better equipped to streamline the identification process, enabling a more efficient search for relatives and selecting the most suitable data for genetic comparison. Moreover, understanding the correlation between the state of preservation of the remains and the quality of the genetic material stored within can guide the choice of the most appropriate portion of bone and/or protocols for future genetic investigations. Ultimately, this presentation could catalyze further research, fostering innovation and advancement in the field of forensic science.

The migratory crisis has recently drawn attention to the need to identify the victims of shipwrecks in the Mediterranean Sea. The present research had the objective of exploring the current methods of geographic origin estimation in forensic genetics and anthropology and contributing to the search in the field of identification. To this end, the remains of 150 victims were subjected to genetic investigation at the affiliated forensic genetics laboratories for identification purposes. Also, on cases with good quality profiles, biogeographical ancestry estimation was performed using different techniques, according to the protocols used in the two different laboratories involved.

Furthermore, having detected differences in terms of recovery of genetic information useful for comparison with the profiles of alleged relatives, the possible correlation between the quality of the results in the analysis of autosomal markers and the taphonomic condition of the cadaveric remains was investigated. The only variable that showed significant variation (p-value <0.01) was the time interval between the shipwreck and the autopsy procedures, during which sampling was performed. Bone samples with optimal analytical results were taken earlier (<200 days) in almost all cases. This finding underlines the importance of early victim recovery and identification procedures.

The genetic samples used for biogeographical estimate were also analyzed by applying morphological methods developed on populations allegedly comparable to those of the origin of the victims. The estimates were then compared with the genetic results obtained. With regard to geographical ancestry, the predictions of the Optimized Summed Score Attributes (OSSA) method appear to coincide with the group with probability percentages related to the higher estimate resulting from the hefneR software. However, the low number of OSSA findings does not allow for any additional considerations to be made.

As far as the non-metric dental characters are concerned, the results of the analysis with the rASUDAS software show little agreement with the cranial morphological predictions, referring in this sense only to the hefneR method. The assessment resulting from the hefneR software show a greater presence of the African and Asian components while the rASUDAS estimates show a European morphological predominance. These findings appear to be related to the type of reference populations chosen in the different methods and, in particular, to the absence of African traits in the rASUDAS and to the paucity of African populations among the references of the hefneR. This selection bias could also explain the many resulting mixed forms. The comparison with the predictions of biogeographical origin using Y-chromosomal Short Tandem Repeat (Y-STR) polymorphisms showed a certain pattern of agreement only with the estimates obtained by the hefneR method. The opportunity to confirm what was observed on a larger sample and when the identification process is completed would be an ambitious goal for future research.

In conclusion, the present research made it possible to identify the potentially most-suitable methods for geographical origin estimation, opening new perspectives in the identification process of the unknown victims of the Mediterranean. The availability of accurate anthropological and odontological methods can complement genetic analysis investigations.

In this sense, the comparison of suspicious identities can be restricted to individuals from a specific geographical area, just as the search for relatives can be directed to certain African countries and, thus, suitable reference data can be selected for genetic comparison. In addition, knowing any links between the state of preservation of the remains and the quality of the genetic material stored in it may allow the selection of the most suitable portion of bone and/or protocols for subsequent genetic investigations.

Migrants; Forensic Anthropology; Forensic Genetics

F59   A Cold Case Homicide Involving Partial Dismembered Human Remains Found in a Dumpster Resolved by a Three-Decade-Old Complex Diligent Police Investigation

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Learning Overview: Attendees will learn that law enforcement, coroner investigators, criminalists, and medical examiners need to work as a team by acknowledging each other's expertise, thus building relationships leading to cooperative ventures. Attendees will learn the value of a diligent initial crime scene investigation by law enforcement and medical examiner-coroner criminalists working together. Attendees will also learn the value of a detailed autopsy on partial dismembered remains.

Impact Statement: This presentation will demonstrate that crimes are solved by the dedication, perseverance, and ingenuity of involved law enforcement professionals working with the community, keeping an open mind, and being attentive listeners.

Dismemberment is usually an attempt to dispose of the body, so usually wounds are all postmortem/perimortem and surfaces (particularly dismembered bony surfaces) should be preserved and examined by a toolmark criminalist expert.

A transient hunting for aluminum cans found a dismembered human head inside a plastic trash bag in a dumpster and notified police. The coroner investigator and criminalist responded and found a second brown plastic bag, which contained one gray bag with two dismembered feet and a white plastic bag with a blood soaked remnant of carpet that were retained by Los Angeles Police Department (LAPD) criminalists as evidence. The torso and upper extremities were never found. The victim, John Doe 243, was identified by his roommate as William Newton.

The autopsy revealed multiple injuries to the head and neck, along with intracranial subarachnoid hemorrhage and supporting evidence for an asphyxia component. Toxicology showed methamphetamine in the blood. The investigation into his death initially hit a dead end until recent developments shed new light on the case. LAPD Detectives will present details of the diligent and thorough follow-up investigation that resulted in them interviewing an adult film actor who confessed to this 32-year-old Hollywood homicide.

One intriguing character in the story is a self-proclaimed private investigator who had been assisting the lead detective, Detective Berndt, for years. This individual, unbeknown to the LAPD, had a hidden secret. He was involved in the gay pornography industry under a stage name. His production company even had an office and editing space that overlooked the very alley where Newton's body parts were discovered. He had been present at the crime scene, blending in with the crowd of onlookers. His double life as an adult film director and his connection to the crime scene raised suspicions.

Further investigation led to the revelation that two of his adult films featured an actor named Billy Houston who was actually Daralyn Madden, a transgender woman with a troubled past. Madden had lived a complex life, engaging in various criminal activities, and adopting different personas.

Madden's criminal record spanned both California and Oklahoma. As a young man named Darrel, Madden had been involved in violent crimes, including membership in skinhead White supremacist gangs, robberies, and assaults specifically targeting gays and minorities. In 2007, Madden was convicted of murdering two men in Oklahoma. During Madden's time in prison, she underwent a gender transition and embraced Orthodox Judaism.

The breakthrough in the case came when a documentary filmmaker and her researcher discovered Madden's connection to the private investigator/adult film director who had been helping the LAPD. This revelation prompted them to dig deeper into Madden's background. They gave that information to LAPD Detectives John Lamberti and Tamara Momayez.

In an interview that detectives conducted in prison, Madden confessed to killing William Newton. Her memory of the events was patchy, given the passage of 32 years and her involvement in other crimes.

Crucially, Madden provided details that only the killer would know, corroborating her confession. Madden also recalled encountering Newton at dusk on Santa Monica Boulevard, aligning with witness accounts. Toxicology reports had shown the presence of methamphetamine in Newton's blood, supporting Madden's claim that he was high at the time of the incident. Additionally, Madden admitted to killing Newton but denied involvement in the dismemberment, which matched the findings from the autopsy report. These details from Madden's confession, previously undisclosed to the public, served as compelling evidence of her involvement in the murder.

With the revived interest in the case, thanks to the documentary, the truth about the case came to light. The unlikely sequence of events that led to the identification of the killer showcased the complexity and unpredictability of criminal investigations, bringing closure and solace to Newton's family and the Hollywood community.

Dismemberment; Crime Scene Investigation; Community Liaison

F60   Homicide Motel: Justice Served After Decades of Waiting

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Learning Overview: After attending this presentation, attendees will understand the value of evidence retention and perseverance even after decades of stagnant investigation and dead ends. Even with a plethora of potential evidence, limited funds, unfortunate circumstances, and technology kept a murderer free for over two decades. Grant funding provided the necessary tools to get not only DNA evidence, but fingerprint and palm print evidence as well. An overview of the 1994 murder scene, autopsy findings, police investigation, and, ultimately, the conviction of the suspect will be detailed.

Impact Statement: This presentation will impact the forensic science community by reminding attendees of the importance of never forgetting history and of asking unanswered questions over and over until a satisfactory result is reached. The forensic community has been and will continue to be full of dedicated professionals who use their intelligence, creativity, and technologies to solve crime, investigate death, and protect the public; this case is an example of all those attributes that make forensic science the backbone of justice in every city, county, and country. The intent is for this case study to be a reminder of the forensic victories that can be won even against all the odds that face the forensic science community today and in yesteryear.

On December 7, 1994, 36-year-old Rita Desjardine was found deceased by housekeeping in a motel room she had been staying at for nearly a week.[5] Motel staff checked on her the day before, but thought she was sleeping due to the placement of blankets over her body.[2] When housekeeping came closer to the body on December 7, they could see that Rita had been assaulted.[4] Men's and women's clothing was found in the room, and bloody fingerprints were noted on the sheets surrounding Rita and on some of the clothing.[2] In the days leading up to her discovery, Rita's social worker, who had set her up with food and at the motel, came by and saw an African American male leaving her room.[4] There were other vague witness statements about other parties in and out of Rita's room, but no names or solid suspects were ever found.[2]

Her autopsy was performed December 8, 1994, and although the pathologist noted blunt force injuries to her head, her cause of death was ruled to be due to smothering, or asphyxia.[2]

Evidence was submitted to the Federal Bureau of Investigation (FBI) in 1995, and the FBI lab noted prints in blood on the bed sheet, but the detail was too poor for any proper comparison. All DNA submitted from Rita did not garner any hits because Cumberbatch had yet to be arrested and convicted of murder. The case went cold for years.

In 2018, the Denver Police Crime Lab received a National Institute of Justice (NIJ) grant to help tackle their backlog of over 80 cold cases, mostly homicides and aggravated sex assaults.[5] Subsequently, the lab tested a gin bottle, some of the men's clothing, and swabs obtained from Rita on that fateful day back in 1994. Male DNA was found and months later, the sample, after being run through the Combined DNA Index System (CODIS), came back with a match, Steven Cumberbach, a male criminal currently incarcerated for murder in the state of Virginia.[1,2]

Once the CODIS hit was received, forensic examiners and police began working to locate and exclude any other possible suspects.[1] There were several men who had been seen in and around the decedent's room, two whom were dead and the third was a drug dealer who wasn't keen on speaking with law enforcement.[4] None of the other collected DNA comparison samples were on, in, and under the body like Cumberbach's.[1] There was more than DNA evidence; the left bloody palmprint that was under the body was re-examined [5], and, with a new chemical process to enhance fingerprint ridge detail, the Denver Police Department (DPD) lab was able to match the bloody print to Cumberbach's palm.[1] This print collaborated the DNA story and solidified evidence against him for charges to be brought decades later.

In June 2021, Cumberbach was extradited to Colorado to face charges. In June 2023, after deliberating for nine hours, the jury sentenced Cumberbach to life in prison without the possibility of parole.[3,6] He is serving out his sentence for the Virginian murder and may never return to Colorado for his life sentence for Rita, but justice was finally served.[1] Rita's daughter, 10 years old at the time of the murder, finally got closure and something tangible to hold close to her heart due to all the diligence needed to bring a killer to justice.[1,6]

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Homicide; Cold Case; DNA

F61 A Cold Case Homicide Solved "The Old-Fashioned Way" After 37 Years

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Learning Overview: By attending this presentation, the attendees will obtain knowledge about the circumstances and the steps taken to solve a homicide that occurred in 1984. The case involved police investigations, forensic science, genetic genealogy, as well as a "death bed" confession.

Impact Statement: This presentation will impact the forensic community by providing actual case information and discussion on the challenges encountered and new technology utilized during the processing of the evidence in this cold case. Interesting case facts will also be discussed, and questions will be addressed.

On February 13, 1984, a 64-year-old woman was brutally stabbed multiple times, strangled, and beaten in a small town in Massachusetts. The suspect then remained in her home, ate her food, and took her car out for a joy ride. Several items of evidence were left behind at the scene that contained blood and skin cells.

The case was assigned to the author on August 15, 2004, during her work as a Criminalist at the Massachusetts State Police Crime Laboratory. The assignment included a review of the case with fresh eyes with updated technology. Numerous items were examined, and DNA processing was completed. DNA profiles were obtained, and some were entered into the Combined DNA Index System (CODIS). Years went by without a CODIS hit. Additional DNA work was performed, but the case remained unsolved. Fast forward to April 2019: a Y-chromosomal Short Tandem Repeat (Y-STR) profile from the crime scene was sent to a private company for comparison to genealogical haplotype databases with the goal of developing a potential surname for the suspect. This provided some leads but did not result in the identification of a suspect.

In December 2019, a crime scene sample was sent out for Forensic Genetic Genealogy (FGG). This sample did not contain enough genetic material to produce searchable Single Nucleotide Polymorphism (SNP) data. Another sample was sent for FGG in September of 2020 and resulted in the identification of an individual who was eliminated as a potential suspect.

In February 2020, police were contacted by a lawyer's office; someone had come forward with information about the case. This information led to the discovery of a "new" crime scene that was processed in June of 2020. New evidence was collected and tested in the Criminalistics Unit by another analyst.

In March 2021, the District Attorney's office made a televised announcement of a "deathbed" confession that was brought forward and verified by forensic science. A suspect had been identified and his involvement in the crime was confirmed with DNA analysis. Finally, this cold case homicide was resolved.

Cold Case; Forensic Science; Genealogy

F62 Resting in Honored Glory: Identifying the Unknown Soldiers of the Segregated 92nd Infantry Division in World War II

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Learning Overview: After attending this presentation, attendees will understand how the efforts to identify those still-missing African American soldiers of the 92nd Infantry Division, a segregated unit in the United States Army during World War II (WWII), is a way for the forensic science communities to promote justice in highlighting their experiences and bringing their families long-awaited answers.

Impact Statement: This presentation will impact the forensic scientific community by emphasizing the need for an inter-disciplinary approach to forensic identifications that must include not only forensic sciences, but also the historical, social, and cultural circumstances of the missing. This collaborative effort provides a holistic approach that acknowledges the challenges under which those individuals were living and serving, the legacy of Jim Crow in America, and how that history of social injustice continues to impact the efforts to find and identify the missing of the 92nd Infantry Division from World War II.

The epithets on the headstones of the Unknown soldiers buried at American World War II military cemeteries read: "Here Rests In Honored Glory A Comrade In Arms Known But To God." At Florence American Cemetery in Italy, several of those headstones mark the graves of Unknown African American soldiers, killed during combat in 1944 and 1945. Despite the reverence of the epithet, African Americans who stood up to fight during the Second World War entered a segregated United States military, fighting for a country that treated them as second-class citizens. The African American soldiers of the 92nd Infantry Division, nicknamed "Buffalo Soldiers," experienced fierce fighting in the rough mountainous terrain of northern Italy, sustaining heavy casualties. Following the identification efforts of the war dead in the 1940s, there were still 53 soldiers of the 92nd Infantry who had not been recovered and returned to their families and communities.

The Defense POW/MIA Accounting Agency (DPAA) has a dedicated project to find, recover, and identify those still unaccounted-for soldiers of the 92nd Infantry. The bulk of that effort focuses on disinterment and modern forensic scientific analysis of 51 sets of remains recovered and buried in Florence in the 1940s, which could not be identified using the science of that time. Since 2016, only 3 of the 53 unaccounted-for soldiers of this division have been identified through these disinterment efforts.

There are several factors potentially affecting DPAA's ability to identify those unaccounted-for soldiers. The history of enslavement and large-scale population migration of African Americans within the United States can make it more difficult for genealogists to find families; families may also refuse to participate, resulting in lower rates of DNA family reference samples on file for comparison to Unknown remains. Incomplete civilian and military records also impact researchers' abilities to reconstruct the events of an individual soldier's loss and negates the ability of scientists to compare remains to antemortem records. In addition, anthropological methods used in remains analysis may be heavily based on and influenced by other reference populations, making them less reliable when applied in these cases. By recognizing and acknowledging the history of segregation and the historical, cultural, and social circumstances that influenced the lived reality and military service of the Buffalo Soldiers of the 92nd Infantry Division, only then can we begin to understand how those past inequalities impact the present identification efforts of the DPAA 92nd Infantry Division Project. With this project, forensic scientists have an opportunity to unite in a multidisciplinary approach to promote justice by reminding people that African Americans were an important part of the "Good War," memorialize their contributions, and bring their families long-awaited answers.

Identify; Unknown; Human Remains

F64   Establishing the Identity of the Dead Through a Complimentary Missing Person Registry With Unidentified Dead Bodies in the Indian Context: An Ethical Imperative

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Learning Overview: After attending this presentation, attendees will be sensitized about the current status of the missing person registry database and unidentified dead body databases in the Indian context. This presentation will raise concerns from the lens of ethical principles regarding unidentified dead bodies and their rights in the Indian context. This study will provide suggestions to strengthen the rights of the unidentified dead through a comprehensive missing person registry and its complement that can be implemented in the countries where it is not practiced today.

Impact Statement: In today's digital era, the scenarios where a missing person is not traceable, and a dead person is not identifiable, show that these two things are not complementing each other. This presentation will impact the forensic community by informing attendees about the need for coordination and the importance of developing empathy for unclaimed dead bodies and missing persons. This presentation will project the need to evaluate and find the lacuna in the identification process in complement with the missing person registry, with some suggestions to mitigate the issue. This presentation will help forensic experts practice in other underdeveloped and developing countries, including India.

We have to accept that even in the era of digitization and advancement in investigation and identification, we have failed to a great extent in identifying unclaimed dead bodies. We conducted this study to raise concerns from the lens of ethical principles regarding unidentified dead bodies and their rights in the Indian context. In addition, it strengthens the rights of the dead and dead bodies through a comprehensive missing person registry and its complement to unidentified dead bodies. In the present study, we reviewed media reports, research papers on unclaimed dead bodies in a forensic context, and articles related to the ethical issues of unidentified dead bodies.

Literature showed that the issues of undignified methods of storing the dead, limited capacity, insufficient mortuary resources, and the challenge of identifying unclaimed bodies are universal. Even the Federal Bureau of Investigation's National Crime Information Center (NCIC) databases used to track missing persons and the unidentified deceased has limitations regarding accessibility, search capability, usefulness, effectiveness, and utilization.[1]

The cause of the unidentified dead due to the lack of resources, improper funding allocations, lack of expertise, and large caseload in context to the developing countries were also found in the literature. Vulnerable populations (homeless persons) are often overlooked. Scholars advocated for a standardized approach to forensic human identification, standardized guidelines, and collaborative agreements with forensic service providers and law enforcement agencies. Emphasized education and awareness and databases aligned to unidentified bodies and missing persons to mitigate the challenges of identification of unknown dead was also quoted as the need of the hour.[2]

In the Indian context, as per the record of National Crime Record Bureau (NCRB) India 2020 data, in India, approximately 600,000 persons are reported as missing and untraced from various states. [3] NCRB reported unidentified dead bodies of 35,857 (2010). According to media reports (*India Today*, June 2021) referring to NCRB, 2,22,446 dead bodies remain unidentified. India's government has taken various steps and initiatives to trace the missing persons and establish identity in unidentified dead bodies.

Policy to report missing persons through the police and update the database shared with other law enforcement agencies already exists. In India, the Crime and Criminal Tracking Network and Systems (CCTNS) is an important networking system through which criminal and unclaimed dead body data can be shared with law enforcement and common citizens.[4]

It was found that the failure to identify the unclaimed bodies on one side and the missing person search on the other side is due to a lack of coordination between police, forensics, and the general public. Coordination between state investigative agencies is also weak due to various practical reasons. In India, CCTNC, because of poor implementations, is weakened as to its purposes. On the other side, the data of unclaimed bodies in the database looks haphazard (i.e., poor-quality photographs and missing other physical and biological information make it impractical). Above all, the lack of sensitization and empathy toward the dead who are not related to us leads us to indifference.

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Ethics; Missing Persons; Death Investigation

F65 Forensic Archaeology Methodologies Developed in Direct Ratio to Practice and Experience: Innovative Drilling Technique for the Well Excavations of the Committee on Missing Persons in Cyprus

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Learning Overview: It is possible to mention the 2002 missing persons during the 1963-64 inter-communal fights and the 1974 events in Cyprus. In this context, the Committee on Missing Persons (CMP) in Cyprus carries out excavations all over the island with the Bi-Communal Forensic Team (BCFT) to find and exhume the aforementioned missing persons. With this presentation, a new method applied in difficult well excavations to find missing persons will be mentioned. The drilling method, which was started to be used as a pilot study, primarily accelerated the difficult and long-term well excavation process and also accelerated the process of searching for missing persons, the exhumation, and identification process. This presentation will discuss the pilot study and results of drilling deep wells with minimal damage to the remains in line with this method applied in Cyprus.

Impact Statement: The main aim of this study is to excavate the challenging deep wells in Cyprus in the name of searching missing persons. The damp environment of the wells makes the process difficult to preserve remains and yield results for DNA. Relatively longer well excavations also make it difficult to find missing persons in this process. With the drill method, which has begun to be used, the wells are dug faster and more cost effectively, and the remains are revealed at an earlier time. For remains that have been waiting to be found for a long time, the faster they are found, the better it will benefit the identification process. In this direction, the method to be explained in the presentation provides benefits by influencing the whole process with the discovery of the method by which the discovery of remains will be accelerated and the approval of use.

The CMP is a bi-communal organization established in 1981 to investigate the missing persons during the inter-communal fighting of 1963-64 and the events of 1974 in Cyprus. Following the deliberations, the committee agreed on the number of missing persons, which is 2,002 (1,510 Greek Cypriots and 492 Turkish Cypriots). Thus, in 2006, the organization became fully functional and started scientific forensic examinations by investigating, excavating, exhuming, identifying, and returning the remains of missing persons to their families.

In this presentation, the archaeological phase will be focused on detailed excavations, techniques, and methodologies that were used and developed, in addition to the other units of the CMP. The main target is to discuss the excavation technique of wells with positive and negative aspects of the success rate achieved. The BCFT of CMP is undertaking excavations in open fields, stream beds and floodplains, forests, caves, and high-altitude slopes in spite of wells. Excavations in different types of locations will not always be accessible, and sometimes it can be so challenging, especially in wells.

In the first years of the CMP, the ramp technique was used for the idea of how to dig wells, which are relatively demanding in terms of risk, cost, and time. The method mentioned above is performed to mobilize the archaeologists to the bottom of the well by creating an access ramp in case human remains are revealed. Long-term well excavations were carried out without being sure whether the remains of missing persons would be found. This process also affects the identification and return of human remains to their relatives. On the other hand, with the practice and experience gained over many years, a new technique has been developed by CMP. The new well excavation method, the "drilling technique," takes a relatively short time, is risk-free for archaeologists, and scientifically reaches the remains faster, thus increasing the chance to get better DNA results. This technique started to be applied as a pilot study in 2018 and is accepted as a main well excavation technique. In conclusion, the results will be presented as to how this technique was developed, its favorable and unfavorable aspects, the success it has achieved, and whether it can be continued to be applied or not in well excavations.[1]

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1. Statistical information gathered from the website of the Committee on Missing Persons in Cyprus (https://www.cmp-cyprus.org).

Forensic Archaeology; Exhumation; Forensic Excavation Methods

F66   Exercising Due Diligence: Utilizing Novel Multidisciplinary Methods in the Recovery of Skeletal Remains

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Learning Overview: This presentation will emphasize the importance of utilizing multidisciplinary methods in medicolegal death investigations involving the recovery of skeletal remains and the effects that such actions may have on the final determination of cause and manner of death. The medicolegal case presented here underscores the adherence to forensic medicolegal standards while incorporating multidisciplinary methods for successful skeletal remain recovery.

Impact Statement: This presentation will impact the forensic science community by informing attendees that while forensic science standards and guidelines facilitate an effective and efficient medicolegal investigative process overall, multidisciplinary methods can be argued to reach further in providing critical information necessary for law enforcement investigations, determination of cause and manner of death, and necessary closure for the family of the victim.

Exercising due diligence with the utilization of multidisciplinary methods may be distinguished as going beyond simply adhering to forensic science standards. It incorporates various resources, information, and personnel in order to obtain evidence essential to the case, not otherwise attainable through current standards and guidelines. A major concern is felt when the pursuit of multidisciplinary methods is weighed against the expenditure of limited resources, potentially resulting in an incomplete investigation, and a public loss of confidence in the medicolegal profession.

Medicolegal investigations involving the recovery of human skeletal remains in the back country pose a unique challenge. Animal predation may mimic trauma, obscure injuries, or result in the fragmentation and displacement of remains. One method of displacement occurs through interspecies competition for a food source. Specific species traits affect such methods, locations, and distances with respect to remains transference (i.e., subterranean, rock outcropping, or hillside locations, each ranging from meters to miles away). This poses unique challenges for medicolegal investigators, as fragmented human remains may be scattered across an immeasurable area. The skeletal remains of medicolegal significance may be absent, and the determination of cause and manner of death may prove challenging if not unobtainable.

The medicolegal case presented here details the skeletal remains recovery of an individual located in an area of Idaho foothills where public lands combine human recreation and diverse wildlife. These human remains were initially discovered in a steep canyon. Years after the death of the individual, a utility crew working in the canyon discovered the remains and alerted officials.

The fragmented remains were discovered within the dense and thorned thicket along the stream at the bottom of a canyon. Also discovered were remnants of the decedent's clothing, multiple personal items, and identification. An initial search by medicolegal investigator and law enforcement officials resulted in the recovery of an incomplete skeleton. This outcome is not atypical, but critically absent skeletal remains included the mandible and skull. Although the investigation suggested suicidal intent, the absence of these significant remains resulted in the cause and manner of death as being listed as "Undetermined."

At autopsy, DNA analysis confirmed identification while radiological imaging and inspection simply revealed evidence of postmortem predation. Void of any other signs of trauma, the forensic pathologist concluded no definitive cause of death.

Further evaluation of the initial recovery effort resulted in the conclusion that the expenditure of limited resources hampered an effective recovery. A determination was made that further recovery actions incorporating multidisciplinary methods were essential to meet due diligence and potentially determine the cause and manner of death.

A multidisciplinary team comprised of medicolegal death investigators, forensic technicians, and an emergency management professional were chosen because of their additional knowledge of local wildlife behavior, seasonal climate patterns, and nearby recreational trails. After an evaluation and search of the initial recovery site yielded no additional results by the team, an onsite discussion and further evaluation of the area provided valuable opinions on potential search areas that were previously believed to be impractical. The refocused effort, combined with in-depth knowledge of the area, wildlife, and weather was successful in the ultimate recovery of forensically significant portions of the decedent's fragmented skull. These portions demonstrated a definitive gunshot wound defect that, upon examination by the forensic pathologist, proved to be both fatal and consistent with suicide.

By utilizing multidisciplinary methods and exercising due diligence in this medicolegal investigation, critical information was provided to assist the forensic pathologist in determining cause and manner of death, thus supporting the law enforcement investigation and helping to bring closure for the family of the deceased.

Medicolegal Death Investigation; Skeletal Remains; Multidisciplinary

F67   Challenges in Identification and Repatriation of Anjala (India) Skeletal Remains: A Humanitarian Forensic Concern

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Learning Overview: After attending this presentation, attendees will be sensitized regarding the challenges and barriers in identification and repatriation of non-scientifically excavated unknown human remains.

Impact Statement: This presentation will impact the forensic science community by informing attendees how the application of a multitude of forensic anthropological techniques can help in the identification of even badly damaged, fragmented, and commingled human remains.

Forensic anthropologists have played a crucial role in recovery and identification of the remains of victims of war or war-related crimes, genocides, racial conflicts, and other heinous and violent cruelties committed against humanity in the past. Various heinous crimes and brutalities have been committed against humanity on the basis of discriminations based on race, caste, creed, gender, power, or greed that have resulted in brutal killings in the past as well as in modern times. Forensic anthropologists endeavor to bring closure, solace, and comfort to the bereaved families of the deceased by disseminating information about the location, exhumation, identification, dignified management, and burial of their mortal remains. The scope of forensic anthropological investigations has transgressed far beyond the realms of the traditional biological profiling casework to the scenarios of humanitarian concerns. Forensic anthropologists have significantly contributed toward amelioration of human suffering, ending the torturous uncertainty of the relatives, and tallowing them to mourn and memorialize the victims of such incidents by applying standardized advanced methodologies and techniques for their forensic identifications.[1,2]

One such incident of mass killings of 282 Indian soldiers and disposal of their cadavers in an ancient abandoned well situated well underneath a religious structure was reported from the Indian suburb Ajnala in Amritsar, India, in early 2014.[3-5] The initial cruelty was committed by the then-administrator who arranged to massacre the weakened, famished, fatigued, and bullied soldiers (who killed British officers and revolted) belonging to 26th Native Bengal Infantry regiment; no legal options like court-martial proceedings were extended to the fugitive soldiers and the cadavers were disposed of in the reported abandoned well near the site of their killings. Third, the non-scientific excavation carried out (to authenticate or refute the written accounts) by the amateur archaeologists resulted in heaps of badly damaged and commingled human remains. The unethical treatment rendered to the mortal remains of Ajnala victims, who were reportedly non-local to the site, further decimated the remains.

The biggest challenge for their forensic anthropological identification was their badly damaged, fragmented, and commingled nature. No grants were provided to the investigators for the biological profiling of thousands of unidentified human fragments; absence any financial support, the collaborative research work helped in establishing their putative identity and geographic affiliations by applying various anthropological, odontological, chemical, and molecular methods.[6-10]

The nature of fractures in long bones and other skeletal elements endorsed the written records that the cadavers were thrown from the top of the well and no ritualistic burial was performed. The toughest barrier in repatriation of 168-year-old Ajnala skeletal remains to their descendants is non-availability of demographic credentials (badge number, name, address, and ethnic attribution) of deceased soldiers, still to be procured from the British government. Efforts have also been made to obtain the cranium of the leader-in-chief (Mr. Alum Begh) of the fugitive regiment from England, who escaped the killings at Ajnala, though he was captured alive and executed a few months after the incident, and his head was gifted as trophy to celebrate the 1857 revolt.

The collection of biological samples of the present-day descendants of the Ajnala victims (provided the list of martyred soldiers is received) is proposed to match the DNA and stable isotopic signatures of identity of Ajnala skeletal remains with the modern-day population to facilitate the repatriation of Ajnala skeletal remains. It is another challenge to reach to the actual descendants of the victims, reportedly belonging to different Indian states.

Various challenges and barriers faced by the author in identification and repatriation of Ajnala skeletal remains will be discussed.

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Forensic Archaeology; Human Remains; Identification

F68   The Ethics of Dead Bodies/Body Remains Following Disasters: A Qualitative Study

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Learning Overview: After attending this presentation, attendees will have insight into ethical issues posed by the dead bodies/skeletal remains following disasters and can develop empathy while dealing with relatives of the dead, as they happen to be vulnerable. It also brings into discussion what ethical issues are specific to Low- and Middle-Income Countries (LMICs). Are they different? This presentation provides a justification for ethical handling of issues.

Impact Statement: While ethical issues on human beings are often discussed, the ethical issues for the dead are not commonly discussed, and there has not been growth in the conceptualization of what the dignity and respect stand for when one dissects the words to make them operationalizable. Through the qualitative grounded theory approach, efforts are made to give a name and develop a hypothesis on ethics of dead bodies/remains in disasters. The hypothesis brought in by grounded theory can be further tested quantitatively through a questionnaire-based study. Since research potential on dead bodies/skeletal remains is immense, but the framework is scant, there is a scope for arbitrary treatment/oversight for research. This presentation will impact the forensic science community by enlarging the scope for discussion of skeletal remains for respectful treatment and their use in research.

The sudden death of many people during disasters requires forensic doctors to identify the bodies and return them to their families. Typically, forensic action during disasters is conducted under considerable time and pressure constraints. In developing countries like India, resource limitations and lack of infrastructure can sometimes result in mismanagement and misidentification of dead bodies, causing a perception of disrespect to the deceased and their families.[1] This is the first study in India to identify ethical issues in forensic practice when dealing with the dead during disasters and carry out an ethical analysis of these issues.

Objectives: (1) Exploration of ethical issues faced by forensic doctors in disaster settings; (2) identification of ethical issues faced in LMICs during disasters; (3) ethical analysis of one issue relevant to a global context and one relevant to the Indian context, and (4) development of a set of recommendations based on this analysis.

Methodology:

Research Objective 1: Reviewed the literature to identify the ethical issues relating to forensic medicine in disasters to understand the ethical issues faced by forensic doctors in disasters.

Research Objective 2: To identify the ethical issues forensic doctors face in disaster situations, we conducted 20 one-on-one in-depth interviews with forensic doctors in India about the ethical issues they faced while providing forensic services during disasters.

Research Objective 3: Based on the results of the in-depth interviews, we focused on the ethical issue most commonly faced by forensic doctors in India and conducted an ethical analysis of this issue using *The Universal Declaration on Bioethics and human rights* (2005).[2] Furthermore, based on the literature review results, we identified the ethical issue most commonly cited by forensic doctors worldwide. We carried out an ethical analysis of the same.

Research Objective 4: Based on analysis of ethical issues relevant to the Indian and global contexts, we developed recommendations to prevent the unethical treatment of dead bodies during disasters.

The study attempts to identify and analyze the limitations of forensic action in the ethical treatment of the dead during disasters. This could help the profession close the gap between forensic medicine services and ethical practices during disasters.

Results: We analyzed the ethical issue of identification of the dead in disasters from Indian in-depth interviews with Indian forensic doctors, focusing on the impact of identification of the dead on treatment, using the United Nations Educational, Scientific and Cultural Organization's (UNESCO's) Universal Declaration of Bioethics and Human Rights (UDBHR) as a tool to examine articles relevant to the dead and their relatives, examining their implications on the question of whether it is ethical to give up identification in disaster settings.

We analyzed the ethical issue that emerged from the literature analysis as the most commonly cited by forensic doctors worldwide, "research on the dead." While antemortem consent or consent from relatives for research was a common theme in the literature, the ethics of research on the unidentified dead was relatively unexplored. Furthermore, we examine articles of the UDBHR for applicability to the dead and their relatives and analyze applicable articles' implications on the research question, "When is it ethical to research on unidentified dead or their body parts obtained in disaster settings?".

Conclusion: A recommendation on planning and vigilance at the site of occurrence of a disaster, at the mortuary, and during research form the framework.

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Cadaver; Research; Disaster

F69   Surfing the Dark Web

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Learning Overview: This presentation will explain how the Dark Web was created, how it provides anonymity, and how it is used both legally and illegally. Attendees will learn to recognize evidence at overdose scenes that can be indicative of drugs that were purchased on the Dark Web. Attendees will also learn how law enforcement has had success in tracking down criminals on the Dark Web.

Impact Statement: This presentation will impact the forensic science community by educating attendees about the Dark Web. The Dark Web is an area of the internet that requires specialized software to access. The websites there are intentionally hidden and cannot be found using common search engines such as Google®. It was designed so that users could access these sites and communicate in secrecy without their online activity being detected and viewed.

It is not illegal to be on the Dark Web, but the common perception is that it is a crime to access it and that it is only used for criminal purposes. Illegal items that can be procured on the Dark Web besides drugs include firearms, sexual services, child pornography, and stolen credit cards and Social Security numbers. While there is a significant amount of nefarious activity that occurs, there are also legitimate reasons that some people use this forum to communicate. People who live under restrictive regimes, journalists, and whistleblowers need to be able to have private conversations online without anyone seeing with whom they are communicating and the websites they are visiting.

The sale of illicit drugs on the Dark Web helps to fuel the drug epidemic. Numerous marketplaces advertise a wide variety of drugs. Cryptocurrency is used for payment, which helps the sellers and the buyers maintain anonymity. The drugs are delivered to buyers in the United States by the United States Postal Service and commercial shippers, such as FedEx® and UPS®. Investigation of the drug death scene may reveal evidence that the drugs were purchased on the Dark Web. Law enforcement can use this information to pursue drug dealers in the United States and in other countries.

This presentation will discuss the different areas of the internet: the Surface Web, the Deep Web, and the Dark Web.[1] It will explain why the Dark Web was created and will list the steps and technical requirements to access it. The Tor browser, the gateway to the Dark Web, will be described as well as the concepts of IP addresses, how they work, and how the Tor browser relays and encrypts internet traffic, which then hides users' IP addresses so they can browse the internet freely, without being tracked and surveilled.[2] Virtual Private Networks (VPN) in relation to the Dark Web navigation will also be explained.[3] A user can indeed be seen logging onto Tor, even though the websites the user visits cannot be tracked. Should there be a need to mask logging onto Tor, a VPN should be used to provide more anonymity.

The Dark Web is all about secrecy and anonymity. Tor's website says it is "run by thousands of volunteers around the world," without sharing any information on that community. Tor can be used for good purposes—whistleblowers, reporters, and activists, for example—but it is certainly a forum that enables users to commit crimes such as illegally buying and selling firearms and illicit drugs, making and posting child pornography, smuggling, and human trafficking.

This presentation will demonstrate how to access the Dark Web from a personal computer. It will show the process of registering with a marketplace, which is required in order to enter many of the websites on Dark Web that sell illegal items, including drugs, and it will simulate how a drug user purchases illicit drugs from one of the websites there. It will also describe how several criminals were tracked and caught by law enforcement officials for business they were conducting on the Dark Web.

Finally, drug scene investigators will be provided with an awareness of how drugs are purchased on the Dark Web and delivered to the buyers. Investigators will be able to recognize evidence at drug scenes that could be crucial to law enforcement efforts to find offenders using the Dark Web to distribute illicit drugs.

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Death Investigation; Drug Abuse; Illegal

F70   Eye Tracking in Evaluating the Decision-Making of Crime Scene Investigators: A Pilot Study

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Learning Overview: After attending this presentation, attendees will form a clearer picture of how Crime Scene Investigators' (CSIs') gaze behavior is associated with their investigation process, exhibits collected, and inferences about the case.

Impact Statement: This presentation will impact the forensic science community by illustrating how wearable eye-tracking technology can detect the dynamic changes of visual attention in complex crime scene investigations. Studying CSIs' decision-making using an eye-tracking approach can assist the empirical study of visual gaze patterns involved in interpreting items at the crime scene and making inference about the case, hence supporting CSI development and training.

CSIs actively learn from surrounding information or traces in the scene to make case inferences, collecting exhibits and sending these to analytical units.[1,2] Eye trackers enable the identification of CSIs' visual preferences, attention, and focus.[2,3] Although eye-tracking has been introduced to study differences of visual and search patterns between expert and trainee CSIs to deduce key elements that may differ in their performance, empirical work is still lacking. This is specifically evident when it comes to modeling decision-making processes and victim interaction during scene investigations.

To address the deficiency, this presentation will show empirical data collected from an ongoing pilot study highlighting similarities and differences in the decision-making processes among ten trainee CSIs. The trainee CSIs were recruited to search for evidence in a mock burglary scene, with an alleged victim present to provide case-related information when asked. Data collected from eye trackers looked at participants' *fixation points* (focusing point), *fixation duration* (time spent on focusing point), *time spent on questioning the victim and documentation*, *visit counts and duration* (regarding areas of interest), *total time on investigation,* and the *visual path* (the order of gaze behavior). Participants were asked to record their decisions in a decision log during their search in addition to taking part in a post-experiment questionnaire regarding their evidence collection, prioritization, and search strategies.

A preliminary decision-making model was established by analyzing the qualitative data from their decision log and questionnaires in addition to the eye-tracking data to look at the similarities and differences in evidence collection and scene approach. The preliminary results show that although trainee CSIs tend to use different systematic search strategies and have slightly different search approaches, they tend to conduct the search roughly following the established strategy rather than their judgment on the relevance between evidence and items at the case in the contact of the case. Those who asked the victim at the beginning about specific items, loss of items, and its location tended to be more aim-driven by targeting their search around that location and areas of interest. However, some trainee CSIs' judgments about the case showed poor understanding of exhibits collected from the scene. Ultimately, the finding demonstrates the importance for CSIs to be aim-driven and the importance of appropriate relationship established between evidence and the case during their search.

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Crime Scene Investigation; Eye Tracking; Decision-Making

F71 The Road to Standard Development: An Update From Dogs and Sensors

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Learning Overview: After attending this presentation, attendees will have an understanding and appreciation of the efforts and progress of the Organization of Scientific Area Committees (OSAC) Dogs and Sensors Subcommittee and the Academy Standards Board (ASB) Dogs and Sensors Consensus Body have made to develop and publish standards related to the various detection disciplines within Dogs and Sensors to be implemented by various agencies, including law enforcement, military, and homeland security.

Impact Statement: The use of forensic science standards is increasing exponentially across many forensic disciplines, both in the laboratory and within field practices utilized in criminal investigations. This presentation will impact the forensic science community by informing attendees about upcoming and existing standards pertinent to Dogs and Sensors. This presentation will also highlight how the standards are being structured and why standards should be adopted across agencies employing canine detector teams.

This presentation will summarize recent standard development progress by the OSAC Dogs and Sensors Subcommittee and the ASB Dogs and Sensors Consensus Body. Since its inception, the OSAC—Dogs and Sensors subcommittee has identified 28 OSAC standards and technical reports making their way through the process map for approval by ASB Consensus Body and ultimately OSAC approval for placing on federal registry. These 28 documents relate to other related forensic disciplines. Updates regarding the status of the various document products will be discussed, including Standards Development Organization (SDO) -published standards eligible for OSAC Registry (Standard for Training and Certification of Canine Detection of Humans: Location Check using Pre-Scented Canines, Standard for Training and Certification of Canine Detection of Humans: An Aged Trail using Pre-Scented Canines, Standard for Training and Certification of Canine Detection of Humans: Patrol Canine Team), recently added OSAC Registry posted standards (Dogs and Sensors Standard for Canine Selection, Kenneling, and Healthcare and Standard for Training and Certification of Canine Detection of Explosives), proposed standards in comment adjudication phase at the ASB (Canine Detection of Human Remains on Land), and forthcoming OSAC proposed standards (Standard for the Systematic Validation of Training Aids for Canine Detection of Explosives, Standard for the Analytical Measurement of Training Aids for Canine Detection of Explosives, Standard for the Canine Testing of Training Aids for Canine Detection of Explosives, and Standard Guide for Canine Detection Professional Progression).

Additionally, this presentation will highlight how the standards are being structured and the importance of implementing standards within the canine detection community. This presentation will project the future of the group's standardization efforts and inform of the current roadmap being used to implement these nationwide. The success and efforts of the Dogs and Sensors subcommittee is dependent on the members and affiliates utilizing the input from the community to help inform and shape these important standards to be ultimately implemented across the wide range of developing detection threats and needs.

OSAC; Standards; Canines

F72   Human Remains Detection Dogs and Residual Odor

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Learning Overview: This presentation uses certified Human Remains Detection (HRD) dogs to detect residual odor of a year prior on a suitcase in an outdoor setting and provide an error estimate. After attending this presentation, attendees will better understand the factors that lead dogs to identify the residual odor of human decomposition correctly and incorrectly. False positives and false negatives rates are also calculated.

Impact Statement: This presentation will impact the forensic science community by informing attendees that this project allows an initial error estimate for cadaver dogs on residual human decomposition odor.

HRD dogs are trained to give a Trained Final Response (TFR) in the presence of the odor of human cadavers. Cadaver dogs may give a TFR in the presence of the residual scent. A TFR has been used in court to show that human remains were at a location, linking a car or house to an alleged perpetrator. This project determined the proportion of certified cadaver dog teams that gave a TFR in the presence of a suitcase that held human remains one year prior.

How long does residual odor last? One study using human blood showed that six dogs had difficulty finding the odor after one month on concrete and one week on varnished wood.[1] Another study used carpet squares exposed to decedents dead less than three hours. The contaminated carpet was placed with six blank carpet squares. Over 65 days, three cadaver dog teams performed 354 searches. Well over 90% of the searches were reported as correct.[2]

These studies focused on varying time and substrates, using small numbers of dogs. This provides a solid error rate for those dogs, but to determine an error rate for dog teams in general, a larger sample size is necessary. This project starts filling that gap.

Colorado Mesa University's (CMU) Forensic Investigation Research Station (FIRS) holds trainings for cadaver dog teams. In one scenario, a suitcase contains human scents. This project used the residual scent on that suitcase, scented a year prior. The research was approved by CMU's Institutional Research Board (IRB-FY23-98) and the Institutional Animal Care and Use Committee.

The scented and two unscented suitcases were placed outside about 60 feet apart. Handlers were not told which was the scented suitcase. The observer recorded the handler's observations and their own assessment of the dog's reaction.

There were a total of 24 runs with 19 different teams. Fifty percent of the time, the teams were 100% accurate, showing a TFR on the scented suitcase and no interest in the blanks. If a false negative is showing neither interest nor a TFR on the scented suitcase, the false negative rate was 0%. If a false negative is showing interest but not a TFR, the false negative rate was 12.5%.

Based on handler observation, if a false positive is a TFR on a suitcase with no scent, the false positive rate was 12.5%. All false TFRs were in Session 1, when the wind was strong. If a false positive is either interest or a TFR, then the false positive climbs to 33%. Both rates are lower when only a TFR, not "interest" is considered. The false readings were by different dogs on different days. Of the four dogs that repeated the exercise, three had different results on the two runs. The false negative rate was less than the false positive; however, in residual scent cases, false positives are a concern.

Conclusions: Nineteen certified cadaver dog teams ran an exercise with one suitcase that had held a body one year prior and two unscented suitcases 24 times. Fifty percent of the time, the teams identified the scented and unscented suitcases 100% accurately. Both false negative and positive rates are reduced when only TFRs, and not "interest" is counted. Further research should test the false positive rate, when there was no residual scent source at all, as wind was an issue.

The results stress the importance of using multiple cadaver dogs when looking for human residual scent, of only using results with a trained final response, of using blanks, and controlling for wind.

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Sensor Dog; Cadaver Dog; Error Rate

F73   **A Multidisciplinary Approach to K-9 Human Remains Detection: The Integration of Medical Examiners, Forensic Anthropologists, and Medicolegal Investigators With the K-9 Team**

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Learning Overview: After attending this presentation, attendees will be informed about the importance of a multidisciplinary approach to scene search and recovery operations when K-9s are used for human remains detection. This model is based on ongoing collaboration and joint training with the New York City Office of Chief Medical Examiner K-9 Unit (NYC OCME K9) and the New York Police Department Emergency Service K-9 Unit (NYPD ESU K9). The incorporation of forensic anthropologists and medicolegal investigators with the K-9 team provides a best-practice model to successfully detect and recover human remains and/or confidently rule out a potential search area.

Impact Statement: This presentation will impact the forensic science community by demonstrating the benefits of including additional medicolegal personnel (e.g., forensic anthropologists and medicolegal death investigators) to work along with certified Human Remains Detection K-9 Teams. Several case studies will be presented to illustrate how a multidisciplinary search team is utilized in New York City.

In 2021, the NYC OCME became one of the few medical examiner offices in the United States to develop a K-9 Unit for Human Remains Detection (HRD) and Recovery. The NYC OCME K9 Team is certified by the North American Police Work Dog Association (NAPWDA). The K-9 Unit enables the NYC OCME, in close collaboration with NYPD ESU K9, to assist in the timely discovery of human remains in various types of investigations, including bodies that have been buried, dismembered, or otherwise concealed. The K-9 Unit can assist when there is suspicion that a body has been moved or when a decedent is believed to be under water and is also ideally suited for fire deaths, explosions, and mass casualty events. The NYC OCME K9 Unit is a multidisciplinary team comprised of staff from Forensic Pathology (K-9 Handler), Forensic Anthropology, and Medicolegal Investigations. Medical examiners (forensic pathologists), forensic anthropologists, and medicolegal investigators are uniquely positioned to staff the K-9 Unit due to their combined expertise in medicine, postmortem changes, human remains recovery, identification, and scene investigation. With this expertise, they can support the various complex issues that may arise during and after the detection and recovery of human remains.

Joint training and collaboration between the NYC OCME K9 Unit and NYPD ESU K9 has led to a well-established process that has proven successful in a wide variety of HRD search and recovery cases. The process incorporates a structured, multi-stepped approach to scene searches that integrates canine odor detection, established forensic anthropology methods, and investigative scene photography and documentation. A typical scene search is initiated by the K-9 team, followed by forensic anthropological review of any areas where the handler communicates that the K-9 showed changes of behavior or a trained final response. Depending on the type of scene (e.g., indoor, outdoor, mass fatality), this may include opening containers, raking debris, carefully probing areas of interest, removing surface soil, and/or digging test pits to search for indications of concealed human remains. The type and scale of a scene determines the complexity of the search response. Any potential remains that are discovered can also be assessed on site by the forensic anthropologist to determine if they are human or non-human. They can also assess if the recovered body is intact or if additional searches are needed in cases of dismemberment or animal scavenging.

Human Remains Detection; Canine Team; Forensic Anthropology

F74   The Effect of Compressed Air Foam on the Detection of Ignitable Liquid Residues on Fire Debris Samples

Victor Omondi*, Crevit Mulier & Co., Nairobi Area, Kenya

Learning Overview: This presentation will inform attendees that no significant interferences were found from compressed air foam when the samples were analyzed using activated carbon strips. The only foam component found was limonene. To date, there has been no research published as to whether the foam causes any interference on subsequent analyses of accelerant analyses, making this study unique.

Impact Statement: This presentation will impact the forensic science community by informing attendees that no interference is brought about by the introduction of foam during fire suppression and in the analysis of ignitable liquid residues from fire debris sample.

Compressed air foam is a substance that is used as an extinguisher delivery system for fire suppression in various fire scene case scenarios. This is due to its high fire extinguishing efficiency, use of less water (hence suitable for areas with no water such as rural areas), and its range of large fire suppression has made it receive considerable attention. Therefore, this technology is widely being accepted as an alternative source to water as a fire extinguishing agent that is commonly used because of its surface tension properties making it more efficient.

Although both water and compressed air foam are being used, they both have certain advantages and also inherent limitations that should be considered. Unfortunately, there is only one study that has been done to investigate whether the introduction of foam to the seat of the fire created any problems in subsequent analyses of fire debris samples using gas chromatography/flame ionization detectors. No significant interferences were found from the foam when the samples were analyzed using activated carbon strips. The only foam component found was limonene. To date, there has been no research published as to whether the foam causes any interference on subsequent analyses of accelerant analyses making this study very unique.

This study's main objective is to prove that no interference is brought about by the introduction of foam during fire suppression and in the analysis of ignitable liquid residues from fire debris sample. This was achieved through the use of gas chromatography/mass spectrometry, which is capable of carrying out extracted ion analysis and is hence able to prove that there are no significant interferences from the foam. This research was carried out in a controlled laboratory environment, International Organization for Standardization (ISO) Certified, and at an accredited facility under the supervision of well-trained staff at the Anglia Ruskin University.

Fire Investigation; Chromatography; Forensic Analysis

F75   **The Impacts of Thermocouple Insulation Failure on the Accuracy of Temperature Measurement Data in Forensic Fire-Death Scenarios**

David Silveira*, California State University, Chico, Chico, CA; Ashley Kendell, California State University, Chico, Chico, CA; Beth Shook, California State University Chico, Chico, CA

Learning Overview: After attending this presentation, attendees will understand how invalid donor temperature measurement data can be produced in fire-death scenarios when thermocouples come in contact with rendered fats and sustain direct fire exposure.

Impact Statement: This presentation will impact the forensic science community by providing examples of the severe effects on temperature measurement error and uncertainty resulting from exposure of inadequate thermocouples to contamination and conditions exceeding rated maximums. Suggestions will be provided for ways to measure temperature in future forensic fire-death scenarios.

Temperature measurements are needed in forensic fire-death scenarios to evaluate the effects of fire exposure on tissue and bone.[1,2] Unfortunately, there are a number of confounding factors in fire research. In many scenarios, thermocouples must pass through fire to reach the donor, which drastically increases the risk of insulation failure. Overheating of glass-fiber insulation causes embrittlement, fragmentation, and exposure of conductors. Contact between exposed conductors creates new thermocouple junctions and temperature measurements at unintended locations.[3,4] High temperatures lower insulation resistance, which allows shunting of electrical signals between conductors even if the insulation remains physically intact.[3-6] Increasing the length of an overheated portion of a thermocouple worsens error magnitudes.[7] Absorption of rendered fat contaminates bare woven-fabric insulation, which further reduces resistance and maximum usable temperatures. Unpredictable movement of measurement locations between the donor and fire, and substantial errors in temperature values are likely to occur.

In this study, glass fiber-insulated GG-K-24-SLE (GG) and HH-K-24 (HH) thermocouple wires, and a BLMI-XL-K-18U-120 Mineral-Insulated Metal-Sheathed (MIMS) probe (BLMI) with a length of 304cm from Omega Engineering (maximum temperature ratings of 482 C, 704°C, and 1,150°C, respectively) were subjected to two controlled burn experiments. For experiment #1, wires passed through a Skutt KM-818-3 ceramic kiln and were heated to 1,093°C. Exposure lengths were 100cm (short) and 339cm (long), with one long HH wire saturated with melted pork fat for comparison to an identical uncontaminated wire. For experiment #2, three wood pallets were burned, with wires suspended above the stack and passing through the middle pallet along with the BLMI probe. Feeding of the fire continued for two hours post-ignition. In both experiments, thermocouples passed through high temperatures to measure an ice bath at 0°C.

In the first experiment, errors due to low insulation resistance appeared on long HH wires at a kiln temperature of approximately 900°C, with increasing electrical noise from heating elements that disappeared upon kiln shutdown. Maximum errors for HH wires after kiln shutdown were 66°C (short), 547°C (long, clean), and 691°C (long, with fat). These errors disappeared by the time the kiln cooled below 800°C, indicating return of the measurement location back to the ice bath. GG wires began showing errors at a kiln temperature of approximately 800°C, with readings from the long wire spanning -270°C to 1,181°C and much higher sensitivity to electrical noise compared to the short wire. Readings from both GG wires never returned to the ice bath temperature and tracked the kiln temperature post-shutdown. Post-test, HH insulation was intact but severely embrittled, and GG insulation had completely melted, which allowed new thermocouple junctions to be created inside the kiln chamber.

In the second experiment, steep error increases occurred on all glass-fiber insulated wires by 1.5 minutes post-ignition of pallets. Errors from wires routed through the middle pallet peaked at 890°C before 6 minutes, with significant errors still present at 3 hours. Peak errors of 1,090°C (GG) and 973°C (HH) from suspended wires occurred before 6 minutes, with HH readings returning to the ice bath 9 minutes post-ignition and GG readings showing fluctuating errors of up to 258°C for 1 hour. The BLMI probe stayed within expected error margins around 0°C for the duration. Post-burn, suspended HH insulation was nearly intact. All other wires showed extensive insulation fragmentation and conductor exposure.

This study highlights the unsuitability of thermocouple wire with exposed woven-fabric insulation for temperature measurements of donors in fire-death scenarios, providing examples of error rates for three different types of thermocouple wire and suggestions for future research.

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Thermocouple; Temperature Measurement; Fire-Death Scenario

F76   ***In Situ* Detection of Gunshot Residue on Fabric Using Attenuated Total Reflectance/Fourier Transform Infrared Spectroscopy**

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Learning Overview: This presentation aims to elucidate the potential for a novel method using Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) micro-spectroscopy for *in situ* detection of Organic and Inorganic Gunshot Residue (OGSR and IGSR) particles on cotton fabric. This presentation will provide attendees with background information regarding the overall relevance of GSR as a form of trace evidence and will further elaborate on the importance of OGSR in particular. After attending this presentation, attendees will have a better understanding regarding the importance of GSR evidence. The novel method for the detection and analysis of GSR particles shall be explained in detail, and future work regarding this proof-of-concept study will be discussed. This research demonstrates the potential for the detection of both OGSR and IGSR on fabric via ATR/FTIR.

Impact Statement: This study demonstrates the capability of ATR/FTIR micro-spectroscopy for non-destructive identification of GSR *in situ* on original fabric without involving any transfer methods. This approach has a potential to greatly impact the forensic science community by increasing the efficiency of GSR detection.

ATR/FTIR allows for confirmatory identification of analytes through their high chemical specificity. The technique is non-destructive, sensitive, and requires little or no sample preparation. The technique has been shown to detect both OGSR and IGSR on adhesive tape. This is contrary to current GSR elemental analysis methods that rely solely on the detection of the heavy metals (lead, barium, and antimony). It has been reported previously that both vibrational spectroscopic techniques, including Raman spectroscopy and ATR/FTIR, allow for the detection and identification of GSR on an adhesive tape. The main objective of this study was to evaluate the possibility of detecting GSR *in situ* on a fabric excluding the need for the GSR transfer on a convenient substrate. To generate the GSR samples, two different bullet types with different calibers were used. Both "traditional" and "lead-free" ammunition were used in this study. It was found that both IGSR and OGSR particles were detected using ATR/FTIR microscopy for both types of ammunition used. In addition, GSR particles embedded in cloth and shielded partially by the cotton threads were also detected and identified. One could hypothesize that such shielded particles may not be collected using an adhesive tape and, as such, could be lost during the traditional GSR analysis.

Gun Shot Residue; FTIR; Microscopy

F77 **An Exploratory Study of 3D Fused Deposition Modeling (FDM) and Polylactic Acid (PLA+) Filament Deposition as Toolmark Signatures for Common Source Comparison and Identification**

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Learning Overview: This research focuses on the additive process known as Fused Deposition Modeling (FDM), utilizing polymer filament materials that are extruded as melted deposition layers onto a 3D printer build platform.[1]

The design of workshop-made 3D-printed firearms (also described as homemade or non-commercial) has been undergoing continuous development since the first appearance in 2013 of a 3D-printable single-shot handgun known as the "Liberator." The proliferation and development of more sophisticated designs, which are manufactured using inexpensive FDM 3D printers, has been confirmed recently by the first Australian and international reports of the prosecution of the 3D printing (i.e., manufacture) of firearm parts as an organized criminal activity.

Impact Statement: This presentation will impact the forensic science community by showing that the possibility of identifying 3D-printed firearms or firearm components (in the absence of a completed firearm) having a similar source origin may assist in developing the ability to link such firearms and components to the same or similar source would strengthen and improve legislation by providing the possibility of identification to a 3D FDM printer (for example, in possession of a suspect) that may have been used to manufacture and distribute multiple illicit firearm components.

3D printing technology using polymer and polymer/hybrid filament materials is being used to construct illicit firearm components with strength and longevity using low-cost FDM 3D printers. Filament deposition build layers and surface characteristics created by Additive Manufacturing (AM) technology produce visible patternation upon a manufactured object and have the potential for forensic comparison and analysis. A determination of same source origin may be developed to establish a threshold of similar origin and source printer identification of illicit 3D-printed components.

Gunmakers produce firearms that incorporate the use of production designs (firearm partonomy) using set manufacturing formats to produce serviceable and reliable components. It is true that many standardized designs for workshop-manufactured 3D FDM-printed firearms are available; however, manufacturers of 3D FDM-printed firearms (licit and illicit) utilize similar production templates and set formats to facilitate production using standardized processing parameters that can be analyzed to determine similarity and same-source correlations.

FDM or AM processes using polymers deposit filament layers that may impart striae upon the surface of a 3D-printed object occurring in repeatable patterns upon other surfaces of the same object. These correlating striae also exist upon consecutively manufactured objects produced by the same 3D FDM printer. Some of the observable "deposition striae" features fulfill the sufficient agreement requirements of the Association of Firearm and Tool Mark Examiners (AFTE) Theory of Identification as it Relates to Toolmarks.

There has been much discussion and conjecture about 3D print technologies on open web forums; however, very little tangible research investigating its use for the production of illicit firearms has been initiated. New polymer and polymer/hybrid materials have become viable and practical options for firearm traffickers and illicit firearm manufacturers.

The forensic examination and analysis of 3D printer deposition striae based upon the mechanical variation within 3D printers has not been fully investigated and its adaption to provide intelligence and evidential data to assist policing agencies to combat the illicit manufacture of firearm components demands further study.

Z-axis topographical signatures are visible on all PLA+ filament 3D-printed objects. Secondary deposition layer build lines occurring on the z-axis are produced by factors within the 3D printer hardware resulting from an accumulation of multiple, variable, or unknown 3D printer characteristics and have been described broadly as Vertical Fine Artifacts (VFA) or as Motor Resonance Rippling (MRR), which is a term first used online in a 2021 website.[2]

The secondary z-axis deposition lines, which are the subject of this presentation, may be related to MRR and similarly occur at right angles to the x-y build plate axis but have less uniform patternation and spacing when compared to motor resonance effects only. They can be observed on images of 3D PLA+ components, regardless of build orientation.

Z-axis artifact pattern repeatability may provide novel identification methodologies that may be suited to implementation by police forensic agencies for the investigation and prosecution of illegal 3D FDM-printed firearm components. The mechanical variations within domestic-grade 3D printers, which create secondary z-axis deposition lines, may present an opportunity for the forensic scientist to explore a "threshold of similar origin" at which the probability of two 3D-printed components having a similar origin 3D printer becomes a realistic possibility.

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3D Technology; Firearms; Toolmarks

F78   Fatal Sexual Assaults Toward Animals (Birds) and Bestiality

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Learning Overview: After attending this presentation, attendees will understand the need for collaboration between a veterinarian and a forensic pathologist in exceptional situations of bestiality regarding fatal sexual assaults of birds.

Impact Statement: This presentation will impact the forensic science community by informing attendees that when faced with special situations (fatal sexual assaults toward animals), we describe the resources needed to answer legal questions.

Introduction: Sexual assaults on humans beings are a common topic of academic research, but they are much less often described when they are committed on animals. The French Penal Code includes sexual violence in its definition of animal abuse—it is a felony. In France, less than six cases a year relate to sexual assaults on animals. They are considered to be akin to torture and acts of barbarism.

Case Report: We report on a case report of fatal sexual assault that was committed on a bird, a great helmeted curassow (*Pauxi pauxi*), in a zoo.

In a zoo, when animals die, an autopsy is performed by the zoo veterinarian in order to eliminate possible infectious causes of death as well as to make informed health decisions with regard to the other animals of the zoo. After four great helmeted curassows died, the zoo veterinarian established that they had died of intestinal perforation complicated by peritonitis. As the cause of death was not infectious in nature, the birds' bodies were cremated. When the fifth suspicious case occurred, the veterinarian kept the body in a freezer and notified the management of the zoo. Judicial services were in turn notified, and a forensic autopsy was ordered, then carried out by a veterinarian as well as a forensic pathologist. The autopsy revealed bruises and wounds on the body, the bird wings, and intestine perforation congruent with sexual assault. DNA samples retrieved from the body determined the involvement of a trainer.

Discussion: Forensic autopsies carried out on animals in order to ascertain the cause of their death and, moreover, to search for stigmata of sexual assault are very rare. It behooves us to compare our findings with the data from the existing literature on the topic. To do so, it is necessary to review criminal data related to sexual violence on animals (dogs, cats, chickens, or horses) resulting in death (definition, penalties incurred), as well as what pertains to acts of bestiality. It is necessary for professionals (forensic pathologists and veterinarians) to collaborate so as to shed light on such cases. Indeed, in this case, the veterinarian knew how to perform the autopsy on animals but did not have the forensic knowledge to collect and analyze the different samples.

Animal Abuse; Sexual Crimes; Autopsy

F79 A Review of Global Egg and Nestling Theft and Collection Practices

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Learning Overview: By attending this presentation, attendees will gain an understanding of the scope of egg and nestling theft and collection, unique dangers posed by that, patterns occurring within it, and current techniques and approaches used to combat it.

Impact Statement: This presentation will impact the forensic science community by generating awareness on a currently under-researched issue in this field and by highlighting a disparity of information on egg and nestling theft compared to general theft of birds.

Theft of any bird can pose a threat to viable populations, but it is doubly dangerous when eggs and nestlings are stolen. When this occurs, birds are less likely to breed or bring hatchlings to adulthood.[1,2] This contributes to declining bird populations, which leads to ecosystem distress.[3] In this study, peer-reviewed and gray literature were examined to determine trends of practices of global egg and nestling theft and areas of concern. A 20-question survey was designed and distributed through Qualtrics (including multiple choice, ranking, and open response questions) and submitted to 28 international conservation/research organizations who then disseminated the survey among their members, at least 1,200 recipients.

There were 113 respondents to the survey, 68% of which were aware of the totality of the issue with 14% expressing no awareness; the remaining were split between knowing only one aspect of the topic. The organization affiliation of respondents greatly varied, but the majority worked in/for a university. Nearly half (46%) of respondents have worked 0 to 5 years in their listed capacity. Additionally, 72% of respondents with little to no awareness of egg and nestling theft and collection had worked 0 to 5 years in their listed capacity, indicating an encouraging trend of increasing awareness with years in the field. A notable difference was environmental scientists who demonstrated a large lack of awareness; however, 92% worked at the same company, so global extrapolations cannot be made. Furthermore, survey results indicated that the International Union for Conservation of Nature (IUCN) Red Listings were not accurate to local threat levels with 26% of respondents indicating that their species of interest did not match. When asked about outreach, 67% of respondents chose "other" and filled it in with various education strategies, which aligned with the results of the desk study, indicating that education outreach is widely utilized and successful.

Collated results showed egg and nestling theft and collection are under-researched in conservation circles and revealed patterns indicating an overall lack of total awareness on the topic. Only a small portion of respondents indicated being keenly aware of the issue. However, even in this case, it cannot necessarily be inferred that the eggs and nestlings are receiving direct attention. When asked about respondent conservation focus, the clear scope was on adult birds with juveniles also ranking about eggs and nestlings. Additionally, IUCN Red Listings were not overall representative of local threat levels. Survey results and the desk review indicated that education outreach is a widely utilized conservation technique that could affect the practice of theft and collection.

In conclusion, global information was compiled on the topic of egg and nestling theft practices in order to disseminate the information and increase overall awareness of its occurrences. This increasing knowledge could be applied to further the conservation purposes of respondents and attendees and promote further interest in research, outreach, and collaboration on this topic.

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Wildlife Forensics; Egg and Nestling; Global

F80   **A Retrospective Study of Displacement and Recovery Patterns for Water Fatalities in British Columbia, Canada**

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Learning Overview: After attending this presentation, attendees will be aware of how often recovery of submerged decedents is successful and how far human bodies are displaced prior to recovery in multiple outdoor aquatic environments in British Columbia, Canada.

Impact Statement: This work impacts the forensic science community by expanding knowledge surrounding body movement in multiple waterbodies, including lakes, rivers, and the coastal ocean, to allow for better prediction of body movement and increasing early recovery success from all waterbodies.

Human body recovery from all waterbodies is a difficult endeavor that can involve multiple search and recovery agencies. Early recovery of decedents from the water requires an understanding of how a body will act when it enters the water. This knowledge is often based on anecdotal or case evidence, along with some large-scale studies. However, these large-scale studies are often focused on decomposition patterns, rather than assisting with the prediction of body movement in the water. There is currently no baseline understanding of how bodies move in outdoor waterbodies in British Columbia, Canada.

This presentation aims to minimize these gaps in knowledge by retrospectively examining the displacement and recovery patterns for all manner of outdoor water-related deaths in British Columbia between 2010 and 2021. One hundred eighty-six water-related fatalities in British Columbia waters, including lakes, rivers, and the coastal ocean, were extracted from the Police Records Information Management Environment (PRIME-BC) for analysis. All manners of death were included in this study, although the majority of cases were unintentional in nature, often caused by drowning. Most incidents occurred in rivers, followed by lakes, and finally the ocean. Sixteen percent of individuals in this study were not recovered. Regardless of the waterbody, the first day was the most successful recovery period. Individuals submerged in lakes and rivers were most often recovered, while submersion in the ocean resulted in the least recovery success. Furthermore, individuals submerged in the ocean were less often recovered after the first day. Delayed recovery success was seen only in lakes and rivers, suggesting that individuals submerged in the ocean may be more likely to remain buoyant to be rapidly moved by currents. Displacement prior to recovery in all waterbodies was minimal overall. Lakes resulted in the least displacement, while rivers resulted in the greatest and most variable displacement. Although displacement prior to recovery in the ocean was minimal, it is likely that unrecovered decedents could have been displaced out of the search area. Decedents in the ocean may have been more likely to float just below the surface to be moved by currents prior to descending, after which it is unlikely they would resurface.

The results of this research suggest that knowledge surrounding human body movement in the water is incomplete and that early recovery is inherently difficult, regardless of the waterbody. Further empirical research based on known data is necessary to continue improving prediction of body movement and increase early recovery success in all waterbodies.

Underwater Recovery; Human Body Displacement; Water-Related Death

F81   The Importance of Proper Underwater Forensics Investigation

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WITHDRAWN

F82   Intimate Partner Violence-Related Strangulation (IPVRS)

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Learning Overview: After attending this presentation, attendees will gain competency in understanding the dynamics of IPVRS incidents and the signs and symptoms of non-fatal strangulation as reported by victims to police and medical first responders. Attendees will also gain a better understanding of the practical implications of strangulation evidence in investigations.

Impact Statement: This presentation will impact the forensic science community by presenting information that will add to the body of knowledge about strangulation in Intimate Partner Violence (IPV) incidents and the implications for evidence and investigation.

Strangulation occurs when the neck and/or upper torso of an individual is compressed in a manner that impedes airflow or blood circulation.[1,2] Although precise IPVRS counts are unknown, estimates have suggested as many as 68% of IPV victims have experienced strangulation.[3-6] The prevalence and rate of injury from IPVRS is also largely unknown because victimization is routinely underreported and only approximately 29% of victims receive medical intervention following strangulation.[7-9]

Victims who report strangulation frequently present with what has appeared to be minor or non-visible, external injuries that may go unrecognized by first responders who do not have specialized knowledge regarding the unsuspecting signs and deleterious consequences of strangulation. Indeed, strangulation victims often suffer from considerably more serious internal injuries that have long-lasting health outcomes, including increased mortality.[7,8,10] Providing law enforcement with the knowledge and skills to detect the range of strangulation signs and symptoms continues to be challenging, given the lack of visible strangulation evidence when victims present to police. Existing research is sparse, though findings have suggested limited documentation of strangulation during the police investigation. In particular, as few as 12% of IPVRS incidents contain explicit references to strangulation in the police report.[2,11,12] These factors compound the challenges that first responders face in identifying IPV incidents involving strangulation. Early detection of strangulation and appropriate medical intervention can provide critical information for first responders to prioritize service decisions, improve victim medical outcomes, and enhance IPVRS evidence collection.[2,10,13]

Tarleton State University's Institute on Violence Against Women and Human Trafficking (VAWHT) Intimate Partner Violence-Related Strangulation Study involves a program evaluation of a Strangulation Ordinance passed in Burleson, TX. The study, "Assessing an Innovative Response to Intimate Partner Violence," is funded by the National Institute of Justice, Office of Justice Programs, United States Department of Justice (award 2018-VA-CA-0005).

This presentation presents content from the process component of a larger program evaluation and examines the prevalence of strangulation in a population of IPV incidents ($N = 583$) reported to the Burleson Police Department (BPD) following implementation of a Strangulation Ordinance (March 6, 2018, to December 31, 2020). Strangulation cases were included in the sample if they were IPV-related and were eligible to receive the new Strangulation Protocol as part of the Ordinance. Data collected from BPD and Burleson Fire Department (BFD) were used along with BPD's Victim Assistance and de-identified client data obtained from the One Safe Place (OSP) for the period of interest. OSP, a Family Justice Center, is a multi-agency network consisting of approximately 22 partner agencies providing coordinated services to IPV victims in Tarrant County, and their involvement in the study provides an independent data source on Ordinance fidelity.[14] Additionally, victim interactions with first responders were explored through qualitative information obtained from a 2020 Victim Survey administered by researchers to Burleson IPV victims.

The sample of BPD IPV strangulation cases ($n = 155$) were used to explore Ordinance fidelity. In addition, a subsample of BFD Strangulation Worksheets ($n = 93$) was used to examine signs and symptoms of strangulation as well as adherence to the Strangulation protocol. Multivariate logistic regression, chi-square, *t*-tests, and qualitative analytic techniques were used to analyze post-Ordinance Burleson IPV incidents. PE results will be presented, and, finally, practical implications for investigation and forensic evidence will be discussed. Findings will also address the nature and extent of physical strangulation signs and symptoms (e. g. bruising, petechiae) and other factors (e.g., number of officers on scene, injuries, witnesses) that provide important forensic science and investigative implications for cases involving strangulation.

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Strangulation; Intimate Partner; Evidence

F83 Sudden Infant Death Syndrome and the Risk Factors of an Unsecure Sleep Environment

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Learning Overview: The majority of cases identified as Sudden Infant Death Syndrome (SIDS), accidental suffocation, and cause unknown had comparable dangerous sleeping patterns, as evidenced by our use of thorough death-scene reports. The risk of SIDS is increased by prone sleeping and insecure sleep surfaces. When an infant's head or face is covered by covering, or when a child shares a bed, the chance of death has increased, according to recent epidemiological studies.

Impact Statement: The purpose of this study is to characterize the frequency of a number of critical risk factors associated with sleeping habits in a selected group of infants who died suddenly and unexpectedly. There may be diagnostic overlap; thus, it's important to think about these conditions together for the sake of public health campaigns.

Background: The risk of SIDS is enhanced when the infant is allowed to sleep in a prone position and when the sleep surface is not secure. According to current epidemiological studies, the risk of death is enhanced when an infant's head or face is covered by a covering or when a child shares a bed with another child. Other risk factors include sharing a bed with another child.

Objective: The goal of this research is to determine the frequency of a number of important risk variables that are connected with sleeping patterns in a particular group of infants who passed away unexpectedly and suddenly.

Results: For the purpose of drawing conclusions about the general population, our study team looked at information from the death records and autopsies that were kept at the Casablanca Medic-legal Institute from January to December 2022. Due to the likelihood of diagnostic overlap, all deaths of infants younger than 2 years old with the diagnoses of SIDS, unintentional suffocation, or reason not determined were included in the study. They had been alive for a total of 109.3 days on average, with a range of 6–350 days. There was a total of seven deaths, five of which were ascribed to the condition known as SIDS, three to unintentional suffocation, and two to causes that could not be determined. It was revealed that some infants were sleeping in prone positions on surfaces that were not intended for use by infants. In other situations, death occurred on a bed that was shared by more than one person.

Conclusion: As indicated by the fact that we used detailed death-scene records, the majority of cases that were diagnosed as sudden infant death syndrome, accidental suffocation, and cause unknown had comparable risky sleeping patterns. Due to the possibility of diagnostic overlap, it is necessary to consider these illnesses simultaneously in order to ensure the success of public health initiatives.

Asphyxiation; Cause of Death; Unexpected Death

F84   Examining Youth Gangs From a Trauma Exposure Perspective

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Learning Overview: At the conclusion of this presentation, attendees will better understand the various trauma exposures experienced by youth gang members. In addition, attendees will be able to identify 12 distinct barriers to treatment and service utilization by youth gang members who experience multiple trauma exposures across their lifespan (i.e., immigration issues, racism, depression, anxiety, generational trauma, fear of death, shame, isolation, age, lack of health insurance, lack of child care, lack of affordable housing, lack of independent income, lack of support from the family/community, cultural fluency, and just not knowing the available resources surrounding trauma therapy).

Impact Statement: This presentation will have a profound impact on the forensic science community as it will present novel and timely information not normally seen when discussing youth gangs. In the past, the forensic science and law enforcement communities have paid attention to the "signs and symptoms" of youth gangs such as tattoos, graffiti, and non-verbal forms of communication. Now, by examining youth gangs through a trauma exposure lens, it elevates the explanations as to why one would join a youth gang, and not only join, but what keeps them staying active.

Exposure to traumatic events among youth is relatively common as more than one-third of United States children report being victims of emotional and physical violence, while many more experience natural disasters, witness violence, have a severe accident or injury, or experience a sudden traumatic loss. Almost all youth experience initial distress as a reaction to such events, but for most, their natural resilience causes the distress to gradually subside. However, for some, they continue to experience distress for months and often years after the trauma exposure, increasing the already challenging transition from adolescence to adulthood. Trauma exposure can lead to a variety of problems, including alterations in mood and behavior and loss of social and academic functioning. Youth gangs are the most vulnerable population as they experience repeated traumatic exposures, often daily, while being exposed to the youth gang itself.

There are over 28,500 gangs in the United States with a total gang membership of 850,000 according to the Office of Juvenile Justice and Delinquency Prevention of the United States Department of Justice. Often overlooked in youth gangs is the pervasive and repeated trauma exposure of sexual violence among female and male gang members before the age of 16 years. In Los Angeles County, CA, alone, there are currently 1,351 documented gangs with gang membership of over 40,000, while in Philadelphia County, PA, there are 340 documented gangs with a gang membership of over 5,000. Demographics show across both counties, a male/female gang member average age of 15 years with a range of 8–22 years.

The author interviewed over 600 gang members in the streets, jails, and juvenile halls using a target trauma exposure questionnaire in Los Angeles and Philadelphia. The author found that over 95% of all female gangs have experienced sexual violence at least once before they turned 16 years old, while 40% of male gang members have experienced sexual violence before 16 years old. Having this disclosure within the youth gang population is rare. What is unique to the youth gang population is the repeated traumatic exposure, often on a daily continuous basis. Consequently, these youth gang members are in constant state of "survival mode" or "chronic stress," which poses a threat to those that respond to them, therefore making this an "officer safety" issue. When one is in "survival mode," these youth gang members will respond by either "flight," "fight," "freeze," or "fawn." Furthermore, with the technical assistance of the Anti-Violence Partnership of Philadelphia (AVP), an anti-violence and trauma therapy non-profit organization, this study identified 12 distinct barriers to treatment and service utilization by youth gang members who experience multiple trauma exposures across the lifespan (i.e. immigration issues, racism, depression, anxiety, generational trauma, fear of death, shame, isolation, age, lack of health insurance, lack of child care, lack of affordable housing, lack of independent income, lack of support from the family/community, cultural fluency, and just not knowing the available resources surrounding trauma therapy).

Youth Gangs; Traumatic Life Events; Violence Against Children

F85   **An Assessment of the Reliability of Subjective Pattern Evidence Disciplines Based on Evidence From Wrongful Convictions**

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Learning Overview: After attending this presentation, attendees will be aware of the experience of wrongful convictions related to specific pattern evidence disciplines, including the impact of bias, subjectivity, standards, and professional standards. The presenter has completed and published in the *Journal of Forensic Sciences* comprehensive research on wrongful convictions related to forensic evidence.[1,2] This session will provide new research and details on the specific issues in four pattern evidence disciplines, including latent prints, firearms, footwear, and hair comparison. Attendees will learn the full breadth of issues encountered in wrongful convictions related to these disciplines, case studies, and root cause analysis. The information will include discussion of scientific research and legal admissibility issues as they relate to lessons from wrongful convictions.

Impact Statement: This presentation will impact the forensic science community by providing unique information about pattern evidence in light of the experience of wrongful convictions. Forensic science improvement should be closely tied to real-world experience and root cause analysis. This presentation provides a grounded, research-based approach to improvement that can be impactful in the pattern evidence disciplines. It closely ties to the conference theme, Justice for All, which can be supported by the adoption of appropriate, evidence-based reforms that improve the reliability and effectiveness of pattern evidence and its use by the criminal justice system.

Wrongful convictions provide useful insight into the sources of error in the criminal justice system, including false and misleading pattern evidence. A comprehensive analysis is presented of wrongful convictions associated with latent print comparison, firearms identification, footwear impression evidence, and hair comparison. Key issues include uncertified examiners, failure to conform to practice standards, underutilization, investigative tunnel vision, and poor understanding of forensic evidence by officers of the court. In some cases, advocates introduced unreliable methods prior to validation. In other cases, examiners opine on issues that are outside their expertise or the scope of their forensic discipline. Identification errors are rare among certified examiners who conform to the standards of their forensic discipline.

Pattern evidence disciplines rely on subjective conclusions from forensic experts. Some critics have maintained that this inherent subjectivity undermines the reliability of pattern evidence and contributes to wrongful convictions. In particular, it has been claimed that contextual bias effects produce unacceptable variability among latent print examiners. Some court decisions have limited the use of pattern evidence on the basis of these concerns. The evidence of wrongful convictions demonstrates that other bias effects may play a bigger role in system errors than contextual bias and should be the subject of research and greater scrutiny. Technical and verification reviews may be biased by deference to colleagues. Investigative tunnel vision may inhibit consideration of potentially probative evidence or exculpatory evidence that points to alternate suspects. Many wrongful convictions could have been avoided if exculpatory pattern evidence was properly considered at the time of trial. Contextual effects do exist and are most commonly associated with types of evidence with low discriminatory power or examiners with inadequate training or in dysfunctional organizations.

These findings support the claim that pattern evidence disciplines are highly reliable but can be undermined in the absence of appropriate governance mechanisms. The reliability of pattern evidence examiners—like other forensic scientists—to work within an organizational framework that supports quality assurance, professional development, and effective management is critical.

In addition to presentation of the incidence of factors associated with wrongful convictions, case studies will be used to elucidate specific issues and trends. Discussion will include wrongful convictions in the United States and internationally and "near miss" cases. All issues will be examined in the context of scientific research related to pattern evidence examination and legal decisions related to the admissibility and scope of pattern evidence.

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Wrongful Conviction; Latent Prints; Firearms

F86   Wrongful Convictions and Jurisprudence of DNA Forensics: An Indian Scenario

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Learning Overview: This presentation deals with various aspects of evidence, jurisprudence of innocence, and reasons for wrongful convictions in the United States. It further explores the possibilities of addressing innocence claims against wrongful convictions in India.

Impact Statement: Impressed with the probity of DNA for human identification, Professors Barry Scheck and Peter Neufeld initiated the "Innocence Project" at the Cardozo Law School, New York, NY.[1] So far, over 3,000 innocent convicts have been exonerated in the United States. This presentation will impact the forensic science community by dealing with various aspects of evidence, jurisprudence of innocence, and reasons for wrongful convictions in the United States. It further explores possibilities of addressing innocence claims against wrongful convictions in India.

Credibility of forensic science has been long established. Scientific evidence is non-partisan, objective, and fair, which is critical for the administration of justice. Despite being corroborative in nature, expert opinion braces the chain of events to prove a fact beyond reasonable doubt. Moreover, several forensic evidence are long-lasting and, in case of absence of an ocular witness, may solely empower the criminal justice apparatus to find out the truth.[2] False allegations and wrongful convictions are evident global challenges for the administration of justice.

For ages, injustices to innocent convicts were reported, but could not receive due attention for various reasons. However, the advent of DNA fingerprinting in the 1980s reversed the ball game, and the very first case of DNA helped to establish the innocence of Richard Buckland in 1986 and also proved that Peter Pitchfork was the actual rapist and killer for both deceased rape victims in the United Kingdom.

This presentation highlights the need for an Innocence Project in India to reform the criminal justice system. First, it examines the need for and working on the Innocence Project in two regions, namely the United States and the United Kingdom. It gives instances of several wrongful convictions and enumerates the reasons why these may occur. Second, it explains the importance of a similar project in India by shedding light on the country's unjust convictions and drawbacks of the criminal justice system. Third, it analyzes how an Innocence Project can be introduced in India. This presentation also mentions the challenges faced by Innocence Projects across the world.

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Forensic DNA; Wrongful Conviction; Criminal Justice System

F87   A Quiet Place: A Case of a Double Homicide in a Corn Field

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Learning Overview: After attending this presentation, attendees will be aware of two fatalities consequent to a run-over incident during pesticide application in a corn field.

Impact Statement: This presentation will impact the forensic science community by explaining the importance of an integrative and multidisciplinary approach in an unusual case of death following an unnoticed accident.

Farm tractor accidents represent a large part of work-related fatalities.[1] The victims are often drivers and/or coworkers, and harming uninvolved subjects is very unusual.[2,3]

On the morning of July 2, 2021, the emergency service received a call from an unknown woman who reported that she was in a corn field with a friend of hers when both of them were run over by an agricultural vehicle, which injured her and killed her friend. The language barrier slowed down the data collection; additionally, the woman was not able to accurately describe their position. Afterward, the mobile phone battery ran out and the call dropped. The search for the two injured women began immediately, based on the little information provided and on the geolocation of the mobile phone. On the evening of July 3, the helicopter finally found the two corpses in a corn field. They were subsequently identified as two homeless Moroccan girls who lived in Milan and who, in the very first hours of July 2, had been seen alive, together with two men.

The corn field was treated with an insecticide application on the morning of July 2, using a special agricultural vehicle with high, narrow wheels and a cabin at a considerable height, designed in order to not damage the corn plants. The tractor driver was questioned about the fact, and he did not remember anything unusual during his work.

For both victims, the cause of death was identified in a contusive/compressive polytraumatic injury, fully compatible with a rolling by a heavy vehicle. The first victim showed severe head and neck injuries, the other presented pelvis and lower limb fractures, with a massive vascular injury that allowed her to survive for probably one-two hours and gave her time to call for help.

The toxicological investigations showed that both girls drank alcohol and ingested drugs (cannabis and cocaine) before death, in non-lethal concentrations. The two insecticides were tested in the blood samples (indoxacarb and chlorantraniliprole), in order to clarify their role in the determination of the two deaths, with totally negative results.[4] In the literature, only one death related to ingestion of indoxacarb is reported, but none to inhalation.

The two young women turned out to have bivouacked with two men. They drank alcoholic beverages, took drugs (cannabis and cocaine), and fell asleep until the unexpected transit of the agricultural vehicle. The driver was acquitted on the double homicide accusation because he could not have noticed the women among the plants, while the two men were sentenced for failure to assist a person in danger.

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Homicide; Pesticide; Autopsy

F88   **Forensic Sexual and Gender-Based Violence Prevention and Response: A Community-Based Approach Pilot**

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Learning Overview: After attending this presentation, attendees will be aware that Gender-Based Violence (GBV) is a very sensitive topic and needs a multidisciplinary approach that fits into different socioeconomic, cultural, and health-seeking barriers considering diversified ideas and solutions in different countries. Despite there being various approaches, this innovative way of customizing and integrating with the Ethiopian Health Extension program will be explored and discussed.

Impact Statement: This presentation will impact the forensic science community by introducing attendees to an innovative approach in forensic science that could lead to societal change and solve forensic challenges and gaps in sexual and GBV response and prevention in a context relevant to a country's health program.

Background: Sexual and GBV is a violation of human rights resulting in immediate and long-term consequences on physical, psychological, and social aspects of survivors, their loved ones, and society.[1,2] An Ethiopian Demographic Health Survey showed 34% of married women aged 15–49 years have experienced physical, sexual , emotional, or spousal violence.[3] The World Health Organization estimates that 38% of women who commit homicide die by their intimate partner. Death may also be due to injuries, a sequel of sexually transmitted disease, and pregnancy-related complications.[4] The Revised Family Law in 2000 and the Revised Criminal Code in 2005 ensures legal measures and actions to address violence against women and children. The Ethiopian Ministry of Health launched a multi sectoral approach of GBV response and prevention.[5] There are also multiple state and non-state actors engaging in the response and prevention of sexual and GBV. However, there are observed gaps such as lack of specialized forensic knowledge among health professionals, a limited number of forensic practitioners in the country, an absence of dedicated forensic science laboratories or infrastructures, a lack of community engagement, and a lack of commitment in governance and leadership. Forensic services are not only interlinked at procedural but also at policy levels, thus necessitating efficient interactions for practical solutions in addressing challenges in Sexual and GBV.[2,6] There are numerous innovative approaches and research in prevention and response of SGBV in low resource settings, particularly in Africa.[1,7] The Ministry of Health of Ethiopia in 2021 launched Health Extension Program Optimization (HEPO) with the goal of accelerating the realization of universal health coverage.[8,9] GBV is one of the HEPO health packages included in the family health packages.[8] Thus, this research will use this opportunity and leverage to successfully implement medicolegal services in Sexual and GBV using this community-based platform.

Goal and Objective: The main goal of this research is to integrate the forensic services of Sexual and GBV in the HEPO packages. The objectives include: (1) promoting medicolegal services; (2) creating competent primary health care professionals with knowledge, attitude, and skills in medicolegal services; (3) increasing demand, access, and utilization of forensic services; (4) creating uniform practice in forensic services; (5) evaluating medicolegal services before and after the research at intervention sites and comparing it with other control sites; and (6) recommending the experience will be gainful to concerned stakeholders.

Methods: This will be a hypothetical study that examines how medicolegal service utilization can be increased through training, mentoring, and referral linkages with primary health care unit professionals. The study will be a prospective randomized cross-sectional study with an intervention and control arm. We will utilize tested and solid grounded research methodology implemented in low resource settings.[1] This will be implemented through: (1) training health and police personnel; (2) recruiting the focal person; (3) mentoring and follow up; and (4) advocacy of GBV with particular focus on the forensic aspect. This will be implemented in the primary health care unit within catchment areas of St. Paul's Hospital Millennium Medical College as an intervention arm and Gandhi Memorial Hospital as a control arm. The studies will have both qualitative and quantitative components. Data will be collected by focused group discussion, in-depth patient and provider interviews, and observation of the service provided.

Conclusion: This study will be the first in its kind in which forensic services will be integrated in sexual and GBV prevention and response at a community level.

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Sexual; Domestic Violence; Forensic

F89   Forensic Medicine Practices in Family Medicine in Turkey

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Learning Overview: After attending this presentation, attendees will know how large the field of forensic medicine practices in family medicine is and the necessity of interdisciplinary work.

Impact Statement: This presentation will impact the forensic science community by informing attendees how special training can be organized by the forensic science community for family medicine and primary health care services. The number of forensic science courses in the residency training curriculum can be increased.

Family physicians and their primary care teams have various role definitions based on local resources, geopolitical factors, and the regional definition of the discipline.[1] Although there are many publications on forensic cases and family medicine in the literature, we have seen that forensic medicine practices in family medicine are not sufficiently mentioned. In this study, we aimed to emphasize the forensic medicine practices in family medicine in Turkey.

Method and Material: Interviews were held with family physicians in various provinces and questionnaires were asked about the forensic cases they encountered. The physicians participating in the study were asked whether they encountered a forensic case in the past 12 months, the age, gender, and forensic characteristics of the case, whether a forensic report was written, the authority that sent the case, and whether treatment was applied to the case.

Results: According to our research, forensic case reporting, forensic report keeping, death examination, burial license, forensic sample taking, and age determination are the leading forensic medicine practices faced by family physicians in Turkey.

Discussion and Conclusion: According to the practice of the legal system in our country, criminal judicial authorities (prosecutor or judge) decide whether a case is criminal or not. Physicians must report suspected or doubtful criminal cases. This is an obligation of physicians.[2] In our country, the first step in the health system and for family physician practices is to have a chance to observe forensic cases and related conditions at the outset. This initial assessment is crucial as it allows the evidence to be evaluated even after it has been lost over time. When family medicine is compared with other branches of medicine, we see that forensic medicine practices are more common.[3] Forensic medicine education should be given more prominence in family medicine, where forensic medicine practices are so common.

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Death Investigation; Forensic; Families

F90   An Unusual Plastic Bag Suffocation Case: Insights From a Comprehensive Forensic Investigation

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Learning Overview: The goal of this presentation is to provide valuable insights into a unique case of suicide by plastic bag, underscoring the importance of meticulous forensic investigations and collaboration among different disciplines in understanding complex fatalities.

Impact Statement: By sharing this case, we aim to impact the forensic community and enhance our collective understanding of uncommon asphyxial deaths

Plastic Bag Suffocation (PBS) is an intriguing form of asphyxia resulting from respiratory blockage caused by a plastic bag. Understanding the nuances of such cases is crucial for forensic practitioners. In this presentation, we will highlight a singular and unusual PBS case from our forensic practice, emphasizing the significance of a meticulous crime scene investigation and a multidisciplinary approach in comprehending peculiarities found on the victim's body.[1,2]

The typical profile of a PBS victim is an adult male, with an average age of 51.3 years, often associated with a history of depression, chronic, or terminal illness based on existing literature.[3]

In this particular case, a 62-year-old man was discovered deceased in his apartment, with no signs of forced entry. The body was found supine on a mattress, with two blue-colored plastic bags secured around the neck by a white-colored rope, and a knot positioned posteriorly, near the left temporo-occipital region. Interestingly, the victim was also wearing swimming pool goggles and two FFP2 masks, with a handkerchief soaked in a strong-smelling substance placed between the masks.

Upon external examination, hypostasis, rigor mortis, and a temperature of 31°C were noted. Several parallel excoriated lesions were observed on the volar surface of the left forearm, along with subungual cyanosis and eye petechiae.

A thorough crime scene investigation revealed a suicide note and documents related to a life insurance policy in the living room. Additionally, a 100ml bottle of diethyl ether and a syringe were found on a nearby table, suggesting premeditation of the act. The autopsy, conducted two days after death, ruled out traumatic injuries and fatal diseases. The cause of death was attributed to cardiovascular arrest secondary to mechanical asphyxia from plastic bag suffocation.

While the autopsy did not reveal any significant findings, the presence of diethyl ether in the victim's apartment shed light on the reasons for wearing swimming pool goggles and double masks. Diethyl ether is known for its anesthetizing and irritating properties, which could explain the victim's protective measures.

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Asphyxiation; Scene Investigation; Medicolegal Death Investigation

F91 Filling the Gaps of Medical Records: The Hidden Truth Behind an ABO-Incompatible Transfusion

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Learning Overview: This presentation highlights the invaluable role of a systematic and in-depth forensic investigation made of merging medical record reports and appropriate medical suspicions to unveiling apparently unresolved clinical cases.

Impact Statement: This presentation will impact the forensic science community by providing useful insights on future forensic investigations and emphasizing the need for standardized investigation protocols from medical record analysis to forensic autopsies.

Any adverse event occurring after a blood transfusion, especially between 15 minutes and 6 hours, but including presentations up to 24 hours, should be considered as a transfusion reaction, until proven otherwise. The diagnosis is often challenging due to a wide range of overlapping symptoms and signs (i.e., fever, chills, muscle contractions, infusion site pain, bleeding, chest, abdominal, and back pain, nausea, vomiting, dyspnea, shock, hemoglobinuria, acute renal failure, disseminated intravascular coagulation) and may present with different degrees of severity ranging from mild to life-threatening events.[1] Among severe reactions, hemolysis resulting from ABO-incompatible Red Blood Cell (RBC) transfusions is the third most common and invariably leads to death.[2,3] Different from other presentations, all ABO-incompatible transfusions occur as a result of a medical error.[4]

Case Report. An 83-year-old woman with blood type O was admitted to the hospital for an elective surgical procedure. During the hospital stay, she received two red blood cell transfusions (i.e., T1 and T2), the former (T1) immediately after the surgical procedure, and the latter (T2) five days later, due to low hemoglobin levels. The second transfusion (T2) was performed at 5:00 p.m., with the medical record documenting the label of an O-positive blood bag, the period at which the transfusion started, but not the ending time. As a result, there is a four-hour descriptive gap during which the patient's vital signs were not recorded. At 9:00 p.m., the medical record shows the patient was described as having tachycardia and dyspnea. On this basis, the anesthesiologist was called, and supportive therapies were provided, along with the request for an urgent complete blood count, and two additional O-positive blood bags from the nearest blood transfusion service. Unfortunately, the patient passed away at 12:40 a.m. the following day.

Through the study of the medical record, a suspicious gap was unveiled in which the patient's conditions (i.e., symptoms, life parameters, etc.) were not described. Interestingly, a thorough analysis of the laboratory parameters raised suspicion of a hemolytic reaction affecting red blood cells, hemoglobin, hematocrit, platelets, and white blood cells.

Given the circumstances, a complete autopsy was performed, but macroscopic examination did not yield a cause of death. Conversely, histological analysis revealed kidney injury by means of acute tubular necrosis. Moreover, the immuno-histochemical investigation found an apparently inexplicable positivity for anti-A antibodies in different organs (i.e., heart, lungs, spleen, and kidneys).

Conclusion: Through in-depth scrutiny of the medical documentation, a suspicious gap emerged in whom the patient's health status was not described. Based on the medical suspicion, along with the careful examination of laboratory parameters compatible with a hemolytic reaction, pathological investigations by means of histological and immuno-histochemical analyses led to the irrefutable discovery that the patient had undergone an erroneous transfusion of a group A red blood cell bag, triggering a pathophysiological cascade of events that ultimately led to the death.

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Autopsy; Document Analysis; Medicolegal Death Investigation

F92   **Unboxing Grandma—PMI Estimation in an Advanced State of Decomposition: A Case Report**

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Learning Overview: After attending this presentation, attendees will comprehend the applicability to a forensic case of new molecular methods in order to establish the time since death in the case of an advanced state of decomposition.

Impact Statement: This presentation will impact the forensic community by presenting the application of new molecular methods in order to confirm the time since death in unusual forensic cases. Particularly, these innovative methods could be used when the corpses are in an advanced decomposition stage.

Estimation of Postmortem Interval (PMI) is a significant focus in forensic medicine, and accurate prediction of PMI is crucial for case detection. Traditional methods for estimating PMI are generally based on physiological changes such as algor mortis, livor mortis, rigor mortis, and supravital reaction, but these methods only provide a rough estimation of PMI and are more effective in early PMI.[1] Nucleic acids such as DNA and RNA, protected in the cell nucleus, were considered to be less damaged by external factors, which may aid a more precise determination of PMI. It was confirmed that RNA in tissues degraded with a delay in PMI, and it was found that microRNAs (miRNAs) and circular RNAs (circRNAs) were more stable than normal reference genes. It was described in the literature as a biomarker selection showing a correlation with PMI (Gapdh, Rps18, U6, and β-actin; these four biomarkers have a higher concentration in liver tissues, heart tissues, and skeletal muscle) as reference genes were identified LC-Ogdh, circ-AFF1, and miR-122 in liver tissues; miR-122, miR-133a, and 18S in heart tissues; miR-133a, circ-AFF1 in skeletal muscle tissues.[2] This study aims to apply a molecular tool in a particular forensic case in order to confirm the time since death in accordance with the circumstantial data collected during the investigation.

In December 2021, an 83-year-old woman was found dead inside multiple bags next to a freezer. The woman used to live with her son, who was a drug addict. The neighbors called the police after being suspicious of not seeing her for a few weeks and alarmed by the presence of bad smells on the landing. When the police arrived, the son tried to escape. During the inspection of the house, in the bedroom on the bed, an envelope was found made up of several plastic bags and sheets tied with the elderly woman inside. A freezer was also found next to the bed, which was empty. During the investigation, the son confessed that her mother died 16 days before and that he tried to keep her in the freezer to continue receiving her pension. At the external examination, the body appeared in an advanced state of putrefaction and showed no signs of external injury. A full autopsy was performed that confirmed death from natural causes. Genetic studies are currently underway for the estimation of PMI through the study of biomarkers within miRNAs on liver tissue in order to confirm the time since death.

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Genetics; Medicolegal Death Investigation; Autopsy

F93   Incised Wounds—Homicide or Suicide? A Case Study

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Learning Overview: The goal of this study is to focus on the importance of site inspection and a multidisciplinary forensic approach in cases of violent death. This presentation describes a case of a 42-year-old man found dead in a garage with several incised wounds and many bloodstains on the floor, on the wall, and on the objects in the room. The particular mode of discovery of the corpse raised the question of whether it was a homicide or suicide.

Impact Statement: This presentation will impact the forensic community by relaying the importance of the site inspection and the usefulness of a multidisciplinary forensic approach by Computed Tomography (CT) scan, autopsy, toxicological investigation, histological/immunohistochemical investigation, and collaboration with the police force, especially in all cases in which signs of violence are found on the body; otherwise, other modalities of death are suspected.

Distinguishing between a suicide and a homicide is a challenging task in forensic medicine. The neck, chest, and wrists are the most usual site for suicide wounds by knife or sharp cutting weapon and, often, in accessible parts of the body, there are other self-inflicted wounds, which are deeper at their start and become shallow during their course, with tailing at the end. Typically, homicide wounds are deeper and spread, while suicide wounds show hesitation cuts, which are more superficial and conterminous with the fatal wound. In the presented case, the complexity of the lesions and the crime scene needed a multidisciplinary approach to determine the suicidal nature of the death.[1-5]

A 42-year-old man was found dead in his own garage after one day of research. The prosecutor's office was immediately alerted, and a forensic pathologist was involved in the crime scene investigation. A cutter stained with blood was found next to the cadaver. The corpse was lying prone on the floor, with a naked torso; a jacket and a polo shirt stained with blood were next to it, and bloody stains were found all around the cadaver. Bloodstains were found on the floor, on many objects and on the wall, most of them splashed from different distances, some of them were finger-shaped or shoe-shaped, generating the suspicion that there had been a struggle. Inspectors didn't find any sign of the presence of other people in the crime scene before their arrival.

A complete postmortem examination was performed on site and for few days after death. At the external examination, 33 incised wounds with regular margins were found on the corpse, 8 on the neck (both sides), 5 on the left side of the chest, 18 on the abdomen, one on the volar face of right wrist, and 2 on the medial face of the right ankle.

Toxicological examination excluded the intake of alcohol and drugs. After the CT scan and the complete autopsy were performed, they demonstrated that most of the wounds were non-lethal, except for the ones on the left side of the neck, which injured some muscles and the vascular bundle at the height of C6. The histological exam on the jugular vein revealed the massive presence of erythrocytes.

Through investigation led by the police, it emerged that the man was left-handed and had previously expressed suicidal ideations. The presence of test cuts (on the right wrist and right ankle), the previous suicidal ideations, the fact that the torso was naked, the deepness and the distribution of the wounds, and the exclusion of the intake of alcohol and drugs lead to the confirmation of a suicidal event.

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Suicide; Homicide; Medicolegal Death Investigation

F94   The Quantitative and Qualitative Analysis of Dye in Fentanyl Tablets Via Ultraviolet Visible Spectroscopy: A Forensic Approach

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Learning Overview: After attending this presentation, attendees will have learned a method that was developed to extract dye from fentanyl tablets via Solid Phase Extraction (SPE). This solution was then run via Ultraviolet/Visible (UV/Vis) instrumentation to determine qualitative and quantitative information regarding the dye component of the tablets. These tablets came from various seizures from the Drug Enforcement Laboratory system, with most of them linked through intelligence to drug trafficking organizations. The goal was to determine any similarities or differences in the dye used in tablet production.

Impact Statement: The analysis of dye in illicit pills has been explored by the forensic science community in order to link seizures and cases together. This research will impact the forensic science community by applying this concept to the pressing fentanyl crisis affecting the United States. A new technique using UV/Vis instrumentation was used to determine the differences found in the illicit fentanyl tablets.

The fentanyl crisis is detrimentally affecting the United States population. The lives of Americans are being impacted and taken by the catastrophic dangers of fentanyl. Law enforcement communities are working together to aid in the protection of the United States from fentanyl.

Pharmaceutical counterfeit drugs are an issue around the world, often leading to the unintentional use of drugs due to their identical appearances. These appear in pills, patches, and ampule forms. This issue only continues to grow. Illicit fentanyl has been seized in multiple forms to include powders, nasal sprays, and tablets. The tablets are most commonly seized in an appearance similar to Mallinckrodt blue M-30 oxycodone tablets. Recently, the appearance of seized tablets has diverged to include rainbow-colored and scented versions. The forensic lab system has acted in combating the rise of fentanyl seen in America by analyzing an abundance of cases in order to expand the knowledge behind the production of fentanyl. Dye analysis of illicit pills has the potential to be a useful tool for law enforcement agencies to link certain cases together. The same concept was adopted for this research on fentanyl tablets. Fentanyl tablets used for this study were obtained from the Drug Enforcement Administration (DEA) laboratory system and most of them linked through intelligence to drug trafficking organizations. The goal of this project was to determine any similarities or differences in the dye used in tablet production.

An extraction technique using SPE was developed to remove the dye component from the fentanyl tablets. The fentanyl tablets were crushed and put into solution. This solution was subsequently placed onto an SPE cartridge and washed with various solvents to expose the dye component. The dye aliquot was run on UV/Vis spectroscopy via a qualitative scanning method and quantitative method. The qualitative method determined the apex absorption of the dye. These values were compared to dye standards. It was determined that the counterfeit M-30 tablets matched FD&C Blue No. 1 Brilliant Blue. In comparison, a real Mallinckrodt tablet was run via this extraction technique and method to result in a match to FD&C Blue No. 2 Indigo Carmine. This suggests that the counterfeit tablet production sites use a different dye than the pharmaceutical companion. The latter part of this research was dedicated to determining the concentration of Blue No. 1 in the illicit fentanyl tablets. A concentration curve was run on UV/Vis using Blue No. 1. The dye from the tablets was extracted, then run via this curve to determine the concentration. The average concentration of dye in the counterfeit tablets was (0.00039 ± 0.00020) mg/mL.

This research determined via qualitative analysis on UV/Vis that the dye used in fentanyl tablets matched to reference standard dye Blue No. 1. This dye was used regardless of seizure location and association to cartel. In contrast, the authentic Mallinckrodt tablet matched to the Blue No. 2 dye standard. Thus, a conclusion can be drawn that the dye used in pharmaceutical production versus clandestinely produced illicit tablets are different. The concentration portion of the experiment revealed with extremely low levels of dye obtained across all tablets. The results for the concentration did not link any cases together. Further analysis of the concentration of dye in tablets may be beneficial to monitor over an extended period of time to see trends between cases.

Fentanyl; Solid Phase Extraction; Dye

F95   Traumatic Brain Injury After Defensive Ball Launcher Use in Law Enforcement Operations

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Learning Overview: After attending this presentation, attendees will understand the importance of lesion profiles and Computed Tomography (CT) scans to highlight the type of injuries and determine the imputability of the several weapons used.

Impact Statement: This presentation will impact the forensic science community by showing the importance of injuries caused by less lethal weapons, but also how the analysis of ballistic lesions in traumatic brain injuries needs to be supplemented by CT scans and a perfect knowledge of the ballistic characteristics of the different projectiles used.

We have recently had several demonstrations in France that have resulted in serious injuries. The use of force by the police during law enforcement operations, and the injury caused by less-lethal weapons, often impact the opinion of the public via the media. Authorities must now justify the use of these weapons and provide a legal framework for their use. The safety of less-lethal weapons such as de-encirclement grenades or defensive ball launchers has been the subject of scrutiny and controversy. The doctrine of the use of weapons is regularly dissected by lawyers following complaints from victims.

These events saw the emergence of a certain number of wounded claiming to be victims of defensive ball launcher fire. The defensive ball launcher has been in use by the French police since 2006. They are loaded with unique defense ammunition called short-range defense ammunition. The projectile is composed of a plastic sabot of cylindrical shape, 40mm in diameter and 65 mm in height, on which is fixed a foam projectile of cylindrical-ogival shape. The launchers provided are equipped with a sight and the preferred firing zones are, in straight firing, the chest and lower limbs. The medical examiner of these injuries will have the task of describing and evaluating the injuries of the victim, but also of determining the compatibility of the injuries with this type of projectile.

We report two cases of brain injuries inflicted with a defensive ball launcher. The first case concerns a 5-year-old child victim of serious head trauma while she was 100 meters from a clash zone between young people and the police. The investigation noted the use of a defense ball launcher, de-encirclement grenades, and the throwing of stones. The second case describes a 48-year-old protester hit in the head by several different projectiles with severe head trauma, loss of consciousness, embarring fracture of the skull, and fracture of the facial bone. In these two cases, we were able to discuss the imputability with the description of the skin wound, thanks to the analysis of the trauma brain injury with the CT scan, but above all by analyzing the ballistic characteristics of the different projectiles used.

These two cases reports show how the analysis of ballistic lesions in traumatic brain injuries needs to be supplemented by a CT scan and a perfect knowledge of the ballistic characteristics of the different projectiles used.

Police Violence; Injury; Weapon Analysis

F96 **Seasonal Soil Microbial Succession During Carrion Decomposition: Applications for Body Translocation and Postmortem Interval Estimation**

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Learning Overview: After attending this presentation, attendees will better understand the succession of soil bacterial communities associated with carcass, or cadaver, decomposition and how such community assembly trajectories vary with season and carcass size. Attendees will also learn how soil bacterial communities can be used as potential evidence during death investigation, specifically regarding body translocation from one habitat to another.

Impact Statement: This presentation will impact the forensic science community by demonstrating that soil bacterial communities underneath carcasses change predictably during decomposition, suggesting they can be used to support minimum Postmortem Interval (minPMI) estimates. Microbes are a critical element in the community of organisms associated with decomposing bodies, so understanding which taxa are present and how the community composition changes throughout decomposition could also greatly inform future studies of forensic utility.

When human or animal remains decompose outdoors, decomposition fluid and organic material are introduced into the soil beneath the carcass, thus altering the microbial communities over time, often referred to as community succession.[1] The trajectory of this community succession is often described using changes in biodiversity metrics such as the number and identities of different taxa over multiple timepoints. These metrics can then be used as time point profiles to model the minPMI.[2] Soil microbes can also be used as forensic indicators to connect an individual to a locality due to the unique community diversity observed at different locations.[3] Samples containing unknown soil sources have been reported to be successfully traced back to the origin city and district.[4] Variations in the surrounding habitat microbiome of a body during decomposition have shown promise as a means for calculating microbiome-based PMI estimates to support other PMI estimation methods.[5-8]

As part of an ongoing decomposition study, soil samples were collected underneath replicate stillborn swine carcasses (n=5 or 6) throughout decomposition to investigate differences in bacterial community successional change during the spring, summer, and fall seasons of one calendar year. During the summer, soil samples were also collected from underneath three adult swine carcasses at the same time points to compare bacterial communities underneath carcasses of varying sizes. Composite control samples were also collected one meter away from all carcasses at all time points. All samples were processed by extracting the DNA and amplifying the V4 region of the 16s rRNA gene with the 515f (5′ GTGCCAGCMGCCGCGGTAA) and 806r (5′ GGACTACHVGGGTWTCTAAT) primers. Extracted DNA was sequenced using 16S amplicon based high-throughput sequencing, and the resulting data were assembled, quality-filtered, and analyzed with the QIIME2™ software.

Preliminary analyses suggest that the fall soil communities significantly changed across time points consistent with trajectories that have been previously reported in other studies of carrion and human cadaver decomposition, and that the taxa and rate of change was different among seasons.[1,8] During the beginning stages of decomposition, Firmicutes has been reported to be the most dominant bacterial phyla of soil communities, but then is replaced by Proteobacteria in communities as decomposition progresses.[9,10] All soil samples from underneath the carcasses had communities that were also significantly different from the control samples, suggesting that soil community profiles under carcasses may indicate if it has been moved (e.g., the soil sample profile from under a carcass is inconsistent with a carcass of advanced decomposition). In the fall season, it took 32 days for the carcasses to become fully skeletonized and in that time, the mean Chao1 value of all six carcasses changed significantly (p=0.001), from 788 on day 1 to 308 on day 32. Observed seasonal weather differences influenced the rate of decomposition, in the fall temperatures ranged from 35.4°C to –3.3°C, in the spring the carcasses were fully skeletonized by day 12 with temperatures ranging from 31.6°C to 3.0°C, and in the summer, carcasses were skeletonized by day 9.

These data demonstrate predictable differences in the soil microbial communities at varying time points during decomposition which could allow for a reliable microbiome-based PMI estimate using soil bacterial communities. These results provide information about how seasonal changes in Michigan affect soil microbes during decomposition and may also indicate body movement when there are soil bacterial community profile mismatches during death investigation.

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Soil Analysis; Postmortem Interval; Microbiology

F97   An Unusual Accident at Work: Fatally Hit by a Chain

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Learning Overview: This presentation will assess the importance of inspecting and 3D reconstructing a crime scene to establish the causes of death and present an unusual case of a work-related accident.

Impact Statement: This presentation will impact the forensic science community by fostering a desire to perform further in-depth studies concerning the inspection of the crime scene and 3D reconstruction techniques.

This study looks at an unusual work-related accident. A 33-year-old man was suddenly struck on the chest by a chain while servicing a wind turbine. The man died instantly. To understand the causes of death, an exhumation was carried out three months after the death.

During the autopsy, a hole was found in the corpse's chest. The hole had an oval shape and dimensions of 4.5 x 4cm. The hole's margins were regular and driven inward. At first sight, it seemed the body had been hit by a large-caliber bullet. Furthermore, the autopsy revealed that a foreign object drilled through the sternum. The object crossed the entire thickness of the sternum, in line with the third and fourth ribs. The object was metallic, with the shape of a parallelepiped. It weighed only 41g. The evisceration of the body revealed a circular hole in the anterior wall of the right ventricle at full thickness. Moreover, a cardiac tamponade was also detected. No other injuries were found on the body.

The main issue was understanding how a metallic object weighing only 41g could have penetrated not only the skin and soft tissues but also the sternum. Unfortunately, no inspection of the scene was carried out. The only additional details on the accident came from a statement released by a colleague of the dead man,

The reconstruction of the accident based on history and postmortem findings throws some light on the dynamics. However, to fully reconstruct the dynamics, a 3D reconstruction was needed. The main issue consisted in understanding how much energy the object needed to penetrate the body, and in which direction the body was in. The 3D reconstruction of the occurrence confirmed the manner of death and the dynamic.

Sudden Death; Trauma; Exhumation

F98   The Evidence for Prosecution of Atrocity Crimes in Ukraine During the Ukraine-Russia Conflict: 2022–Present

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Learning Overview: The goal of this presentation is to use academic resources to clarify the role of the international legal system in analyzing the potential commission of atrocity crimes. This is then applied to the current conflict in Ukraine, in which atrocity crimes have been widely alleged.

Impact Statement: This presentation will impact the forensic science community by aiding in the understanding of the currently developing conflict in Ukraine and the strength of the accusations of genocide, crimes against humanity, and war crimes. It may also potentially aid in the general understanding of international humanitarian law.

The invasion of Ukraine by the Russian Federation in February 2022 sent shock waves around the world and caused a humanitarian crisis on a scale unseen in Europe since the Second World War. Despite not being a member of the International Criminal Court (ICC), Ukraine accepts the jurisdiction of the ICC for the three so-termed atrocity crimes. These three crimes are genocide, crimes against humanity, and war crimes. There have been widespread reports and accusations of all three atrocity crimes during this armed conflict, which have been principally directed toward Russian troops across many different locations.

This work aims to summarize the legal scope of atrocity crimes for application to the conflict, especially with regard to the ICC; the geopolitical context which underlines the conflict itself and the potential crimes committed therein; and to undertake a study of the evidence for the commission of these crimes, including those potentially perpetrated by Ukrainian forces. This work was done via a review of the existing English-language literature, primarily using Google® Scholar and Queen Mary University of London Library Search. Only peer-reviewed sources, such as academic journals and publishers, were used, in addition to a variety of newspaper articles, which were also in English.

This revealed that although individual elements of the atrocity crimes have been undoubtedly committed, ranging from acts such as killing and rape to torture and forcible displacement, there is a difference of opinion as to whether these acts qualify as the crimes themselves. This is because there is debate as to the required mental and contextual elements for each of the three crimes—genocide, for example, requires a specific element known as the *dolus specialis*, which is not required for either crimes against humanity or war crimes.

ICC; War Crimes; Rape

F99 Cannabinoid Hyperemesis Syndrome-Related Death: An Emerging Public Health Threat

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Learning Overview: After attending this presentation, attendees will have learned about potential side effects of cannabinoid heavy use, including, but not limited to, Cannabis Hyperemesis Syndrome (CHS), how to recognize CHS, and how to attribute the cause of death to it. Attendees will also learn about the public health impact of cannabis legalization.

Impact Statement: This presentation will impact the forensic science community by discussing the case of a cannabis hyperemesis syndrome-related fatality and aims to deliver insight into the possibility of misdiagnosis leading to delayed treatment and possible fatal outcomes. This presentation will also discuss the risks of legalizing cannabis and the reported chronic effects of long-term Tetrahydrocannabinol (THC) use thus far.

CHS is characterized by recurrent episodes of cyclical nausea and vomiting associated with heavy cannabis consumption that may be accompanied by compulsive hot baths for relief of symptoms.[3-5] CHS is considered to be an extension of cyclical vomiting syndrome, which is a functional gastrointestinal disorder, by the ROME IV criteria.[4] CHS is usually initially misdiagnosed and only recognized after several recurrent emergency department visits, which leads to delayed prevention of its potentially life-threatening long-term sequelae.

An example of a medicolegal autopsy involving CHS has come through the District of Columbia Office of the Chief Medical Examiner. The case is of a 39-year-old male who presented to an Emergency Department (ED) with the complaint of a three-day duration of an intermittent, dull, epigastric pain, nausea, and vomiting that worsened with food and drink consumption. A medical record review revealed similar symptoms over the past year, and he was diagnosed with gastroesophageal reflux disease and was discharged with reflux and pain medication with minimal relief. He reported a long history of smoking marijuana and cigarettes, and socially drinking alcohol. IV fluids and pain medications were given in the ED, and he was then discharged on proton pump inhibitors. He subsequently visited emergency departments periodically for the same complaint and was eventually discharged after symptomatic therapy. His symptoms were attributed to THC use, and he was advised to abstain from THC consumption. One day following his last medical encounter for the same complaint, he was found unresponsive at home and was pronounced dead. At autopsy, there was a 5.3-centimeter perforated gastric ulcer with 1,300 milliliters gastric content within the abdominal cavity. There were no other significant natural diseases or injuries.

The discussed case exemplifies a detrimental complication of a delayed diagnosis of CHS and misdiagnosis for a long period of time that potentially can lead to patients and families developing medical mistrust. There are limited literature reports of fatalities attributed to chronic heavy THC use. A recently published case series included three cases that described the death of three young adults, all of whom had similar histories to the above discussed case. The cause of death was attributed to CHS.[3]

The long-term effects of cannabis use that have been described in the literature thus far include impairment of long-term memory, inattention, increased risk of anxiety and depression, and cannabinoid hyperemesis syndrome.[1,2,6] Cannabis is the most-used recreational drug worldwide and its consumption is increasing following its legalization in many countries. It is of utmost importance to recognize the chronic effects of THC use and to spread awareness to the general public and medical personnel. Appropriate recognition of symptoms to achieve better medical care and avoid complications is imperative to prevent negative outcomes. It is also crucial for forensic pathologists to recognize the symptoms of CHS to better characterize the cause of death for medicolegal cases.

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Cannabis; Public Health; Autopsy

F100   **Unlocking New Insights Into Inorganic Gunshot Residue Analysis Through Single Particle Inductively Coupled Plasma Time-of-Flight Mass Spectrometry (spICP-MS)**

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Learning Overview: This presentation will demonstrate the capabilities and advantages of spICP-MS in the analysis of Inorganic Gunshot Residue (IGSR).

Impact Statement: This presentation will impact the forensic science community by its ability to unlock new perspectives on IGSR sample identification, comparison, and characterization. Using association rule mining, the study has revealed a broader elemental association in Gunshot Residue (GSR), expanding the understanding of the potential sources and origins of particulate matter found at shooting scenes.

GSR analysis is a critical aspect of forensic investigations involving firearms, aiming to link the source of particulate matter to shooters, observers, or passersby. While scanning electron microscopy with energy-dispersive X-ray spectroscopy has been the gold standard for IGSR analysis, its limitations in identifying the origin of individual particles hinders its full potential for forensic purposes. In this study, we explore the capabilities of spICP-MS, a novel technique offering rapid analysis of undigested sample for number, size, and composition of submicrometric particles.[1,2]

Single particle ICP-MS enables the atomization and ionization of colloidal particles suspended in a solution as they reach the plasma. The technique offers high throughput, enabling the collection of signals from hundreds of particles per minute. Unlike traditional techniques, spICP-MS goes beyond the detection of characteristic IGSR particles containing Sb, Ba, and Pb, revealing a more comprehensive elemental association in gunshot residue.

The method's efficiency lies in its ability to analyze more than 100 samples in a single day, thanks to the absence of sample preparation and fully automated sample introduction.[3] By employing association rule mining, we unveil local dependencies between elements and particles in IGSR samples, providing a deeper understanding of their composition and determine the strength of the similarity between potential IGSR sources.[4,5]

The time-of-flight capabilities of spICP-MS are particularly crucial as they enable the analysis of the full mass spectrum of detectable elements per particle. This comprehensive approach broadens the perspective of IGSR sample identification, comparison, and characterization, going beyond traditional IGSR analysis limitations.

This work demonstrates the significant potential of spICP-MS in advancing research on IGSR analysis. The technique's rapidity, sensitivity, and ability to provide a more extensive elemental association pave the way for future breakthroughs in forensic investigations related to firearm incidents. Ultimately, spICP-MS holds promise in enhancing the association of IGSR particles with specific individuals or scenarios, contributing to the pursuit of justice and the establishment of robust evidence in firearm-related cases.

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Firearms; Gun Shot Residue; Cartridge Casings

F101   Understanding Volatile Odor Signatures From Entomological Samples

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Learning Overview: After attending this presentation, attendees will have a better understanding of the odor profile of insect samples commonly encountered in entomological applications within the forensic practice.

Impact Statement: This presentation will impact the forensic science community by depicting a laboratory approach exploring headspace odor sampling methodologies for the odor profiling of maggot samples. Different sample treatment methods will be explored and related to the chemical odor profile obtained to understand how sample handling affects observed volatile odor markers. The information gained can further enhance the use of volatolomics for routine entomological applications and showcase an added tool for processing insects found at the crime scene.

Forensic entomology proves to be an important tool in helping criminal investigations. Insect development along with patterns of insect succession can be utilized to estimate the Postmortem Interval (PMI). Given that certain insects appear on the body at certain times or in specific locations, their presence along with other evidentiary material can demonstrate potential links to times and geographic locations where crime scene events may have occurred. As it relates to the chemical characterization of insect odor volatiles, studies have focused on profiling cuticular hydrocarbon composition as a function of pupae age/growth, behavioral assays for chemical signaling, or larval odor print as a function of decomposition stage. An unexplored area lies in the minimal understanding of insect odor analysis from a method treatment perspective. Identifying optimal methods for maggot sample collection, treatment, and analysis is an important first step to understand what compounds are innate to the maggot specimen itself and leverage a volatile odor repository from laboratory-controlled conditions. In entomological studies, maggots are routinely boiled for accurate length measurements. Hence, understanding the odor print from live versus boiled samples is critical to enhance volatolomic uses in entomology.

In this study, the blow fly (*Cochilomyia macellaria*) was utilized to compare Volatile Organic Compounds (VOCs) between boiled and non-boiled maggots using a headspace vapor sampling approach. For this purpose, 60 maggots (30 non-boiled and 30 boiled maggots) were employed for a 24-hour headspace extraction using Solid Phase Microextraction (SPME) coupled with Gas Chromatography/Mass Spectrometry (GC/MS). Insect samples were reared at a consistent temperature (25ºC) using the same rearing source (chicken liver) to provide a standardized platform for insect collection and monitoring. All the materials used in the headspace analysis, including the sampling vials, tweezers, and related boiling containers, were pre-treated to make sure that no other volatiles would interfere with the procedure.

SPME-GC/MS proved to be a viable method for the detection of volatile odor markers from individual maggot samples of the targeted species. While same compounds were identified in both treatment groups (i.e., boiled vs. live), differing ratios of chemical compounds were highlighted in both groups as a function of treatment. Preliminary results suggest that chemical odor profiles are distinctive based on sample handling and are currently being investigated to extrapolate further variables from the generated odor profile, such as sex identification of the immature stages. The benefit of this study is enhanced knowledge in the realm of optimal odor profiling of maggots for entomological studies, which can prove to be an added capability to routine insect sampling and analysis methodologies.

Entomology; SPME-GC/MS; VOC

F102   The Findings of Criminal Judgements Related to Driving Under the Influence of Drugs During the 2018–2022 Period in Taiwan: Using District Courts as Examples

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Learning Overview: The goal of this presentation is to help attendees gain insights into: (1) the quantitative research of criminal judgements related to Driving Under the Influence of Drugs (DUID); and (2) trends and a retrospective review of DUID in Taiwan during the 2018–2022 period.

Impact Statement: This presentation will impact the forensic science community by providing rare research of a DUID empirical study in Taiwan. The findings resulting from this study will change the vision about the practice of DUID and be helpful to the improvement of public safety.

DUID has become a global problem in recent years.[1] Compared with the effects of alcohol, fewer people have been concerned about the DUID cases in Taiwan. Car accident-related death statistics in Taiwan, regularly provided by the Ministry of Justice's Institute of Forensic Medicine, indicated that sedatives, hypnotics, and illicit drugs were the most commonly detected drugs in these cases, except for alcohol. Furthermore, prescription drugs involving death would also be a potential threat.[2] The purpose of this study was to examine some information about the criminal judgements related to DUID (Subparagraph 2, Paragraph I, Article 185-3 of the Criminal Code of the Republic of China) by district courts during the 2018–2022 Period.[3] These criminal judgements were excluded in the offense resulting in death or serious physical injury, which is against another paragraph. Hopefully these findings would be helpful to the administration and prevention of traffic policies in Taiwan.

Statistics of DUID judgements (277 total) during this five-year period revealed: (1) the numbers beginning from 2018 were: 82, 76, 53, 39, and 27, and the conviction rate was 96.03% (266); (2) the range, mean, and median of sentences to imprisonment were 2–10 years, 3.28 years, and 3 years, respectively, where the mean value was identical to driving under the influence of alcohol.[4] Forty-one point five percent (110) of offenders were sentenced to imprisonment for three years; (3) there were 197 judgements with toxicology testing reports in urine or blood; however, the concentrations of drugs in the reports were cited in only 41 judgements; (4) the usual types of motor vehicle were cars/motorcycles (196/58); (5) there were only 9 judgments that showed the combination of alcohol and drugs. Category 3 narcotics[5] were the most-found drugs (41%), especially ketamine and flunitrazepam.[5] This was followed by category 2 narcotics (22%), such as methamphetamine, then category 1 narcotics (12%) in which heroin was included and category 4 narcotics (6%), which were prescription drugs, for example, benzodiazepine and zolpidem, in sequence. Some drugs were not regulated by law, but they were used by drivers: nitrous oxide, super glue, and antidepressants. One thing worth noticing is the pattern of multiple drug abuse is different between criminal judgements related to DUID and deaths. The former showed about 77% pointed out single drug abuse in contrast with autopsy cases, which involved abuse of multiple drugs, even new psychoactive substances.[6]

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DUID; Retrospective Study; Court

F103   **An Analysis of Inorganic Gunshot Residue (IGSR) Nanoparticles by Conventional and Single Particle Inductively Coupled Plasma/Mass Spectrometry (spICP/MS) Using Design of Experiments (DOE)**

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Learning Overview: The goal of this presentation is to present the results of the investigation on the influence of different factors on the sampling and analysis of IGSR nanoparticles by conventional and spICP/MS using DOE. The results for the number of IGSR nanoparticles detected and the characterization of the peaks obtained by spICP/MS will also be presented.

Impact Statement: This presentation will impact the forensic science community by proposing the use of spICP/MS as a promising alternative for the forensic analysis of IGSR. The results obtained indicate the possibility of identifying individual IGSR nanoparticles and analyzing their composition, giving some information on particle size and number concentration.

IGSR is identified as a spherical nanoparticle containing a mix of Pb, Ba, and Sb, which can provide useful information for the reconstruction of events involving firearms incidents.[1] spICP/MS performed in single detection mode is a promising alternative for forensic analysis of IGSR nanoparticles. The technique is able to determine characteristics such as inorganic composition, concentration, size, and size distribution. Signals from hundreds of nanoparticles can be collected in 1–2min per sample.[2] Thus, spICP-MS can be used as a screening-level technique for the analysis and characterization of IGSR nanoparticles in addition to the recommended standard method (Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy [SEM/EDS]) that requires two to six hours per sample to obtain combined information on the morphology and elemental composition of the particles.

This work evaluated the factors that influence the sampling and analysis of IGSR particles by conventional and single particle ICP/MS. Two saturated fractional factorial designs 2(7-4), one for the shooter and the other for the observer, were used to evaluate the influence of the investigated factors on the concentration of Pb, Ba, and Sb in the samples. The following factors were investigated: detection mode (conventional and single particle ICP/MS); sampling time; gun caliber; room ventilation; number of shots; and gun grip (shooter) or shooting distance (observer). A group of volunteers, shooters, and observers performed the eight trials of each saturated fractional factorial design performed in triplicate at the ICMG shooting range. Shooter (0m) and near observer (0.45m) were positioned below an exhaust, while the distant observer (1.45m) was positioned below another exhaust. Swabs with plastic rods and cotton tips were used for collections. The best conditions to determine the presence of the characteristic elements of IGSR were obtained: using the conventional ICP/MS analysis; performing the collection immediately after the shot(s); and using the exhaust during the shot(s). Furthermore, for analyzes involving shooters, hand sampling and three shots resulted in greater detection of characteristic elements of IGSR when compared to nasal sampling and one shot, respectively. The results obtained in the analysis by spICP/MS received a statistical treatment using a program developed in the R Studio software for detection and characterization of IGSR nanoparticles.

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Cartridge Casings; Firearms; Gun Shot Residue

F104 **Maxillary Sinus Dimensions as Predictive Markers for Age and Sex in Northwest Indian Subjects: An MRI-Based Study**

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Learning Overview: The goal of this present study is to investigate the utility of maxillary sinus dimensions and additional parameters as a predictive marker for age and sex determination in Northwest Indian subjects using Magnetic Resonance Imaging (MRI) as the radiological modality. By analyzing the MRI-based dataset and correlating maxillary sinus measurements with age and sex, the study seeks to provide valuable insights into the potential applications of this non-invasive approach in forensic and anthropological contexts.

Impact Statement: This presentation will have a multifold impact on the forensic science community by validating the maxillary sinus as a reliable marker and valuable tool for age and sex determination in the forensic identification process. Exploring the knowledge pool related to maxillary sinus and its correlation with parameters like age and sex can help the researchers to contribute to improving the forensic identification techniques and inform the future anthropological and medical fields toward this advancing region.

Introduction: Forensic anthropology as a discipline is concerned with discerning the variations associated with skeletal morphology. It rarely ever happens that a body is found without a skull, albeit there can be numerous scenarios where the individuals are involved in mass disaster, natural calamities, bio-terror activities, outbreak of conflict, political genocide, etc. resulting in heaps of unidentified cadavers or badly damaged and commingled skeletal remains in their wake.[1] The objectives remain the same (i.e., dignified repatriation of the remains). In such situations, rather than finding the complete set of skeletal remains, there are instances where only fragments or incomplete skeletal remains are discovered.[2] Under such conditions, forensic anthropologists rely on other parts of the skull such as the maxilla that remain intact despite any significant damage caused to the skull and that can be used to identify individuals.[3] The maxillary sinus, located within the maxillary bone of the skull, is an air-filled cavity that exhibits sexual dimorphism and undergoes age-related changes.[4,5] However, to date, limited research has been conducted in specific populations such as the Northwest Indian subjects to validate the potential of this approach.

Methodology: Bilateral maxillary sinus MRI images obtained from 200 adult subjects (males=95, females =105) aged 18 to 72 years, were considered for measuring different parameters like length, width, height, area, perimeter, and volume of the maxillary sinus. The study involved a prospective selection of randomly chosen healthy individuals attending the outpatient department, and informed consent was obtained from each participant. The exclusion criteria focused on identifying subjects with maxillary sinus showing changes due to malformations, obliterations, and/or trauma. The sex and age-dependent differences in these parameters were estimated and statistically analyzed using SPSS (version 21.0) software and the results were interpreted to highlight any forensic significance of such differences.

Results and Conclusion: The results of the study yielded evidently visible sexual dimorphism; males exhibiting more values than the females. Present study findings revealed that maxillary sinus dimensions and the associated parameters like its area and volume display anatomical differences between two sexes and different age groups and, thus, can be a useful indicator of the sex and age of an individual when only this portion of the cranium is available for forensic identification purposes. The MRI images provide accurate measurements of maxillary sinuses more than other alternative methods. The detailed results will be presented in the textual, graphical, tabular forms in the presentation.

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Forensic Imaging; Maxillary Sinus; Sexual Dimorphism

F105   **A New Frontier in Postmortem Interval (PMI) Estimation: The Assessment of Yellow Bone Marrow Changes Using Dual-Energy Dual-Layer (DECT)**

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Learning Overview: The goal of this presentation is to propose a completely innovative method to perform a more precise estimation of the PMI with the aid of radiological techniques and, in particular, of Computed Tomography (CT). More specifically, this study will focus on the post-mortal alterations of the yellow bone marrow using a particular type of CT, the DECT. After attending this presentation, attendees will better understand the importance of considering this type of evaluation to perform a more precise estimate of the PMI, also considering that CT is an examination frequently performed on corpses.

Impact Statement: This presentation will impact the forensic science community by demonstrating the post-mortal alterations that the yellow bone marrow undergoes following a specific temporal trend, evaluating, as in the case of the Hessnge nomogram, all the correction factors.[1] More specifically, we will focus on the possibility of identifying a water component within the yellow bone marrow itself, which normally cannot be identified even by machines with the highest sensitivity in living humans.

It is known that the estimate of the postmortem interval is currently based on the use of dated and potentially biased methods, which in any case allow a correct estimate only within 48–72 hours of death: these are represented by the Henssge nomogram, and from the assessment of rigor and livor mortis. For this reason, in recent years, the scientific community has tried to develop new methods that allow us to overcome the limits existing today, with a consequent significant increase in forensic research directed in this field. In fact, to date, the relevant literature offers studies on the use of forensic entomology, the variation of the microbiome, the modifications of bone and parenchymal structures by means of imaging, and still on the analysis of the degradation of proteins, DNA, and RNA.[2,3]

The study of the yellow bone marrow that we propose is completely innovative: in fact, until today, bone marrow study has never been considered for this proposal. The evaluation of yellow bone marrow, which is possible with almost all CT methods, can be performed in a much more specific way using DECT, as this allows us to acquire quantitative information on the individual chemical components that make up a tissue. Literature evidence has shown that normally, in living humans, it is not possible to identify water inside the yellow bone marrow, not even with the use of machines with higher sensitivity. On the contrary, as we have demonstrated, an amount of water is identifiable in the yellow bone marrow of subjects who died even a few hours after the radiological examination. Thanks to this evidence, after the acquisition of a sufficient series, we will try to identify a pattern of degradation of the yellow bone marrow to attempt to perform a more precise estimation of the postmortem interval.[4]

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Warly PMI Estimation; Forensic Radiology; Bone Marrow

F106   **The Detection of the Yellow Fever Virus in Human Remains Using Mass Spectrometry-Based Protein Analysis of Dental Pulp**

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Learning Overview: This presentation will showcase the ability of dental pulp to be used in pathogen detection, specifically for the detection of the yellow fever virus. After attending this presentation, attendees will better understand the importance of dental pulp analysis, the difficulties that exist surrounding current yellow fever diagnosis and detection, and how modern proteomic techniques can be used on historic remains.

Impact Statement: This presentation will have a large impact within the fields of archeology and paleomicrobiology as well as the forensic science community as a whole by showing that the analysis of dental pulp, an often forgotten tissue, can be used to detect the yellow fever virus decades after traditional diagnosis techniques became unusable.

Typical diagnostic methods for detecting the yellow fever virus involve a histopathological examination of liver tissue of laboratory blood tests, where neutralizing antibodies or the viral RNA sequence are targeted.[1,2] Both liver tissue and blood are unrecoverable in historic, skeletal remains, greatly limiting the potential of pathogen detection. In these instances where blood and liver tissue are no longer recoverable, dental pulp can prove to be an ideal target for the detection of infections found in the blood. Dental pulp, a loose connective tissue located at the center of the tooth, has a natural protection from the outside environment, allowing the biological information contained within the tooth to be recoverable for much longer than other tissues.[3,4] Blood vessels are contained within the dental pulp, meaning that the pulp is exposed to bloodborne agents that are contained within the vessels.[5] Yellow fever, a vector-borne disease, is transmitted by mosquitoes and following a mosquito bite, the virus enters a person's bloodstream.[6] This presence of the virus in the bloodstream means that the virus can also be detected in a person's dental pulp. Although the viral RNA genome is likely unrecoverable in dental pulp after a matter of days, the viral proteins are recoverable for much longer, as long as hundreds of thousands of years.[3,7]

The ability to use dental pulp to detect pathogens that have previously been thought of as undetectable in historic remains can have great impacts. This type of analysis can point toward a possible cause of death or a possible identification for these remains. By following a protocol of analyzing modern teeth (to confirm the presence of blood proteins), teeth from a known yellow fever victim (to confirm the presence of yellow fever proteins), and historic remains, this presentation validates the use of dental pulp as a diagnostic tissue for the purpose of yellow fever detection.

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Proteins; Dental Pulp; Yellow Fever Virus

F107 The Challenges and Scope of Iris Biometrics in Forensic Science: A Systematic Review

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Learning Overview: This presentation will explain the possibility of iris recognition to enhance security and privacy in various applications, including access control, authentication, and identity verification in the normal iris, ocular disease, and corpses. Following this presentation, attendees will have a greater understanding of the relevance of iris biometrics, which is not only restricted to live humans but is also used as a password for a human identity even after death, and iris deidentification, a privacy-preserving approach.

Impact Statement: This presentation will have an impact on the forensic science community as providing iris scans will be a valuable tool for law enforcement groups, resulting in more accurate and trustworthy identification of suspects and criminals and reducing the likelihood of false arrests or mistaken identities. Iris' biometric data serves as persuasive evidence in court. It may help to establish a strong link between a suspect and a crime scene.

Introduction: Forensic science aims to scrutinize the identity and authenticity of criminal perpetrators, and biometric tools have played a vital role in this endeavor. Among the multiple physiological and behavioral biometrics options available to forensic experts, iris patterns serve as the best forensic tool, even superior to DNA or dental records as the former have about 266 unique spots/features compared to 16 to 20 distinct characteristics of other biometric modalities.[1,2] The iris patterns are impressively unique, exceptionally complex, hard to alter, and even not shared by identical twins.[3] The iris biometrics method is more acceptable than other biometrics because of its non-invasive and non-contact method. The human iris can be used for biometric authentication even after death, within a specific period.[4] Applying iris biometrics in criminal investigations has been a game changer for forensic investigators and law enforcement agencies.

Method: For the present systematic review, the Boolean search methodology was used to search the databases like Embase®, PubMed®, the Web of Sciences®, and Scopus® to find relevant studies that have been published and addressed the topic of iris biometrics. Keywords included "iris biometrics," "forensic identification," "challenges," "scope," "forensic science," and related synonyms and combinations. This search was restricted to articles published within the past ten years to ensure the relevance of the findings.

Results: Out of 281 potentially related search articles identified from different databases, only seventeen (N=17) articles discussed iris deidentification, postmortem iris recognition, iris identification in ocular disease, and iris decomposition after death that were finally selected and included in this review. Improving the iris biometrics approach in ocular disease and cadaver eyes was the main objective of the outcome measurement. The users need an iris deidentification method to access social networking sites and other internet resources without worrying about losing their data.

Conclusions: This review explains the fascinating future possibility of iris recognition in the normal iris or with ocular disease and in corpses. In addition to identifying the iris pattern, the iris identification technique may also be used to pinpoint minute variations in the iris and its vasculature.

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Iris Biometric; Identification; Cadaver

F108   Child Sexual Abuses in Chandigarh, India: Dimensions and Causes

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Learning Overview: After attending this presentation, attendees will have a glimpse of the prevalence of Child Sexual Abuse (CSA) in a modern Indian city and the remedial measures offered for its prevention.

Impact Statement: This presentation will impact the forensic science community by providing deep insights into the socio-demographic profile of the perpetrators and the social and legal implications of this crime in India. At the same, the presentation wishes to sensitize attendees about children's rights, especially in cases pertaining to CSA and the role of forensic experts in such cases.

Introduction: CSA is a cruel and tragic occurrence and a serious infringement of a child's rights to health and protection.[1] Every child is the future of any nation and needs to be protected against all sorts of sexual offences. CSA is defined as "the involvement of a child in sexual activity that he or she does not fully comprehend, is unable to give informed consent to, or for which the child is not developmentally prepared and cannot give consent, or that violates the laws or social taboos of society."[1] A large number of CSA cases have been reported from India indicating its high prevalence in Indian society. The Protection of Children from Sexual Offenses Act 2012 (POCSO) mandates to protect children below 18 years from sexual assault, abuse, harassment, and pornography. A sharp 12% increase in CSA cases was reported during the COVID-19 pandemic lockdown during 2020-21 as reported by the National Crime Records Bureau (NCRB).[2] The national guidelines for the prevention of child abuse should be put into place to begin with, followed by their effective implementation and translation into programs and action plans in order to have a broader impact at a national level.[3]

Material and Methods: The present study was conducted to estimate the causes, nature, and prevalence of CSA as a criminal activity to be investigated forensically and medicolegally. For this purpose, the retrospective data of child abuse cases reported in different police stations of Chandigarh, India, from 2016–2021 were accessed by obtaining permissions from the concerned competent authorities, and the collected information was scrutinized and compiled to add up to the existing knowledge on the topic.

Results and Discussions: Analyses revealed that in 16 out of these 24 cases (i.e., 70.6% cases), the victim and the perpetrator were mutually aware of each other's identity prior to the assault as a relative, neighbor, or acquaintance of any family member and very few were unknown to the victim. The age profile of victims was found to be within the age range of 12–15 years, tantamount to 41.7%, indicating that a higher proportion of victims were young teens. In contrast, the age profile of offenders varied from 12–40 years. Overall, 48.3% of the sexual assault offenders were adults and belonged to an age profile of 18–25 years. Whereas 10.3% juveniles made up to the offender's list, in 17.2% of cases, the sex of the perpetrator was unknown to the victim. Also, 77.6% of victims were medically examined to verify the criminal act and this can certainly be used as a parameter to analyze the behavior extended toward the victims.

Conclusions: In a majority of cases, perpetrators approached the victim through stalking and eve teasing before committing the actual crime. Educational disparities and lack of sex education were major reasons for the victims to fall prey to the perpetrators. Educating children about the allurement by criminals is essential to prevent and combat sexual abuse against children, sexual violence, and sexual exploitation.

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Child Abuse; Violence Against Children; False Allegation

F109   Self-Strangulation Using Electrical Cable Ties: A Case Report and Literature Review

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Learning Overview: After attending this presentation, attendees will be informed of a very unusual case of self-strangulation, including the victim's chosen tool, psychological profile, and circumstantial data. Also, thanks to the review of the literature, attendees will be able to correctly collect and interpret forensic data in the event of similar cases.

Impact Statement: This presentation will impact the forensic science community by tracing the manner of death in atypical suicide circumstances, such as self-strangulation by ligature. This presentation will allow attendees to analyze in detail and schematically all the forensic data useful for the differential diagnosis between suicide and homicide in these circumstances of death. This will be achieved by describing our case and comparing it with the very few similar cases in the literature.

Self-strangulation by ligature, most frequently employed for homicidal purposes, is not a common method chosen in suicidal scenarios.[1,2] Therefore, when faced with such circumstances of death, it is crucial that the forensic doctor makes an accurate differential diagnosis using all available data at best. The first peculiarity of the described case is relative to the tool chosen by the victim, that is, an electrical cable tie. The other few similar cases present in the literature will be compared to that of our experience.[3-5] Moreover, our case is emblematic because it is rich in circumstantial data that allows detailed reconstruction of the planning of suicide, personological factors, the family, and social context of victim. These data should always be researched and carefully analyzed by the forensic doctor as they allow us to not only define the manner of death more easily, but also can provide important contributions in the prevention of suicide.

We describe a case of a 50-year-old woman discovered with an electrician's cable tied tight around her neck on the side of a cycle path along a Roman riverside. Based on the medical records of the victim and the testimonies of family members, the victim suffered from depression linked to economic problems and severe bereavement stress and had repeatedly expressed suicidal intentions.

As regards the tool chosen for suicide, according to the family, the woman had bought the cable ties about three months before the event. On the very morning of the woman's suicide, the mother had also attempted suicide at their home by tightening an electrical cable tie of the same type around her neck, and then asked her son for help to free herself. These and other circumstantial data, as well as clinical and anamnestic data, have been critical in reconstructing the psychological profile of the victim and in defining the dynamics of death. Moreover, the external examination, the autopsy findings, and the toxicological tests were crucial to exclude intoxication and assault by third parties.

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Asphyxiation; Suicide; Strangulation

F110 Fatal Systemic Aspergillosis in a Puerpera: A Rare Case Report and Review of the Literature

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Learning Overview: After attending this presentation, attendees will understand and evaluate the forensic approaches in the management of a natural death due to a rare fatal fungal infection dissemination in a puerpera.

Impact Statement: This presentation will impact the forensic science community as it presents a rare case by increasing clinical suspicion of disseminated aspergillosis in severe sepsis of unclear origin and suggesting alternative tests such as investigation.

Introduction: Fungal infections are an increasing threat to global public health. There are more than six million fungal species worldwide, but less than 1% infect humans. Most of these fungal infections are superficial, affecting hair, but some species are capable of causing life-threatening diseases. The most common of these include *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Candida albicans*. These fungi are typically innocuous and even constitute a part of the human microbiome, but if these pathogens disseminate throughout the body, they can cause fatal infections, which account for more than one million deaths worldwide each year. The development of diseases and their presentation depend on the relationship between host immune status and Aspergillus burden. During pregnancy, a decrease in the response of microbial elimination by neutrophils leads to an increase risk of susceptibility to infections.[1,2]

Invasive aspergillosis is an infrequent clinicopathological entity that is difficult to diagnose, since it requires tissue samples, where the pathogenic effect of the fungi may be seen, and thus an important number of cases are not found until autopsy.

Case Report: We report the clinical case of a 41-year-old obese woman affected by reactive depressive anxious syndrome, diabetes mellitus treated with insulin, who underwent previous Revision Uterine Cavity surgery (RCU) for spontaneous abortion. She was 39 weeks pregnant, underwent subarachnoid anesthesia for a duly completed caesarean section, and was discharged after three days. The same evening, she was taken to the emergency room for abdominal pain and intestinal diarrhea, and was subjected to laboratory and instrumental examinations.

Computed Tomography (CT) examination showed massive abdominal effusion. For this reason, she underwent exploratory laparotomy surgery that evidenced a picture of peritonitis. The patient was then transferred to the Anesthesia and Intensive Care Department with a diagnosis of coma and severe respiratory failure with septic shock (for which antibiotic therapy was started with piperacillin/tazobactam 1f 4.5g every 6hrs and ciprofloxacin 400mg every 12hrs; this therapy antibiotic was strengthened in the following days with infectofos 4gr x 4 and linezolid 600mg x 2). After 18 days, death occurred.

Multiple abscesses in both cerebral hemispheres, thyroid glands, myocardium, lung (pseudonodular and some with excavations in context), stomach, spleen, pancreas, kidney, and uterus were presented at autopsy.

Histological examination showed multiple necrotizing mycotic micro abscesses, consisting of septate hyphae and spores disseminated at the level of all organs; picture of early cerebritis; numerous necrotic-hemorrhagic petechial foci in the context of micro abscess areas in the cerebral cortex, vermis and cerebellar cortex; to lungs, congestion of the subserosa, massive hemorrhagic edema and diffuse alveolar damage, fungal, septic necrotizing vasculitis, and multiple abscess foci, centered by tangles of fungal hyphae attributable to aspergillus; multifocal septic thromboembolism. Visceral serosa pleurisy and micro abscess septic subserositis.

Conclusion. This case demonstrates that fungal diseases can be difficult to correctly diagnose or differentiate from other infectious agents; thus, often resulting in a lack of diagnosis in these patients before their death. For this reason, feedback to doctors and coroners would also be appropriate for autopsies performed for forensic purposes. The case also demonstrates the desirability of suspecting a fungal infection, even in patients not known to be immunosuppressed.

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Aspergillosis Disseminate; Puerpera; Infection Disease

F111   Almost to Lose Your Heads: A Case Series of Accidental Internal Decapitation

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Learning Overview: The goal of this presentation is to report a cause of accidental death that is little described in scientific literature. In fact, in the forensic field, complete and incomplete decapitations are known, much rarer are the deaths caused by a complete detachment of the head from the cervical column with spinal cord resection and complete preservation of the skin and soft tissues.

Impact Statement: This presentation impacts the forensic scientific community by highlighting that some violent indirect forces (e.g., occurring in traffic and air accidents) can lead to internal decapitation, even in the absence of suggestive external signs of traumatic injuries in the fracture site. This should encourage forensic doctors to carry out postmortem radiographic examinations, which are useful to ascertain this cause of death and to reduce the risk of misinterpretation.

Complete decapitation means detachment of the head from the trunk with separation of all vascular, muscular, nervous structures, full thickness fracture of the cervical column, and laceration of the soft structures of the neck. In case of incomplete decapitation, the cervical column is detached, but there is a variable conservation of tissue connections between the trunk and the head.[1] Both the complete and the incomplete decapitation (regardless of whether they are suicidal, homicidal, or accidental) occur by direct action of strong blunt force on the fracture site resulting in external injuries to the neck.[2,3] On the contrary, in the internal decapitation, only indirect forces act (mostly acceleration and deceleration forces of the head), resulting in a full thickness fracture of the cervical spine; this mechanism explains why in internal decapitation, soft tissues and skin can remain totally uninjured.[4]

In this presentation, we describe a case series of deaths from accidental trauma: two from car accidents and one from a plane crash. Regardless of the circumstances of death, in all three cases, the external examination did not reveal any external damage to the neck or spine. However, in all cases, the autopsy showed full thickness fractures of the cervical vertebrae (at various levels) resulting in complete internal detachment of the spine from the head. In our work, we will reconstruct the circumstances and dynamics of lethal trauma. In addition, any involvement of organs and soft tissues adjacent to the fracture, together with any other cranial-encephalic-rachidal injuries, will be revealed by macroscopical and histological examination.

Finally, in view of the impossibility of carrying out X-ray examinations before autopsies, the problems that such a gap may cause in the diagnosis of internal decapitation will be discussed, such as the risk of aggravating the fracture during the mobilization of the deceased during the autopsy.

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Accident; Forensic Science; Violent Deaths

F112 The Contribution of Forensic Sciences in the Study and Monitoring of Mycotoxin Levels for the Protection of Public Health

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Learning Overview: After attending this presentation, attendees will understand the role of forensic science in combating exposure to toxic levels of mycotoxins on foods or environments.

Impact Statement: This presentation will impact the forensic science community by demonstrating how this topic can benefit from the figure of the coroner in a multidisciplinary context aimed at contrasting crimes involving mycotoxins.

Introduction: Mycotoxins are toxic substances produced by some types of fungi such as *Fusarium*, *Asperigillus*, *Penicillium*. Some of the better-known mycotoxins include aflatoxins, ochratoxin A, fumonisins, trichothecenes, zearalenone, and patulin. These toxins can grow in a wide variety of environments, including foods, manures, soils, and other organic substrates, thus easily entering the food chain. Several families of mycotoxins have been identified, and each of them can have acute and chronic toxic effects on human and animal health such as gastrointestinal disorders, immunodeficiency, and cancer. For this reason, competent international bodies have drawn up well-defined maximum levels of mycotoxins in feed and food. To date, the scientific literature has shown a growing interest in the analysis of mycotoxins through the development of a large number of studies relating to the methods of extraction and analysis of mycotoxins with the creation of *in vitro* and *in vivo* models. However, in the face of a large number of papers concerning the direct toxicological analysis of these molecules, little is known with respect to the impact that this issue could have on the forensic scientific community. The purpose of this work is to evaluate what the forensic implications of the study of mycotoxins are today and the contribution that forensic sciences can offer in this area which is so important for the protection of public health.

Materials and Methods: A review of the scientific literature was carried out through the analysis of the PubMed® NCBI, SCOPUS®, and Google® Scholar search engines by inserting the keywords "forensic and mycotoxins." Subsequently, the works that did not concern the forensic analysis of mycotoxins or that did not show implications of medicolegal interest were removed.

Results and Discussion: The analysis of the literature shows that there are very few forensic or medical examiner works on this topic. However, we have outlined various aspects relating to the study of mycotoxins in which the contribution of forensic sciences can prove to be decisive. Some of these include: (1) autopsy and postmortem toxicological investigation of organs and biological fluids in cases of acute and/or chronic exposure with development of appropriate protocols; (2) toxicological analysis of contaminated feed or agricultural products; (3) identification of violations of food safety regulations; (4) forensic investigations into possible sources of contamination; and (5) exposure to toxic levels of mycotoxins in the workplace with assessment of the effects on health and related liability profiles. In forensic pathology, we point out that the literature has demonstrated the presence of very few works concerning the postmortem identification of mycotoxins from biological samples extracted from human cadavers. Among these, we cite a case of identification of satratoxin H in an intoxication with mushroom poisoning (*Podostroma cornu-damae*), the identification of aflatoxins from kidney samples in a series of minors affected by kwashiorkor, the identification of aflatoxin B1 in a case of hepatocellular carcinoma, and the identification of aflatoxins in cases of hepatitis in India.

Conclusions: We believe that forensic sciences can offer a decisive contribution in the control of mycotoxin exposures in a large number of diversified contexts through appropriate standardized control systems. Mycotoxicology could benefit from the contribution of the coroner to address the challenges associated with the spread of mycotoxins, including the fight against crimes involving mycotoxins, and to develop effective prevention and risk mitigation strategies to protect public health.

Toxicity; Toxins; Forensic Analysis

F113 Human Body Dissection and Donation for Research Purposes: An Overview of Current Legislation in European Countries

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Learning Overview: The goal of this presentation is to provide an overview of the regulations regarding the postmortem donation of the human body for research currently in force in European countries. In addition, the focus will be on the analysis of recent Italian legislation on the subject. Finally, bioethical issues inevitably related to the topic will be addressed, such as, among others, the formulation of informed consent and the limits (if any) to the use of human bodies for scientific purposes.

Impact Statement: This presentation will have a positive impact on the forensic science community by providing the first comparison between the regulations on human body dissection and donation in European countries. A similar comparison is so far absent in scientific literature, to the best of our knowledge. Moreover, any critical findings will be a starting point for an inspiring bioethical debate on the issue in the scientific community.

As is now widely recognized internationally, postmortem donation of the human body to research is a valuable tool for scientific progress. In fact, anatomical dissection is an indispensable tool for the training of students and surgeons, for the testing of new operating techniques, and for the improvement of those already known.[1-3] Although today technology and artificial intelligence are increasingly used for the design of anatomical simulators, dissection is an irreplaceable tool for the realistic experience it guarantees. In addition, anatomical dissection of human bodies offers the indisputable advantage of ensuring scientific progress even in university facilities that do not have economic funds to invest in simulators. Therefore, adequate regulation on the donation of bodies to science is also an urgent need to ensure a fair possibility of formation between all university structures.

Recently in Italy, a Law and an Implementing Decree concerning the donation of one's own body and postmortem tissue for the purposes of study, training, and scientific research have been issued.[4,5] These regulations, poorly disseminated in our country, have some operative and bioethical problems that severely limit the accession of donors, whose incidence is currently very low. For the donation of the body to science to be a resource for scientific progress, it is necessary that it be regulated by solid and appropriate regulations. Moreover, it is important that the rights of the donor are respected in the procedure, through an adequate compilation of informed consent, also tailored on the needs of subjects incapacitated. In addition, the regulations should clarify the roles of third parties in the donation process (such as family members and/or legal guardians), the modalities and times allowed for the use of the body for scientific purposes, and the possibility of donating the body only partially and with limitations.

The comparison and study of the regulations on the subject currently in force in Europe will be an interesting starting point to analyze and discuss the position of the various countries on the delicate operational and bioethical issues that are related.

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Forensic Science; Forensic Professionals; International

F114   The Concealment of Bodies by Charring: A Case Series and Multidisciplinary Approach

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Learning Overview: After attending this presentation, attendees will understand that corpse concealment by charring is a practice often applied by the murderer to make investigation more difficult, so proper methodology must be applied when faced with such events.

Impact Statement: This presentation will impact the forensic science community by highlighting a case series of concealment of bodies by charring and by showing the need for proper multidisciplinary approaches.

The concealment of the body by charring following a homicide is an event that can bring different challenges to the forensic examiner and pathologist. Especially in cases in which the corpse is retrieved in altered conditions, forensic pathologists often have to deal with skeletal remains and putrefied corpses, so there is the need to involve different professional figures within a multidisciplinary approach who can each contribute, adding a small piece to the resolution of the case. More complications arise in the face of burned bodies and corpses by the willingness on the part of the murderer to destroy the evidence of the crime. The interesting cases that have come to our observation are reported in which different homicidal methods are described, all followed by the intent to destroy the corpse by burning and charring to make medical-legal analysis impossible, according to the concept of "no body, no crime." At the same time, attention is drawn to the postmortem pyre following the murder by determining the general characteristics of a series of burnt corpses.

Based on the forensic investigation (crime scene analysis, circumstantial data, Postmortem Cardiac-Magnetic Resonance [PMTC], autopsy, histology, toxicology) and through the implementation of a multidisciplinary approach also involving an entomologist, odontologist, anthropologist, and geneticist, it was possible to identify the victims and reach the resolution of the judicial case and establish a possible reconstruction of the dynamics and methods, showing the cruelty and supporting the extreme violence with which the crimes were committed, demonstrating the malicious concealment through the absence of vital wounds on the dead body.

Conclusion: Our cases are in line with what is shown in the literature, where the method of combustion and carbonization, together with dismemberment, are the most widely described with bloody and heinous crimes in an attempt to hide and conceal the murder and to disperse of the traces.[1-3]

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Charred Bodies; Concealment; Multidisciplinary Approach

F115   A Multidisciplinary Forensic Approach in Two Complex Cold Cases and a Review of the Literature

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Learning Overview: After attending this presentation, attendees will understand how to evaluate the forensic approach in cold cases, particularly in the management of severely altered bodies due to decomposition, skeletonization, or dismemberment. Attendees will also learn about the role of combining all evidence to reconstruct events accurately and to reply to all the investigative questions.

Impact Statement: This presentation will have a significant impact on the forensic science community as it presents a systematic review on the multidisciplinary approach necessary to solve complex cases. By analyzing two cold cases, it emphasizes the importance of integrating various procedures and collaborating with different professionals in forensic investigations.

Introduction: Frequently, certain crimes are classified as "cold cases" when they remain unsolved. In situations where the corpse is severely altered (decomposed, skeletonized, charred, dismembered, etc.), obtaining crucial information about the characteristics and injuries indicative of the dynamics leading to death can be challenging.[1] In addition, questions may arise about the manner of death, the Postmortem Interval (PMI), and any attempts to conceal the crime. In such cases, a comprehensive investigation can only be achieved through a multidisciplinary approach starting from the judicial investigation at the place of discovery and extending to a thorough examination of the corpse, including investigations into the causes of death and PMI.

Materials and Methods: This presentation includes a literature review that highlights the significance of a multidisciplinary approach in reconstructing/solving forensic cases.[2] It emphasizes the importance of collaboration with various professionals, including experts in anthropology, biology, chemistry, radiology, engineering, computational science, entomology, odontology, genetics, veterinary medicine, geology, and more. This multidisciplinary approach is illustrated through two "cold cases" from the Institute of Forensic Medicine of Messina.

Results: The multidisciplinary approach in the first case enables the identification of the skeletal body belonging to a 27-year-old woman who had disappeared about a year earlier. It helped in distinguishing between a road accident and a homicide. In the second case, which involved the discovery of a partially skeletonized body and the dismembered remains of a child's body, the importance of a multidisciplinary approach was highlighted in identifying the bodies (the remains belonged to a 33-year-old woman and her 4-year-old little boy who disappeared 20 days earlier after being involved in a road accident) and evaluating the time, the cause, and manner of death. This comprehensive approach led to a correct reconstruction of the facts and the resolution of the case, distinguishing between suicide and homicide.

Conclusion: These cases demonstrate that a multidisciplinary approach is essential in complex forensic cases to ensure a complete and accurate scientific evaluation that supports the investigation. A proposed procedural flow scheme for the management of complex bodies summarizes the value of each investigation that can be conducted.

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Cold Cases; Forensic Pathology; Multidisciplinary Approach

F116   "I Can't Find the Remote": The Ability to Act After a Single Gunshot Wound to the Head

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Learning Overview: After attending this presentation, attendees will better understand the complexity of a case of gunshot wound to the head without immediate incapacitation. Our aim is to stress the importance of blood pattern analysis and to remind attendees of the steps of a meticulous crime scene investigation for a thorough reconstruction of the event's dynamics.

Impact Statement: This presentation will impact the forensic science community by showing an extremely unusual case in which blood pattern analysis and careful forensic examination was crucial for the understanding of the event, which was digitally reconstructed to evaluate the hypothesized dynamics.

Gunshot wounds to the head should be rapidly fatal, with more than 90% of penetrating cranioencephalic gunshot wounds immediately resulting in the death.[1] However, despite this high mortality rate, numerous publications have reported not only the survival of the subjects, but even the presence of residual activity after the gunshot.[2] In particular, during the interval between the wound and death, the person may be capable of performing certain voluntary actions, such as walking, talking, or even firing additional shots.[3] The forensic pathologist must be aware of the conditions supporting the hypothesis of the absence of immediate incapacitation following a gunshot wound to the head as this assessment is crucial for distinguishing between a homicide and a suicide.[4] In this scenario, the careful analysis of the crime scene, as well as the meticulous examination of the brain and other autopsy findings, are particularly important. They allow for a precise assessment of the overall data and, consequently, provide a correct understanding of the case.[1,5] The details of the investigation may suggest a self-inflicted gunshot wound, but further analysis is needed to confirm the circumstances surrounding the incident and exclude a homicidal dynamic.

The present case concerns an unusual suicide involving a rifle with which the man fired a shot directly to the head. Subsequently, the victim engaged in a relatively prolonged and methodical premortem activity. The body of a 79-year-old man was found in his bedroom, lying on the bed in a sitting position. The man lived with his family but had limited interaction with them. Both man's hands were stained with blood, and he was holding a television remote control in his right hand. A .22-caliber rifle was found on the right side of his body. Blood stains of various shapes were detected on the floor, suggesting the man had moved between the bed and the drawer after the gunshot. Further examination revealed that the man had a stellate entrance gunshot wound in the right temporal region, and cranial X-rays showed fragmented bullet pieces in the right anterior cranial fossa with a comminuted fracture of the right frontal bone. The bullet's trajectory was from right to left, without passing through the interhemispheric fissure, leaving the left cerebral hemisphere intact as well as the right occipital, parietal, and temporal lobes.

Based on evidence at the scene and bloodstain analysis, it was postulated that the man had survived for some time after the shooting, and the complex motor tasks were digitally reconstructed: he fired a single shot while placing the rifle on a cabinet flap, ejected the cartridge case, and then moved to the right side of the bed. He then sat down briefly before getting up to reach the left side of the bed, where he eventually laid down and attempted to wipe the blood away with a paper tissue. Finally, he picked up the remote control with his right hand and died.

The ability to act after a gunshot to the head presents significant investigative and judicial challenges. Post-shooting activity and motor tasks are unlikely and can be perplexing. The evaluation of such cases requires precise implementation of neuroanatomical and physio-pathological knowledge to understand the phenomena of functional ability and accurate reconstruction of the crime scene to exclude the presence of another person or aggressor.

Brain injuries don't always cause immediate incapacitation, making it crucial for forensic pathologists to accurately identify affected areas to determine if fundamental structures were damaged by the initial gunshot. In this case, blood pattern analysis was fundamental to recognize voluntary motor activity and reconstruct the antemortem movements of the man.

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Shotgun; Autopsy; Crime Scene Investigation

F117   The Forensic Point of View of the Serial Murders at the Holmes' Labyrinth Hote

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Learning Overview: After attending this presentation, attendees will have learned of the reconstruction of the historical case of the serial killer Holmes.

Impact Statement: This presentation will impact the forensic science community by demonstrating how this case is unique in the history of serial killers, considering the construction of the labyrinthine structure of the castle and the systematic methods of numerous serial murders and tortures that were carried out.

Introduction: According to some authors, the serial killer is a subject who performs three or more homicides through an emotional cooling phase in order to satisfy a sexual impulse. The psychologist Norris has identified various phases: (1) aura phase: the murderer imagines the homicide; (2) trolling phase: the killer tries to find a victim; (3) wooing phase: the killer gains the victim's trust; (4) capture phase: the chosen victim is captured; (5) murder phase: the homicide is performed; (6) totem phase: excitement gives way to a depressive state; and (7) depression phase: the killer suffers a strong emotional disappointment.

Case Report: We report the case of the serial killer Holmes. As a child, Holmes had strange perversions of death; in fact, he killed and dissected animals. With money from some insurance fraud in 1889, he built a huge building that served as his home, pharmacy, shop, and hotel. With a series of changes in the internal structure of the building, Holmes made the second and third floors a real labyrinth; about 100 rooms were in fact connected to each other thanks to secret passages, sliding walls, peepholes, armored doors, secret rooms, soundproofed rooms, stairs and corridors that ended up against a wall, and trapdoors on the floor that opened on command and slid the victim into the cellar. There was a huge swimming pool filled with corrosive acid where Holmes immersed the bodies. Between 1892 and 1894, a huge number of clients of all ages died in the "castle." His method was to rent a room to the victim, then, after trapping the victims, he had them asphyxiated in the room or gassed or charred them. The body was then eviscerated and stripped to resell the skeleton; dissolved completely in the pool with acid; or used by Holmes for dissections and experiments without scientific purposes. The frequency of the murders was high, and the income obtained from the sale of the skeletons was very profitable. Several weeks later, an accomplice burned down the building, furious at the loss of his lover, his sister, and his illegitimate daughter. After a series of insurance scams, Holmes was arrested in Boston on November 17, 1894, where he confessed to 27 murders. The police found about 15 skeletons inside the castle, but he was found guilty of only 9 murders and sentenced to death.

Discussion: The forensic literature demonstrates that in the context of serial killers, it is possible to define a series of common characteristics such as mental pathologies, lack of remorse, a history of childhood abuse, the presence of previous petty crimes, manipulation, construction of morbid fantasies with obsessions, and the fascination with power and control. According to some sources, Holmes had a difficult relationship with his father, a violent and alcoholic man. Before engaging in his serial killings, Holmes was involved in several scams. With respect to the characteristics outlined, we point out that the case of Holmes is unique in the history of serial killers for the materialization of the idea about the capture of the prey through the construction of the labyrinthine structure of the castle. Certainly, the case is characterized by a systematic method of killing, motivated, at least in part, by the economic motive as the killer often derived economic benefits of an insurance nature from the death and the sale of the skeletons. To date, the real number of victims is controversial since Holmes confessed to only 27, taking into account that he had in any case used methods of eliminating the corpses with acid and that the structure was set on fire.

Serial Killer; Forensic Analysis; Criminalistics

F118   Deaths Due to Suicide by Falling From Bridges: The Role of Architectural Barriers and Video Surveillance Systems

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Learning Overview: After attending this presentation, attendees will understand that bridges are infrastructures often used to carry out suicides.

Impact Statement: This presentation will impact the forensic science community by demonstrating, on the basis of the cases analyzed at the Institute of Forensic Medicine of the "Magna Graecia" University of Catanzaro in Italy, the importance of setting up architectural barriers and video surveillance systems near bridges as instruments of containment and dissuasion aimed at discouraging suicidal behaviors.

Introduction: Suicide is a major public health problem. Reducing the death rate from suicide is one of the target objectives of the United Nations 2030 agenda for sustainable development. Falling from great heights is one of the most frequently used methods of committing suicide. As regards high-rise places, bridges are infrastructures often used to commit suicide.

Case Series: A series of ten suicides that occurred by falling from a bridge higher than 60 meters was examined.

Results: In all the cases performed, the autopsy showed injuries compatible with falling from a great height. In particular, the external examination revealed multiple bruises related to the presence of shrubs in the falling area, with the absence of lesions from active and/or passive defense. In all cases, at autopsy a polytrauma with multipolar injuries, including head trauma, thoracic and vertebral lesions and hemoperitoneum were identified. In 40% of the cases, the suicide left a suicide note containing farewell messages, drawings, or wishes. In one case, an autopsy was even requested in the suicide note for suspected malpractice of the doctors who had performed a surgical operation indicated as the reason for the extreme act. In 30% of the cases, toxicological tests showed positivity for psychotropic drugs. In all cases, the testimonial findings revealed previous disorders such as depression, schizophrenia, and hallucinations with paranoid psychosis in the absence of suitable compliance with pharmacological or psychotherapeutic treatment.

Discussion: Statistics on suicides show that falling from height is the second cause of death regardless of age, in fact the 2030 United Nations Agenda has considered the reduction of the suicide rate among the objectives to be achieved, starting with the development of global health prevention strategies and socio-health. While the link between suicide and mental disorders (particularly depression and alcohol use disorders) is well established in high-income countries, many suicides occur in times of crisis due to an inability to cope with stress over issues such as finances, broken relationships, chronic pain, and illness. Additionally, experiencing conflict, disaster, violence, abuse, or loss and a sense of isolation are strongly associated with suicidal behavior. Falling from high places is one of the most frequently used methods of committing suicide. Among the elevated places, bridges are infrastructures often used by suicides to carry out their extreme gesture, some bridges more than others because they are considered symbolic.

With this work, we want to demonstrate the importance of making bridges and viaducts safe through the preparation of architectural barriers and video surveillance systems as dissuasive tools against anti-conservative acts. The argument that "If they don't do it from the bridge, people will find another way," in fact, seems to be denied by some research and authoritative opinions, which show that the suicidal impulse is in many cases momentary. The installation of suitable parapets, horizontal protection nets, presence detection devices with sound activation, and the upgrading of street lighting systems are some of the deterrent systems already used in various countries, the use of which should be implemented through collaboration between authorities' health care and policy makers.

Suicide; Forensic Analysis; Autopsy

F119   The Role of Patient Management in Nursing Homes in the Genesis of Death: Critical Issues in Rehabilitation Programs and Medicolegal Liability Profiles

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Learning Overview: After attending this presentation, attendees will understand the main causes of death and the comorbidities present in the elderly patient, especially within the social-health structures, and the necessary measures to adopt in order to avoid the development and progression of themselves.

Impact Statement: This presentation will impact the forensic science community by demonstrating how neglect of the elderly is one of the main causes of mortality in patients living in social and health care facilities and by proposing possible measures to adopt in order to prevent the development and progression of such conditions.

Introduction: Nursing homes are structures designed to provide health and social care services in favor of non-self-sufficient patients who are carriers of chronic-degenerative diseases. The welfare objectives are aimed at safeguarding clinical stability, with consequent limitation of hospital admissions.

Materials and Methods: In this work, at the Institute of Forensic Medicine of the "Magna Graecia" University of Catanzaro, ten cases of deaths of elderly guests in health facilities were analyzed, for which a motor rehabilitation project was foreseen, with complaint for alleged malpractice of the structure. The following were examined: health documentation with particular attention to the comorbidities present at the entrance to the structure, pharmacological therapies, and use of aids for non-self-sufficiency. A judicial autopsy with histopathological examination was then carried out in all cases.

Discussion: In the cases analyzed, we found a high incidence of "invisible" patients (i.e., untreated ones) with progressive development of immobilization syndrome, not present at the time of admission to the facility, complicated by the presence of advanced-grade pressure sores caused by the failure to mobilize and implement the foreseen rehabilitation projects, by the lack of aids for non-self-sufficiency, and, finally, by the lack of internal and external health assistance to the structure. Management shortcomings attributable to the structure and consequently to the medical director have been identified; the medical director remains the figure in charge of supervising and managing health personnel and patient outcomes. In order to prevent such events and therefore reduce the number of legal disputes concerning these structures, it would be advisable for the competent bodies to carry out a more careful analysis of the accreditation procedures as well as the periodic evaluation of the health services provided through strict monitoring of quality indicators of assistance (number of hospital admissions, number of deaths, turnover of guests, recovery objectives achieved). In this regard, it could prove useful to set up a figure in charge of evaluating the services provided as a "mystery guest" (i.e., trained third parties who, anonymously, can evaluate what is actually carried out in the context of rehabilitation projects for fragile patients).

Conclusions: In this work we have analyzed the health care provided to guests of health facilities mainly dedicated to the rehabilitation of debilitated patients. From our assessments, numerous critical issues have emerged attributable both to the clinical state of the guests, who appear to be carriers of numerous comorbidities, and attributable to the structures often not adequate to the standards of care, where a shortage of personnel and equipment has unfortunate results as regards outcomes in patients. Therefore, in the light of what has been described above, it is appropriate to establish specific evaluation criteria for the assistance provided as well as to strengthen the structures themselves, in order to improve the services provided and consequently result in a reduction in legal disputes in this area.

Elderly; Neglect; Forensic Analysis

F120   Intrafamilial Homicide: A Series of Cases From Southern Italy

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Learning Overview: The goal of this presentation is to increase the understanding of Intrafamilial Homicide (IFH) by analyzing incidences, motivations, relationships between family members, age and gender groups, weapons mostly used, types of injuries, causes of death, and psychiatric disorders.

Impact Statement: This presentation will impact the forensic science community by helping attendees better understand the incidence of IFH in Italy and its comparison with larger studies.

IFH, or domestic homicide, is considered when the perpetrator and decedent were both members of the same chosen or biological family.[1] It is a complex crime that has significant implications for the family and the community. This definition includes intimate partner homicide, feminicide, filicide, fratricide, and parricide, each one with unique challenges for investigators. Domestic violence, jealousy, money, mental health issues, and triviality may drive family members to commit this heinous crime. According to the 2022 report of the Italian Institute of Statistics (ISTAT), Italy shows the lowest homicide rate among the European countries (0.48 homicide per 100,000 inhabitants compared to the European median value of 0.89).[2] In 2021, only 303 homicides have been accounted in total, among which 139 IFH are reported (45.9%). In comparison with the Italian data, in United States only 13% IFH occurred in 2019. Most of the Italian victims are females (100 out of 139 IFH). In most cases, the motivations for the crime were trivial reasons (45.9%) followed by relational conflicts (11.6%), financial issues (6.9%), and others (24.1%). The main cause of death was related to sharp force injuries (39.3%), followed by gunshot wounds (30.0%), blunt force trauma (42.2%), and asphyxia (15.2%).

Materials and Methods: Nineteen cases of IFH occurring in Campania from 2012 to 2022 have been reviewed. Campania is the third-most populous Italian region located in the southern west coast with a population of 5,800,000 people. Data from police reports, autopsy findings, and medical records were analyzed. Age and gender of the victims and perpetrators, the intrafamilial relationship, history of drug/alcohol abuse and mental illness, motivations, and cause of death were considered.

Results: Sixteen offenders killed 19 victims in total. Most of the offenders were male (17/19, 89.5%). Female offenders were involved in only two cases (10.5%). The offenders were mostly partners or ex-partners (47%) with an age range of 17 to 72 years, followed by sons (21%), parents (16%), and other family members (16%). The victims were mostly female (12/19) from 7 to 69 years of age. In four cases, the victims showed a history of psychiatric disorders and drug/alcohol abuse in six cases. The most common motivations were money (42.1%) and relational conflicts (42.1%). In three cases, the perpetrator was affected by mental disorders. The most common cause of death was related to firearms (42.1%), followed by sharp force injuries (31.6%), blunt trauma (10.5%), and asphyxia (10.5%). In one case, the cause of death was due to fall from height. In 4 out of 19 homicides, the perpetrator was unable to stop his homicidal impulse by using excessive force or action that went further than necessary. In three cases, the number of stab wounds ranged from a minimum of 8 to a maximum of 100. In one case, the total number of gunshot injuries was six.

The study of IFH provides valuable insights into the incidence, the motivations, and the forensic aspects of these violent crimes. In our series, IFH by gunshot injuries were more frequent compared to the 2021 national data. This research may contribute to the development of effective law enforcement strategies and forensic procedures, ultimately contributing to public safety and justice in society.

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Homicide in Family; Parricide; Filicide

F121   To Kill or to Be Killed—A Differential Diagnosis in Sharp Force Injuries to the Neck: A Case Serie

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Learning Overview: After attending this presentation, attendees will be aware that Sharp Force Injuries (SFIs) of the neck are uncommon in the forensic practice; hence, the importance of a state-of-the-art background for the medical examiner. The main challenge in these cases could be the differential diagnosis between homicide and suicide. Forensic pathologists should be very accurate in dissecting neck's muscular layers to highlight which structures have been injured, both vascular and cartilaginous. In fact, there could be some differences in the interested structures in homicidal events compared with suicidal ones. Through a retrospective review of the SFIs observed in the Forensic Department of the Sapienza University of Rome and Foggia University, we collected 13 cases in which the differential diagnosis between suicide and homicide was achieved by performing an accurate autoptic technique in dissecting the neck muscles, so as to identify the injured vessel(s) causing the hemorrhagic shock.

Impact Statement: This presentation will impact the forensic science community by demonstrating the importance of details, especially when the dissection of anatomical layers is involved. Through a comparison from the available data in literature, the use of a standardized technique should be applied in the common forensic practice.

SFIs to the neck have been extensively studied in forensic sciences since trying to ascertain the means of death (suicide v. homicide, if not accidental) is not always straightforward.[1,2] Recently, a statistical approach was suggested, but its application is long from being incorporated in the common practice.[3] The issue is particularly relevant since the means of death are often difficult to state even when all the stages of the forensic investigation (crime scene investigation, autopsy, histological examination, circumstantial evidence, toxicological reports) are performed.[4,5]

We performed a retrospective, observational study, including 13 cases collected from two large Italian Universities (Sapienza University of Rome and Foggia University) where the cause of death was acute hemorrhagic shock by SFIs to the neck. In each case, crime scene investigation followed by autopsy and histological examination were performed, together with the study of both the toxicology report and the medical charts, when available, especially regarding psychiatric disorders. Eight out of the 13 cases were ultimately recognized as suicides, 4 as homicides, while 1 was filed as accidental.

The most common finding while distinguishing between suicide and homicide, beyond circumstantial evidence and crime scene investigation details, was the difference in the vascular lesions: internal jugular veins were the most common source of hemorrhage in the suicides, while the common carotid artery was injured in the homicide cases. Laterality of neck's region was confirmed as expected by literature (i.e., the left-handed tend to reach the right region of the neck, and vice-versa).

Some exceptions were the following. In suicides, since the victim is committed to end their own life and tends to produce more than one lesion on the same region, other anatomical structures, such as the trachea, were likely to be involved as well. In these cases, blood was found in the lung's bronchi, together with signs of asphyxia; this process probably produced a synergic effect with the hemorrhagic shock, resulting in the death of the subjects. On the other hand, in homicides, lesions were larger and more numerous when compared to those in the suicide group, reflecting the tendency of the aggressor to hit the victim multiple times in order to incapacitate the subject.

Microscopically, all the collected skin and vessels specimens showed the characteristic appearance of vitality, with interruption of the epidermal layer and extensive erythrocytes infiltration.

While crime scene investigation and circumstantial evidence is useful in the differential diagnosis, the exact identification of the vascular lesions by SFIs, especially in the neck, could be an aid in the forensic determination of the cause of death, especially when suicide-staging simulation is set by the assailant.

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Sharp Force; Vascular; Crime Scene Investigation

F122   The Most Dangerous Games: A Ten-Year Retrospective Review of Homicidal Deaths Associated With Dice and Domino Games in Shelby County, Tennessee

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Learning Overview: The goal of this presentation is to highlight the features of 20 incidents of dice and domino game-associated homicides, demonstrating cause of death trends, victim demographics, weapons involved, number of victims and fatalities, as well as associated drugs, etc.

Impact Statement: This presentation will impact the forensic science community by showing that, although deaths associated with games of chance, including dice and dominoes is low, the commonalities between cases can help identify at-risk populations.

Dice games and other games of chance have a history of leading to violence and death across the United States.[1-7] Shelby County, TN, is not exempt and though the incidence is rare, with only approximately two dozen cases reported in the past ten years, the victims of such crimes have similarities. The main purpose of this study is to present a retrospective review of dice and domino game-associated homicidal fatalities to identify trends in causes of death, victim demographics, weapons involved, number of victims and fatalities, as well as associated drugs, etc.

In this ten-year retrospective study, 20 incidents and 23 fatalities related to dice or domino games were identified in Shelby County, TN, from January 2013 to July 2023. Of the types of games identified, 17 were dice games and 3 were dominoes. The causes of death in all cases were related to gunshot wounds, to include single and multiple wounds. The majority of decedents were male (21/23, 91%) and Black (23/23, 100%). The age range of the victims was between 2-49 years, with the median age of 24 years. The majority of victims were active participants in the game (21/23, 91%). Many of the games involved multiple injured parties or fatalities (14/20, 70%), with most involving a single fatality (17/20, 85%). Most of the incidents involved handguns (12/20, 60%) with seven of the incidents involving multiple weapons (7/20, 35%). The victims were most often shot multiple times with the median gunshot wounds being three, with a range between 1–22 wounds. The most common locations of the injuries were the torso (34/105, 32%) and the head/neck (25/105, 24%). Review of the circumstances of each incident shows arguments directly related to the game were often the probable motivation for the shootings (13/20, 65%) and most often happened during the game (15/20, 75%). Toxicology analysis demonstrated metabolites of marijuana in 74% of cases (17/23) and alcohol in 26% of cases (6/23).

This study provides an initial look at dice/domino game associated fatalities in Shelby County, TN. Although deaths associated with such games may be relatively rare, roughly 1% in our jurisdiction, there are many commonalities between the cases, which may help identify at-risk populations. A limitation to the study is that the inciting event in many homicides is unknown. We aim to continue to document cases in the future and to consider incorporating deaths associated with other recreational games and sports in future analyses

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Dice; Homicide; Retrospective Study

F123   Artificial Intelligence (AI) -Based Automated Facial Recognition Systems and Contributions of Cone-Beam Computed Tomographs (CBCTs)

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Learning Overview: After attending this presentation, attendees will be sensitized to the importance of CBCT scans and other radiological modalities in development of AI-based models toward automatic forensic facial recognition systems.

Impact Statement: This presentation will impact the forensic science community by informing attendees that AI-based algorithms can crucially help in facial developing recognition systems for high security and surveillance areas.

Technological advances have revolutionized science for human welfare, be it physical, chemical or biological sciences. AI refers to the simulation of human intelligence in machines that are programmed to think and interpret like humans and mimic the human brain and its actions. It has positively impacted the data and medical sciences for the efficient analysis of larger datasets, contributing significantly to diagnosis, monitoring, and treatment of diseases.[1-4] AI is both science and engineering that covers various fields of processes like reasoning, natural language processing, planning, and machine learning. AI models are primarily developed and designed to overcome variabilities caused by human errors due to cognitive bias and are trained and then applied for problem-solving and decision-making based on logical mathematical reasoning.[5]

AI can prove to be a powerful tool in forensic facial research for developing automatic facial recognition systems. Various automated cephalometric software utilizing AI have been developed and claim to be reliable. Automated facial recognition has become an integral part of our day-to-day personal (access to ATM machines, mobile devices, bank lockers, high security zones) and national (entry to airports, nuclear reactors, music concerts, business malls) security requirements, including for many other purposes. Such systems have been developed by experts based on the parameters like AI and computer-generated advanced analogs and algorithms. The facial soft-tissue thickness, craniometrics, and facial profiles/analytics play an important role in developing built-in facial recognition technologies by computer professionals like the Federal Bureau of Investigation (FBI) facial recognition software.[6] Increased use of facial biometrics in the legal system has necessitated the need for examiners to perform facial identification using the automated facial recognition systems. The technology of facial biometrics can help the sister-biometric tools like iris patterning, retinal scans, fingerprints, footprints, lip prints, ear prints, etc.

The automated AI algorithms for detecting skull damage on CBCT scans and approximating face (based on soft-tissue thickness, musculature and fat layer) from the skull has raised forensic facial research to new heights.[7-10] The latest developments in the applications of AI-based algorithms generated using CBCT scans for forensic facial recognition systems will be discussed at this presentation.

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Artificial Intelligence; Facial Identification; 3D Technology

F124 Do Gender, Age, Body Mass Index, and Height Affect Eye Biometrics in North Indian Adults?

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Learning Overview: The goal of this presentation is to explain the average values of ocular biometry dimensions and investigate their inter-correlations and their association with gender, age, Body Mass Index (BMI), and height among Indian adults.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the eye biometric traits are vital for ophthalmology diagnostics and therapies, as well as for identifying individuals, estimating ages, and defining population-specific differences. These findings highlight the importance of considering these aspects when studying ocular features in various human societies, which improves our understanding of human variation and adaptation.

Each eye has its own set of biometrical features. As a result, these characteristics differ between individuals and populations. Variations in biometrical qualities can be related to factors such as height, weight, gender, heredity, and others. A diagnostic procedure that evaluates the anatomical aspects of the eye is known as eye biometry.[1] The assessment of the biometrical aspects of the eye is crucial in ophthalmology diagnostic and therapeutic procedures. The human eye has particular potential as a tool for human identification, and eye biometrics has emerged as a viable field within forensic anthropology.[2] Eye biometrics is the study of numerous eye traits such as iris patterns, retina features, and eye dimensions in order to determine an individual's identification. As improvements in ocular biometrics continue, it is critical to understand how characteristics such as gender, age, BMI, and height may influence its accuracy and dependability.[3]

The present narrative review is carried out to relate the eye ocular biometric features with gender, age, BMI, and height, This is the first sort of study that will aid in the extraction of ocular biometric features in the North Indian population. This not only aids in anthropology research, but also in the realm of ophthalmology.

For the current study, we searched databases such as Embase®, PubMed®, the Web of Sciences®, and Scopus® for relevant studies that addressed how the problems with gender, age, BMI, and height affect eye biometrics and related synonyms and combinations. Following the Helsinki Declaration guidelines, 60 patients were enrolled in this study. All participants with a history of ocular injury, ocular inflammation, or other eye disorders such as strabismus, amblyopia, significant myopia, or hyperopia were excluded from the study. The study of ocular anatomy and its changes among various human populations is an essential element of anthropology and eye data.

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Eye; Biometry; Gender

F125   Understanding the Crisis of the Persons Reported Missing in East Tennessee

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Learning Overview: After attending this presentation, attendees will have a more in-depth understanding of the prevalence of persons reported missing in East Tennessee, some factors that can affect it, and the proposed solution to alleviate it.

Impact Statement: This presentation will impact the forensic science community by incorporating a new approach in working with the missing person reporting system to redistribute governmental funding and resources to prevent the increased number of cold cases and unidentified human remains.

In the United States, Missing Person (MP) reports are filed at local and national venues. Local venues can consist of police stations and sheriff's offices. As for the national venues, a missing report can be reported to the Federal Bureau of Investigation's (FBI) National Crime Information Center (NCIC) or National Missing and Unidentified Persons System (NamUs).[1,2] NamUs provides government and public accounts where an individual can access data on cases of missing, unidentified, and unclaimed persons. Due to the lack of universal databases, there can be some inconsistencies between the different agencies' protocols in acquiring, documenting, and storing the information of an MP. These inconsistencies can lead to additional issues such as negative interactions between the affected families and the criminal justice system, delay in locating the person, or identification of unknown human remains. It is essential to understand that various social, political, and legal reasons can prevent an individual from being reported missing, and these reasons are interconnected on both the local and national levels. In the United States, two reported databases were recently created by forensic anthropologists to reflect their communities' needs.[3,4] But, it is necessary to initiate local databases to contextualize the magnitude of the MP crisis in each region.

Due to the lack of local databases in East Tennessee, the author focused on East Tennessee's MP cases reported in NamUs from August 2022 to April 2023. The data consists of public profiles of MP cases from January 2010 to April 2023, totaling 2,076 cases. The research questions explored the relationship between location, age, and sex of the MPs' cases in East Tennessee per population density at the county and city levels. Data were spatially analyzed using ArcGIS® to highlight current plans' efficacy in distributing local governmental funding in East Tennessee to alleviate the MP crisis.

The results showed a consistent association between the number of MP cases reported in NamUS and counties population density in highly dense areas such as Knox (0.227%) county. However, Hamilton County had lower reporting cases (0.071%), the second most highly populated county in East Tennessee. Two counties stood out, with low population density and higher reporting percentages compared to other counties, Claiborne and Johnson counties, with 0.094% and 0.148%, respectively. The age distribution of cases in the 255 cities was mainly between 19–64 (adults), with only 11 cities having a majority of missing children (1–18) cases and one city (Jacksboro-Campbell County) with a majority of elderly missing persons (65–74) cases. The numbers of female and male cases were comparable, 415 and 378, respectively, during the studied period. However, their distribution geographically showed distinct trends, with some cities and counties having a prevalence in males (Jefferson, Claiborne, and Rhea) and others with a prevalence in females (Bradley, Loudon, and Cocke). Larger counties with higher density had an almost equal distribution of MP reports for females and males.

These results provide a perspective on the factors involved in the reporting process of MP. The ratio between the number of reported cases in smaller communities highlights factors contributing to higher reporting, such as social ties between the community and the reporting agencies and family dynamics. In allocating more resources to struggling communities, the reporting system can be enhanced to reflect a realistic number of missing individuals.

This research is the first step toward a large project to understand social ties and interaction between the communities and the criminal justice system. It is part of a project investigating the shortcomings that lead to cold cases and the role of bureaucracy and funding in the MPs' crisis.

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Missing Persons; GIS; Interdisciplinary

F126 Fatal Entrapment Under a Lift-Up Storage Bed: A Rare Case of Accidental Death by Positional Asphyxia

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Learning Overview: Through a case description, this presentation aims to provide useful elements in the interpretation of the Death Scene Investigation (DSI) in the diagnosis of positional asphyxia in forensics. In addition, this presentation also aims to highlight dementia as an intrinsic risk factor for this type of death in older people, with a view to increasing their supervision strategies.

Impact Statement: This presentation will have a positive impact on the forensic science community by providing data obtainable from the DSI that could lead to the hypothesis of death by positional asphyxia, even in atypical cases such as the one presented.

Positional or postural asphyxia is a type of violent mechanical asphyxiation caused by an unusual position of the body (i.e., neck and/or chest) resulting in kinking of the airway and impaired aeration.[1,2] Accidental deaths from positional asphyxia are little described in scientific literature and are more frequent in infants due to their inability to move independently and to free themselves from the fatal position.[3] However, in adults, reduced independence, dictated by various causes such as dementia and intoxication with abuse substances, may be a risk factor for positional asphyxia.[4,5] During the DSI, positional asphyxia may be suspected when the deceased is found with the body in a forced position that may have concretely prevented breathing. In addition, the victim, for various reasons, could not have been able to free himself from the lethal position for at least a few minutes. The autoptic signs of positional asphyxia are not specific and compatible with other forms of violent mechanical asphyxia. Therefore, the scene of death investigation plays a key role in the diagnosis of positional asphyxia. The DSI is crucial to ascertain the position of the victim's body and the ability to wriggle from the restraint position, as well as to correctly interpret any external signs found on the deceased. In addition, it is critical to identify any risk factors for death from positional asphyxia. With this aim, it is essential to collect medical history, clinical data, and a psychological profile of the subject and to carry out toxicological examinations to ascertain the intake of drugs or alcohol.

We present an unusual case of death by positional asphyxia during a domestic accident. The victim was an 85-year-old woman suffering from dementia who was discovered trapped with her neck under a lift-up storage bed. The findings of the DSI, together with the clinical-anamnestic collection of the elderly victim and postmortem changes (i.e., hypostasis), have allowed the assumption of death by positional asphyxia. These data, in concert with the absence of assault injury as well as with autoptic findings compatible with asphyxiation, made possible a definitive diagnosis of accidental death by mechanical positional asphyxia.

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Asphyxiation; Death Investigation; Autopsy

F127 Ready Investigator One: The Use of Computer-Based Simulation Games for Medicolegal Investigation Training and Education

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Learning Overview: After attending this presentation, attendees will understand the utility of computer-based simulation games as a tool that can augment training and education for medicolegal investigators.

Impact Statement: This presentation will impact the forensic science community by introducing a technologically accessible and multipurpose method that can be utilized by multiple roles and agencies involved in medicolegal investigations.

Training and continuing education for medicolegal investigation requires not only written and verbal instruction but also interactive training within a scene environment. Often it is difficult to cover every scenario that an investigator may encounter in their career. By using computer-based simulations, a trainee could be exposed not only to the basics of a scene investigation but also to scenarios they may not routinely experience.

For this study, two computer-based simulations based on routine scene investigations were created using game engine software, 3D model assets, and 3D model-rendering software. The simulations were compiled into a single game package and were loaded onto portable and downloadable drives for distribution. Volunteer participants, which included investigators, autopsy technicians, and forensic pathologists, were asked to play the game and fill out a questionnaire of their findings and an evaluation of the game. In the first simulation, the participant as a first-person player would explore the environment of an apartment scene investigation, try to find six relevant objects related to scene investigations, and note relevant findings that would typically go in an investigative summary report. Drawers and doors in the apartment could be opened, and their contents examined. There were numerous selectable objects of varying relevance also in the scenario. The decedent could be externally examined and turned to assess lividity. In the second simulation, the participant would explore the same apartment with evidence of potential violence and note findings of forensic relevance, much like in a nutshell study. The participants then completed an evaluation of five Likert type and numerical scale questions related to ease of play, understanding of goals, effectiveness, and level of recommendation to other investigators as a training tool and as a tool for investigation education.

For the first simulation, the investigators all found two to four of the six relevant objects. They noted the positioning of the body, presence of blanching lividity and the information provided by the discoverable medical records, medications, and other relevant objects in the room. For the second simulation, the investigators noted the positioning of the body, location of wounds, location of blood or blood-like substances, the locations of weapons (a knife, handgun, and broken bottle). Participants also offered free comments regarding their experience with the game and suggestions for expanding its potential.

When evaluating the game, the participants favorably rated the ease of play (3.29 on a five-point scale), understanding of the game's goals (4.71/5), effectiveness as a teaching tool (4/5), level of recommendation to other investigators as a training tool (4.29/5), and level of recommendation to other investigators as a tool for investigation education (4.43/5).

In conclusion, medicolegal investigators of varying experience found computer-based simulations as an engaging and useful tool for training and education. With the widespread availability of personal computers and increasing familiarity of computer and console video games in current populations, computer-based simulation games can be a readily accessible, versatile, and reusable tool to supplement medicolegal investigation training and education.

Medicolegal Death Investigation; Training; Simulation

F128   Safety Considerations for Medicolegal Death Investigators

Taylor Lowry*, Denver Office of the Medical Examiner, Denver, CO

Learning Overview: The goal of this presentation is to examine considerations for the physical safety of medicolegal death investigators in the field, outside of the normally considered risks such as contagious diseases, bloodborne pathogens, and illicit substances. After attending this presentation, attendees will have ideas on new trainings and policies to consider implementing at their offices in order to better ensure the safety of their investigators.

Impact Statement: This presentation will impact the forensic science community and be beneficial for all those working in the medicolegal death investigation system, as it highlights risks that may not be considered when thinking of scene safety for death investigators. Following the theme of the conference, "Justice for All," can apply not only to victims but to those working in the field as they cannot do their jobs properly and effectively if they are unsafe or injured in the line of duty.

On May 14, 2023, an investigator for the Denver Office of the Medical Examiner was followed approximately 15 miles from a scene back to their office and shot at. The motivation for this incident is still currently unknown, as the aggressor has not yet been caught; however, the implications remain the same. Safety risks to medicolegal death investigators in the form of illicit substances, contagious diseases, and blood-borne pathogens is not a new concept. Unfortunately, the Denver Office of the Medical Examiner was forced to learn the hard way about this potential risk to investigators. All manners of death scenes, even natural deaths, can present with high emotions and extreme and fluid variability. Medicolegal death investigators work in the public eye, which can call for unwanted attention and volatile situations. While entirely dependent on the jurisdiction, many medicolegal death investigators are not sworn peace officers and may not have received associated training. Additionally, some jurisdictions have policies allowing their investigators to carry a firearm while performing their duties, while it is prohibited in others.

Denver is not the only jurisdiction where the safety of its investigators has been called into question. For example, investigators with the San Francisco Medical Examiner are proposing they receive firearms to carry in the field after one of their investigators was forced to disarm someone who pulled out a gun following a death notification.[1] Unique dangers are presented responding to scene. Training recommendations for investigators include topics such as situational awareness, "verbal judo," and self-defense tactics in order to be better equipped for any situations that may arise on scene that puts them in jeopardy. Investigators should be trained in proper radio communication with dispatch, should the need to use the radio arise. Offices should make safety tools, such as panic buttons, readily available to investigators in the office.

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Death Investigation; Scene Investigation; Medicolegal Death Investigation

F129   Medicolegal Death Investigation: Organizational Climate and Job-Related Stress

Julie A. Howe*, Saint Louis University, Saint Louis, MO; Steve Clark*, Occupational Research and Assessment, Inc., Big Rapids, MI; Amy M. Hawes, Tennessee Office of the Medical Examiner, Clinton, TN

Learning Overview: After attending this presentation, attendees will better understand several organizational factors that may ultimately affect the perception of job-related stress among medicolegal death investigations.

Impact Statement: This presentation will impact the forensic science community by making attendees aware of how organizational complexities such as jurisdictional type, reporting hierarchies, in-house versus subcontracted service offerings, etc. should be considered in personnel hiring and continuing education planning.

The Medicolegal Death Investigator (MDI) is an individual essential to all death investigation systems in the United States. The medicolegal organization, and its staff, provide the foundation for performing professional death investigation in Medical Examiner and Coroner (ME/C) offices charged with the responsibility of determining cause and manner of death in sudden and unexpected deaths. Their work affects criminal justice and public health policy decisions in both subtle and profound ways. Traditionally known as the eyes and ears of the forensic pathologist for on-scene death investigation, today's MDIs are functioning as part of a larger organization (state, regional, county, public, private, etc.) that may have unexpected stressors and interpersonal challenges not typically associated with on-scene investigation.

As with any organization, adherence to professional standards and consistency in performance are paramount to success. To ensure and maintain quality, medicolegal office accreditation and individual death investigator certification are considered as benchmarks. The two existing accrediting bodies for ME/C offices, National Association of Medical Examiners (NAME) and the International Association of Coroners and Medical Examiners (IACME), both specify ME/C facility specifications, procedural and staffing standards, including performance standards for forensic pathologists. The American Board of Medicolegal Death Investigators (ABMDI) assesses the skill and knowledge requirements for the MDI job globally at the basic level (Registry) after specific on-the-job requirements are met and advanced with skills assessment (Board Certification) available after additional job experience is attained. However, there are few post-employment performance standards for MDIs hoping to ascend a somewhat illusional career ladder, and fewer organizational supports to ensure MDIs are retained in a changing and dynamic work environment, where non-specific educational requirements abound and both leadership and personnel changes may be a part of the legislative cycle.

As the MDI organizational and operational requirements increase, job-related stress may be unavoidable, resulting in interpersonal conflict within the organization's staff members, the possibility of decreased on-the-job performance, and a high rate of MDI employee turnover. In addition, current challenges in many ME/C offices due to increased case referrals and autopsy caseload restrictions appear to be shifting many non-investigative tasks to MDIs, pathologist assistances, technologists, and others to compensate for the shortage and availability of board-certified forensic pathologists.

The purpose of this research is to assess levels of job-related stress perceived by medicolegal death investigators. Multiple dimensions of an organizational structure studied include: (1) how the organization itself is structured (e.g., public agency, private company, etc.), (2) how individuals within the organization interact with one another (e.g., chain of command, responsibilities, job requirements, etc.), and (3) how individuals in the organization perceive the climate within the organization (value, respect, support, expectations, etc.). The data used to describe these indicators have been collected through online survey to individuals certified by the ABMDI, affiliate members of the NAME, members of the IACME, and members of the Society of Medicolegal Death Investigators.

Medicolegal Death Investigation; Certification; Accreditation

F130   Death Investigation: Factors Associated With Scene Investigation and Autopsy Completion

Jennifer R. Snippen*, Lane Community College, Albany, OR

Learning Overview: This presentation utilizes data from cases recorded at two large United States medicolegal death investigation offices over a four-year period to explore the process of death investigation. Specifically, factors associated with scene investigation and autopsy completion were examined. Attendees of this presentation will better understand: (1) the variability in how death investigations are conducted, and (2) factors associated with scenes being investigated and autopsies completed.

Impact Statement: This presentation will impact the forensic science community by contributing empirical data to help address the paucity of scientific research in death investigation and provide a foundation from which to build a more consistent and rigorous national response to this critical public health and public safety function.

United States public health, criminal justice, and other social and scientific processes are affected by the quality of data obtained during medicolegal death investigation. Yet, little research has focused on how these investigations are conducted. Specifically, we have yet to adequately examine the interface between death investigators and the information used to make cause and manner of death determinations. Lacking this knowledge, we can neither measure the true magnitude of the variation between death investigation systems, assess reliability of death investigation outcomes, nor design effective interventions to improve death investigation practice.

A variety of sources may be useful during a death investigation, but two are central: the body and the scene. Examination of the body provides important information about the estimated time of death, medical history, recent physiological changes, evidence of drug use, and other potential influences on the individual's health that should be considered when determining cause of death. Examination of the scene(s) may provide key information about the decedent and the event, reveal contextual information that may support or disprove assumptions drawn from the examination of the body, and identify additional sources of information germane to the circumstances of death. Lacking adequate information from the scene and body, death investigation outcomes may be incomplete or inaccurate.

The overall goal of this research was to examine the process by which information was obtained from the scene and body during death investigation. The study utilized data from 12,386 cases recorded at two large United States medicolegal death investigation offices over a four-year period: 2018–2021. These data were analyzed to determine how often medicolegal death investigators directly examined scenes and how often autopsies were completed by forensic pathologists. Factors associated with these outcomes were identified

Findings indicated that 41% of the scenes were investigated and 31% of bodies were autopsied. Factors significantly associated with both scene examination and autopsy completion included decedent age, reason for reporting, and location of death. Deaths occurring outdoors were more likely to have scenes investigated and autopsies completed than those occurring in any other location. Deaths reported as Natural/Sudden/Unexpected deaths were less likely to receive scene investigation or autopsy than deaths reported as suspected homicides, accidents, or suicides. Decedent sex and the time-of-death reporting were significantly associated with scene investigation, but not autopsy completion.

The results of this study suggest the need for a closer look at factors that influence the decisions to directly examine scenes or complete autopsies. Leaders within the fields of public health, public policy, and criminal justice would benefit from understanding precisely what the death investigation process entails, as well as potential biases that may influence how deaths are investigated. Other entities that utilize aggregate death investigation data would benefit by better understanding the sources of data provided to them and factors that may impact data collection and interpretation in medicolegal death investigation.

Medicolegal Death Investigation; Scene Investigation; Autopsy

F131   Medicolegal Death Investigation Data Modernization

Margaret Warner*, Centers for Disease Control, Takoma Park, MD

Learning Overview: After attending this presentation, attendees will understand the progress of a Medicolegal Death Investigation (MDI) data modernization initiative that includes identifying information commonly collected during an investigation, understanding how data are stored in MDI offices, learning how offices currently utilize data, and envisioning future practices, including borrowing concepts of standards-based approaches used in other fields such as health care and transportation.

Impact Statement: This presentation will impact the forensic community by serving as a resource to understanding the steps taken thus far to make MDI data more accessible to MDI offices for tracking deaths within a jurisdiction, managing office caseload, and measuring quality assurance and forensic science research as well by answering requests from others who utilize MDI data for preventing future deaths.

Modernizing the way information collected during a medicolegal death investigation is stored and shared is necessary in medical examiners and coroners' offices in order to efficiently track deaths within a jurisdiction, office caseload, respond to data requests, and measure quality assurance.[1] Currently, inconsistent practices in the types of information stored and ways of storing and utilizing the information has led to inefficiencies. In addition, many outside agencies are requesting information from offices as MDI data plays a pivotal role in understanding mortality patterns and informing public health and safety strategies. This presentation will explore aspects of a data modernization initiative, including identifying data commonly collected and stored within death investigation, describing the landscape of computerized case management systems, describing data standards utilized for interoperability between data systems, as well as the MDI community activities related to the initiative.

There is certain key information collected during every information death investigation that have been identified as Common Data Elements (CDEs) by a workgroup made up of medical examiners, coroners, death investigators, forensic scientist, public health and safety, and other stakeholders.[2] These CDEs form a foundation for data collected and encompass demographic, geographic, clinical, and cause of death information. These CDEs are essential for each office and can be mapped between and across various jurisdictions and will ensure consistent information reporting. Using modern methods, these data collected during medicolegal death investigations can be harmonized between offices in order to create consistency between offices. Data collected in offices are either stored in paper files or are stored in case management systems that have certain features in common, which helps to make data more accessible.[3]

Data standards are fundamental to the creating of a more modern approach as they are key to consistency and seamless data exchange. Focusing on the CDEs to create data standards will help ensure that efficiencies and ideas between offices can be shared. The Medicolegal Death Investigation Fast Healthcare Interoperability Resources (FHIR) Implementation Guide is a data standard for bridging the gap between information management systems and medicolegal death investigation.[4] FHIR facilitates seamless data exchange and integration, enabling real-time access to critical information such as autopsy reports, toxicology results, death investigation information, and death certification. Through FHIR, the sharing of standardized data becomes more efficient, enhancing collaboration among forensic experts, law enforcement, and public health professionals.

Currently at least seven MDI offices within five states are exchanging data with their electronic death registration systems using the FHIR standard. This standards-based approach to data can facilitate inter-operability with other types of information management systems. It can also assist with forensic science research and facilitate quality assurance measures. To further work in this area, an MDI FHIR Implementation Collaborative initiative was established with offices of medical examiners and coroners to help design, build, and test standards based inter-operable solutions[5]. This collaborative approach will promote innovation within the community and help make the concepts scalable.

In conclusion, data modernization through standards-based approaches will pave the way to data standardization, inter-operability, and quality. By harnessing technology and engaging the broader implementation community, medical examiners and coroners can unlock new possibilities in understanding mortality patterns, informing office management, promote forensic science research as well as share data to help save lives.

References:
1. United States Department of Justice Office of Justice Programs (DOJ) National Institutes of Justice. (2019). *Report to Congress. Needs Assessment of Forensic Laboratories and Medical Examiner/Coroner Offices*.
2. Ropero-Miller, Jeri, Nichole Bynum, Kelly Keyes, Erica Fornaro, and Micaela Ascolese. *Data Exchange Practices of Medicolegal Death Investigation*. U.S. Department of Justice, National Institute of Justice, Office of Investigative and Forensic Sciences, December 2022.
3. Vernon, E., Sorrell, C., Shute, R., & Ropero-Miller, J. D. (2022). *A landscape study of electronic case management systems (CMS) for medical examiners and coroners*. Forensic Technology Center of Excellence, U.S. Department of Justice, Office of Justice Programs, National Institute of Justice, Office of Investigative and Forensic Sciences
4. Health Level 7 Fast Health Interoperability Resources Medicolegal Death Investigation (FHIR MDI)(2023), Version 1.0.0: Standard for Trial Use. See HL7.FHIR.US.MDI\Home Page - FHIR v4.0.1.
   Wiser G, (2023) CDC Foundation Announces Awardees For Collaborative Aimed At Improving Data. *CDC Foundation Blog*. See https://www.cdcfoundation.org/blog/cdc-foundation-announces-awardees-collaborative-aimed-improving-data-health-systems-medical.

Data; Retention; Automation

F132   Medicolegal Death Investigations: Interactive Mapping of Key Data and Organizations

Jeri D. Ropero-Miller, RTI International, Creedmoor, NC; Nelson A. Santos, RTI International, Research Triangle Park, NC; Jennifer R. Snippen*, Lane Community College, Albany, OR*

Learning Overview: This presentation will provide attendees with a detailed understanding of the relationships and flow of data between data producers and data users in medicolegal death investigations.

Impact Statement: This presentation will impact the Medicolegal Death Investigation (MDI) community by providing an interactive graphical process map to communicate the complexity of MDI investigations and the complex data exchange processes involving medical examiner and coroner offices, ultimately impacting research, legislature, and other policy or science-driven efforts and organizations. The information in this process map can guide process improvements, data standardization, and the implementation of data modernization systems.

MDIs are complex and multidisciplinary processes that involve the collaboration of various professionals, including medical examiners, coroners, law enforcement, first responders, forensic pathologists, toxicologists, and other experts. Data collected during an investigation influences evidence recognition, hypotheses generation, diagnostic differentials, and identification of leads. Data can come from various sources, including physical evidence, witness statements, documents, surveillance footage, and digital records. These data are authorized, shared, and exchanged by numerous data producers and data users. Data sharing allows professionals in the investigation to collaborate, analyze, and make informed decisions.

The interactive process map was designed and created by the National Institute of Justice—in partnership with its Forensic Technology Center of Excellence at RTI International and the National Center for Health Statistics, Centers for Disease Control and Prevention MDI Working Group (MDI-Data-WG). The MDI-Data-WG of 30+ practitioners, researchers, and representatives from governmental agencies associated with the MDI community used iterative rounds of development and revision to refine and arrive at a consensus product.

Understanding the flow of data in an MDI investigation and the complex relationships between the entities helps to better identify and characterize areas for improvement. By using this interactive process map, users can explore specific details and access additional information and resources. This format reveals the relational and multitude of processes and entities reliant on data generated during a death investigation and informs much-needed process improvement and data standardization at the local, state, national, and international levels.

The presentation will provide an overview of methodology used to determine data sources, data users and data producers and entity relationships. The presentation will also discuss the four primary sources of information, the exchange of data derived from the sources, and the flow of data through the MDI system. A demonstration of the interactive graphical process map will be provided. This presentation will help to validate how presenting the data flow and entity relationships in an interactive graphical process map provides a high-level global understanding of the magnitude, complexities, and diversity of data needs and requirements.

Death Investigation; Data; Process Mapping

F133   Infrastructure-Related Insights About the Medicolegal Death Investigation Community From the Bureau of Justice Statistics' (BJS) Census of Medical Examiner and Coroner Offices (CMEC)

Kelly Keyes*, RTI, International, Yorba Linda, CA; Kathryn E. Greernwell, RTI International, Durham, NC; Hope M. Smiley-McDonald, RTI International, Research Triangle Park, NC; Jeri D. Ropero-Miller, RTI International, Creedmoor, NC; Connor Brooks, Department of Justice, Bureau of Justice Statistics, Washington, DC; Heather G. Meier, RTI International, Holly Springs, NC

Learning Overview: After attending this presentation, attendees will understand the data available from the 2018 CMEC.

Impact Statement: This presentation will impact the forensic science community by providing updated information on infrastructure, resources, and practices of Medical Examiner and Coroner offices (MECs) across the United States who participated in the survey.

In the United States, MECs fill critical public health and public safety roles. These professionals are primarily charged with determining cause and manner of death, often serving as first responders to crime scenes and mass fatalities and are on the front lines of identifying trends in public health crises through medicolegal death investigations. Despite their instrumental role, they are organized in disparate systems with varying makeups of governing functions, staffing, caseload, budget, and access to resources.

The 2018 CMEC was conducted by RTI on behalf of BJS (2017-MU-CX-K052) from June 2019 through March 2020.[1] The 2018 CMEC questionnaire was developed based on review and update of the 2004 CMEC, expert panel review, and pilot testing. Mixed mode collection of data was conducted by mail, online, and email. A total of 1,648 MECs submitted the 2018 CMEC surveys, for an overall response rate of 80.9%. RTI conducted an analysis of the 2018 CMEC data using the publicly available file. The 2018 CMEC data provided by the responding offices was examined in terms of budgets, staffing levels (including any full-time employees, autopsy pathologists, and death investigators), caseloads, responsibilities, and percentage of reported cases accepted and autopsied.

As preparations are underway for the 2023 survey (to be conducted in 2024), the data are again being reviewed to see how improvements can be made in the survey instrument and how the collected information can best be used by the community. This presentation will highlight how key metrics of agency type, population, budget, staffing, and caseload, including performance of autopsies, are interrelated and will examine regional variations. For example, the data show that only 26% of MECs reported they employed autopsy pathologists, with 20% of MECs reporting that forensic pathologists determine what cases will be sent for autopsy and 4% of offices report that forensic pathologists perform death scene investigations, with the remainder of offices reporting others as performing these duties. Data also show that the number of cases reported to offices varies from 1 (in both the South and Midwest census regions) to over 30,000 (in the Northeast) with a median of 138 reported cases.

This presentation will also include early information about the 2023 CMEC so that offices can be aware of key milestones and expectations of their offices as that process roles out in 2024.

Reference:
1.   Brooks C. Medical Examiner and Coroner Offices, 2018.

Medical Examiner; Coroner; Medicolegal Death Investigation

F134 Standards Development Activities in Medicolegal Death Investigation, Including Disaster Victim Identification

Kelly Keyes*, RTI International, Yorba Linda, CA; Michelle Rippy, California State University, East Bay, Hayward, CA

Learning Overview: After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of medicolegal death investigation, including disaster victim identification.

Impact Statement: The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to medicolegal death investigation, including disaster victim identification. This presentation will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development in medicolegal death investigation will be discussed. These include: (1) recent standards that have been added to the OSAC Registry (ANSI/ASB Standard 125, *Organizational and Foundational Standard for Medicolegal Death Investigation, 2021, 1st Ed.*; ANSI/ASB Best Practice Recommendation 108, *Forensic Odontology in Disaster Victim Identification: Best Practice Recommendations for the Medicolegal Authority, 2021, 1st Ed.); and (2) o*ther OSAC proposed standards (OSAC 2023-N-0004, *Standard for Interactions Between Medical Examiner, Coroner and All Other Medicolegal Death Investigation Agencies and Organ and Tissue Procurement Organizations and Eye Banks; 2023-N-0022-BPR for Communicating with Next of Kin during MDI-NON-STR-Registry Approval for OSAC Proposed Standard;* OSAC 2022-N-0027, *Medicolegal Death Investigation Response to Death Locations and Incident Scenes: Best Practice Recommendations;* OSAC 2022-N-0020, *Standard for Mass Fatality Incident Management)*; (3) published standards from the Academy Standards Board (ASB) that have yet to go through the Registry approval process, or standards published by other SDOs (HL7 FIHR Medicolegal Death Investigation) (4) documents currently in development at the OSAC or by the ASB (Body Inspection of a Decedent, In Custody Death Investigation; Daily Operation Resource List; Commonly Exchanged Data in Medicolegal Death Investigation; Best Practice Recommendations for Data Management in a Mass Fatality Incident; Ethics in a Mass Fatality Incident; Best Practice Recommendations for Pandemics); and (5) priorities for new documents or work products (Family Engagement Following a Mass fatality Incident; Victim Information Center Best Practice Recommendations for Medicolegal Authority; Mass Fatality Management During a Pandemic; Best Practice Recommendations for Managing Disaster Victim Identification with Chemical, Biological, Radiological, Nuclear, and Explosive Trauma; Ethical Considerations in Disaster Victim Identification; Next of Kin Notification; Daily Operations Identification and other highlights, including reference documents).

Opportunities for supplemental training related to discipline-specific standards will be presented as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

Medicolegal Death Investigation; Coroner; Medical Examiner

F135 **Fostering the Role of Forensic Epidemiologists in Medical Examiner and Coroner Offices: A Collaborative Partnership for Enhanced Public Health Response**

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Learning Overview: After attending this presentation, attendees will understand how medical examiners and coroners can utilize the wide-ranging skill sets of forensic epidemiologists to benefit their offices and the communities they serve.

Impact Statement: This presentation will impact the forensic science community by illustrating the link between public health and medicolegal death investigation while providing an overview of abilities and strengths that forensic epidemiologists bring to medical examiner and coroner offices.

Medical Examiners, Coroners (ME/C), and medicolegal death investigators play a vital role in public health. However, the linkage between ME/C offices and public health partners is often missing, resulting in untimely reporting, inaccurate information, and missed opportunities to prevent premature death. Forensic epidemiologists can close this gap by making ME/C data accessible to public health partners in a timely, accurate manner. This essential linkage also highlights the foundational roles of ME/Cs in public health infrastructure.

This presentation will feature epidemiologists working in a variety of jurisdictions, commonly referred to as forensic epidemiologists. These public health professionals will share their experiences and highlight the benefits of employing a forensic epidemiologist in ME/C offices. Given that ME/C offices are often understaffed and overworked, the addition of a forensic epidemiologist can expand the reach and responsiveness of ME/Cs by allowing them to share Medicolegal Death Investigation (MDI) data with relevant stakeholders, such as local health departments and fatality review boards.

Forensic epidemiologists can take on a multitude of new and existing responsibilities within ME/C offices, including periodic surveillance reports and the generation of annual reports to aid in accreditation efforts. Additionally, they can create interactive public-facing dashboards, allowing for real-time responses to data queries. Depending on the size and mandate of the jurisdiction(s), forensic epidemiologists may serve as liaisons for mortality reviews, respond to data inquiries, conduct epidemiologic analyses to identify risk factors and populations at risk, collaborate in all aspects of research, and train the next generation of forensic epidemiologists through mentoring and field experience for students. Forensic epidemiologists may engage directly with the next-of-kin and others to close gaps in knowledge about the circumstances leading up to an individual's death. By leveraging their expertise, forensic epidemiologists can help ME/C offices make the most of the data they collect and play a crucial role in improving public health outcomes.

To illustrate, as the opioid epidemic worsens, forensic epidemiologists are critical to the surveillance of opioid-involved deaths. Forensic epidemiologists relay MDI information, including substances involved and populations impacted, to public health partners. Forensic epidemiologists can also identify grant opportunities and successfully secure funding for additional personnel, expanded toxicology testing, and establishing enhanced overdose mortality surveillance. Forensic epidemiology positions within ME/C offices are increasingly recognized as being crucial to strengthening the role of, and contributions to, ME/C offices in public health.

Forensic Epidemiologist; Surveillance; Public Health

F136   Modern Morgue Design: How Current Architecture and Technology Help Mortuary Personnel Do Their Job

Denise C. Murmann*, DDS, Naperville, IL

Learning Overview: This presentation will reveal the new Will County, IL, morgue to highlight the features in the design made to be more efficient as well as to be supportive of the people who work there. After attending this presentation, attendees will have ideas for technology, workflow, and architecture design that can be utilized to serve the decedents, while assisting the living that serve them.

Impact Statement: This presentation will impact the forensic community by inspiring attendees to know not only what is possible, but what has been done in other morgues. This presentation will help attendees know what high-tech solutions to operational problems are now available. In addition, in a field that has a strain on the emotions of the workers, there are helpful elements to create a sense of calm and well-being.

Will County, IL, was formed in 1836. In the 1800s, autopsies were performed at local funeral homes. In 1895, Silver Cross Hospital, Joliet, IL, was opened. After that, county autopsies were performed in the basement of the hospital. When Silver Cross decided to build a new hospital in New Lenox, IL, the morgue was moved to a temporary morgue in a county building complex in Crest Hill. The area was small, so the administrative staff worked in Joliet in office buildings. For 22 years, the staff worked out of the temporary morgue. What was not ideal in the beginning became worn and more cramped over time.

The COVID-19 pandemic created a terrible strain on the Will County coroner's office. However, the pandemic did make federal, state, and county funding available to design and build a new, contemporary building to meet the needs of the county for day-to-day issues, as well as another pandemic or mass fatality incident.

The greatest need was more room, not only for the autopsy suites and refrigeration, but office space to bring the administrative staff into the same building as the morgue. The architects at Wight and Company planned for a circulation corridor between the front half of the building housing office staff and the back half of the building containing the morgue. This ensures not just pleasant air quality, but safety throughout.

To assist the employees and visitors, the concept of biophilia was applied to the design. Biophilia design recognizes that humans love and connect with nature and because of that using natural daylight, colors, and textures that are found in nature helps to result in creating a sense of calm and well-being in people. The blueprints were entered into a computer program and a 3D digital model of the building was built. Then another computer program was used to measure how much daylight would enter the building over the course of a year. Two things were evaluated: the glare and the percentage of time the office was exposed to light, aiming for 50% of the time.

Thoughtful design, high-tech equipment, and an efficient layout has made the new Will County Morgue an ideal place to take care of the employees that daily take care of others.

Morgue; Design; Equipment

F137   Forensic Laboratory Needs Technology Working Group: The History and an Opportunity

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Learning Overview: The Forensic Laboratory Needs Technical Working Group (FLN-TWG) is a communication channel between forensic science service providers, researchers, the National Institute of Justice, and other federal partners. This working group is maintained to ensure technologies and best practices keep pace with the increasing demand for forensic laboratory services. During this presentation, attendees will learn the history of the FLN-TWG, receive an overview of work products produced by the working group, and be informed on how to participate in the group.

Impact Statement: The demand for forensic science services is increasing throughout the United States, impacting state, local, and federal forensic laboratories; these entities need to exchange information to keep pace. The goal of the FLN-TWG is to provide objective ideas, independent knowledge, and alternative perspectives to ensure that research and implementation of forensic technology meets laboratories' operational needs nationwide.

The National Academy of Sciences (NAS) 2009 Report, *Strengthening Forensic Science in the United States: A Path Forward*, called for strengthening forensic science through validated and reliable science.[1] In 2014, the National Commission of Forensic Science (NCFS) was formed to address the recommendations laid out in the NAS Report. NCFS served as a Federal Advisory Committee to the United States Department of Justice (DOJ) in partnership with the National Institute of Standards and Technology (NIST). It represented a broad range of individuals with an interest in forensic science, including Forensic Science Service Providers (FSSPs), prosecutors, defense counsel, victim advocates, judges, law enforcement, academics, and members of the broader scientific community. NCFS provided recommendations and advice to the Attorney General of the United States and gave opinions for forensic policy consideration at the federal level. NCFS had several successes, including positively influencing changes to discovery practices and adopting a new code of professional conduct for those working in DOJ laboratories, advocating for postdoctoral fellowships to foster collaboration between emerging forensic science researchers and FSSPs, and supporting grant funding to promulgate quality assurance programs for forensic laboratories. In 2017, the NCFS charter was not renewed, ending its activities.

In 2018, the FLN-TWG was formed to continue selected efforts of the NCFS, with respect to technology adoption, operational challenges, and barriers to success. The FLN-TWG was created pursuant to 6 U.S.C. § 162(b)(2) to assess the forensic technology needs of federal, state, local, and tribal law enforcement agencies. The statute authorizes the National Institute of Justice (NIJ) to create working groups, like the FLN-TWG, to assess, advise, and provide relevant information regarding the law enforcement technology needs of federal, state, local, and tribal law enforcement agencies, as assigned by the Attorney General, to accomplish NIJ's mission. The FLN-TWG is housed at the NIJ and is supported by NIJ's Forensic Technology Center of Excellence. To date, the group has generated several work products, including five implementation strategies for new technologies, a guide for improving technology transition for forensic science, and a review of technical needs for implementing standards in forensic laboratories.

In the next chapter of the FLN-TWG, NIJ is looking to refocus the working group on exchanging information with federal agencies and organizations such as the DOJ Forensic Science Working Group and the Council of Federal Forensic Laboratory Directors. NIJ intends the working group to remains focused on: (1) improving coordination of technology transition or standardization; (2) identifying localized or system-wide operational gaps or impediments to adoption, not including resource limitations; and (3) increasing the capacity and quality of forensic science services within an ever-increasing demand for services nationwide.

NIJ is putting out a call for new members to join the working group. How to apply and the preferred qualifications of members will be discussed during this presentation as well as a deeper dive into the FLN-TWG history and planned future.

Reference:
1.   National Academy of Sciences (2009) *Strengthening Forensic Sciences in the United States: A Path Forward*. ISBN: 0-309-13131-6. https://www.ojp.gov/pdffiles1/nij/grants/228091.pdf. Access July 3, 2023.

Forensic Science; Laboratory; Technology

F138   **Crime Laboratory Quality Assurance Insights From the 2020 Census of Publicly Funded Forensic Crime Laboratories**

Jeri D. Ropero-Miller*, RTI International, Creedmoor, NC; Hope M. Smiley-McDonald, RTI International, Research Triangle Park, NC; Erin P. Forry, RTI International, Research Triangle Park, NC; Connor Brooks, Department of Justice, Bureau of Justice Statistics, Washington, DC

Learning Overview: This presentation will provide attendees with an overview of the quality assurance policies and practices followed by United States crime laboratories based on data from the Bureau of Justice Statistics' (BJS') 2019/2020 Census of Publicly Funded Forensic Crime Laboratories (CPFFCL).

Impact Statement: This presentation will impact the forensic science community by providing updated information about the quality assurance policies and practices of the nation's crime laboratories.

The CPFFCL is part of the BJS' forensic portfolio that provides the only comprehensive information about the infrastructure of the forensic services provided by federal, state, and local crime laboratories across the United States. Since 2002, BJS has administered the CPFFCL five times (2002, 2005, 2009, 2014, and 2020). There are four primary goals for the 2020 CPFFCL, including: (1) the administrative characteristics of crime laboratories (e.g., staffing, training, population served, resources, and caseload); (2) policies related to quality assurance, data, records, and evidence retention; (3) levels and methods of interaction with law enforcement agencies covering shared jurisdictions; and (4) changes in demands on crime laboratories and other emerging trends in the field.

The 2020 CPFFCL provides an update on the 330 United States publicly funded forensic crime laboratories and multi-laboratory systems. The 2020 CPFFCL was conducted by RTI International on behalf of BJS (2018-85-CX-K037) from July 2021 through February 2022. The 2020 CPFFCL questionnaire was developed based on a review and update of the 2014 instrument, an expert panel, and pilot testing. Mixed mode collection of data was conducted by mail, online, and email. Data from the 2020 collection will be compared to the 2014 CPFFCL as it relates to quality assurance metrics, including proficiency testing, competency testing, laboratory accreditation, and certifications. In total, the 2020 CPFFCL included 12 questions pertaining to quality assurance measures used in forensic laboratories. From 2009 to 2014, there was a 5% increase in accreditation (88% vs. 83%), while blind proficiency testing across crime laboratories remained unchanged at 10%.[1,2] The percent of crime labs that employed one or more analysts with external certification increased about 20% between 2009 and 2014 (60% vs 72%).[1,2] This presentation will build on these results and present trends through 2020 and on the findings of the 12 questions pertaining to quality assurance measures used in forensic laboratories that were included in the 2020 CPFFCL.

This presentation will discuss the information gathered by the 2020 CPFFCL to provide an update of the current state of laboratory quality assurance policies. These findings will enhance understanding of how the nation's publicly funded forensic crime laboratories are providing quality assurance to the jurisdictions they serve as they seek to promote justice while maintaining scientific integrity.

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Quality Assurance; Proficiency Testing; Accreditation

F139   Building Laboratory Infrastructure—Recent Findings From the Bureau of Justice Statistics' 2020 Census of Publicly Funded Forensic Crime Laboratories

Connor Brooks*, Department of Justice, Bureau of Justice Statistics, Washington, DC; Hope M. Smiley-McDonald, RTI International, Research Triangle Park, NC; Jeri D. Ropero-Miller, RTI International, Creedmoor, NC; Kathryn E. Greernwell, RTI International, Durham, NC

Learning Overview: This presentation will provide attendees with an overview of the state of publicly funded forensic crime laboratories in the United States, including staffing, workload, budget, and available services, based on data from the Bureau of Justice Statistics' (BJS') 2019/2020 Census of Publicly Funded Forensic Crime Laboratories (CPFFCL).

Impact Statement: This presentation will impact the forensic science community by providing updated information about the infrastructure and needs of this nation's crime laboratories.

The CPFFCL is part of the BJS' forensic portfolio that provides the only comprehensive information about the infrastructure and the forensic services provided by federal, state, and local crime laboratories across the United States. There are four primary goals for the 2020 CPFFCL, including: (1) the administrative characteristics of crime laboratories (e.g., staffing, training, population served, resources, and caseload); (2) policies related to quality assurance, data, records, outsourcing, and evidence retention; (3) levels and methods of interaction with law enforcement agencies covering shared jurisdictions; and (4) changes in demands on crime laboratories and other emerging trends in the field.

The 2020 CPFFCL provides an update on the 330 publicly funded forensic crime laboratories and multilaboratory systems. The 2020 CPFFCL was conducted by RTI on behalf of BJS (2018-85-CX-K037) from July 2021 through February 2022. The 2020 CPFFCL questionnaire was developed based on a review and update of the 2014 instrument, an expert panel, and pilot testing. Mixed mode collection of data was conducted by mail, online, and email. The 2020 response rate was similar to previous administrations. The 2020 and 2014 CPFFCL data provided by the responding laboratories were compared in terms of budgets, staff, workloads, forensic services offered, backlogs, and outsourcing. Over time, there have been significant changes in the caseloads for forensic laboratories. From 2014 to 2016, labs experienced a 7% decrease in received forensic requests (3.8M vs. 4.1M); a 36% decrease in backlogged requests (570,100 vs. 895,500); and a 9% increase in full-time personnel (14,300 vs. 13,100).[1] This presentation will build on these results and present trends through 2020.

This presentation will discuss the information gathered by the 2020 CPFFCL to provide an update for the current state of the nation's forensic laboratory infrastructure. These findings will enhance understanding of forensic services and operations and provide data to track key performance metrics relevant to the field and show operational needs.

Reference:
1.   Burch, A.M., Durose, M.R., Walsh, K.A., Tiry, E. *Publicly Funded Forensic Crime Laboratories: Quality Assurance Practices, 2014.* Bureau of Justice Statistics. Office of Justice Programs. November 2016. https://bjs.ojp.gov/library/publications/publicly-funded-forensic-crime-laboratories-resources-and-services-2014.

Laboratory Infrastructure; Outsourcing; Backlogs

F140   The Search for Collateral Deaths Derived From the Events of the 1921 Tulsa Race Massacre

Phoebe R. Stubblefield, PhD*, University of Florida, Gainesville, FL; Angela Berg, Office of the Chief Medical Examiner's Office, Oklahoma, Tulsa, OK; Robert B. Pickering, R.M. & Ida McFarlin Dean of Library, University of Tulsa, Tulsa, OK

Learning Overview: After attending this presentation, attendees will be able to describe the death documentation history of the 1921 Tulsa Race Massacre and consider the likelihood of collateral deaths recorded in adjacent counties. This study contributes to modern death investigation by highlighting the significance of contributing or secondary events in death certification and demonstrating the historical and social impact of death certification. This study demonstrates the role of historical narratives or documentation when reconstructing past events featuring significant loss of life.

Impact Statement: This presentation will impact the forensic science community by demonstrating the progress of the forensic anthropology community to identify as many decedents of the 1921 Tulsa Race Massacre as possible, using in this example historical documentary evidence. Illustration of past and present death certification practices and coding systems is included. This presentation is accessible by vocabulary and content to all members of the Academy. The subject matter involves multiple deaths derived from a race-based conflict on American soil and may be disturbing to some viewers.

The number of deaths resulting from the 1921 Tulsa Race Massacre remains speculative. Thirty-nine deaths were certified in Tulsa County, but Maurice Willows, director of Red Cross operations in 1921 Greenwood, noted in his final report that the hospital housed one Arthur Morrison, orphaned when his mother died as a result of her injuries in the riot. We found Mrs. Ella Morrison's death certificate in the adjacent Muskogee county. A June 12, 1921, *Tulsa Tribune* article indicated the Red Cross was transporting wounded victims to adjacent cities unless they had family to manage their care in Tulsa. This information led us to examine the June 1921 Muskogee County death certificates for additional deaths possibly associated with the race massacre event.

We hypothesize that Muskogee deaths of interest will be associated with the following features: gunshot wounds; burns; surgical shock; post-surgical infection associated with early June 1921 dates; or puerperal infections associated with early June 1921. We examined 69 Muskogee County death certificates from Oklahoma Vital Records. The race distribution of the decedent population is 28 Colored, 1 Indian, 36 White, and 4 undesignated. As in Tulsa County, each certificate had been coded using the International List of Causes of Death, Revision 3 (1920), and we used this system to summarize the causes of death. The Tulsa County riot deaths by gunshot wound were uniformly coded "183 accidental injury by firearms." No such code was observed in the Muskogee sample, nor were there any gunshot wound deaths for the month.

The majority of Muskogee deaths derived from infections (approximately 38%) and stillbirths (17%). In contrast, 12% of Tulsa deaths were infectious; the majority of deaths were "accidental" (27%) after combining the firearm injuries with coded drowning/crushing and burn accidents. Six Muskogee certificates distributed across the first week of June 1921 were not coded, representing deaths for five Colored and one White individual, and of these, one death was of interest. John Colis (*sp?*), a 14-year-old Colored male, died June 7, 1921, due to empyema following rib resection surgery June 2, 1921. The reason for this rib resection will likely never be known, and our attempts to trace the Colis family have been unsuccessful. However, the race, surgical date, and death date support including John Colis as a possible transported race massacre victim.

Mrs. Morrison's and John Colis' death certifications highlight the difficulty of accurate or meaningful documentation of cause of death when contributing or secondary factors apply. This is a problem that persists for modern death certification without the significance of a racial conflict. Our Muskogee County review indicates death certification for other counties with large African American communities must be examined for suspicious deaths associated with the 1921 Tulsa Race Massacre.

Human Rights; Death Investigation; Victim Identification

F141   The Fernanda Maciel Case: The Results of Forensic Reconstruction

Carlos A. Gutierrez*, Chaminade University of Honolulu, Honolulu, HI

Learning Overview: After attending this presentation, attendees will learn the results of a high-profile case that involved a missing seven-month's pregnant woman, how the forensic reconstruction helped to find her body and discover what happened with her and her baby, and how all the evidence found and analyzed helped to convict those responsible for the crime.

Impact Statement: This presentation will impact the forensic science community by informing attendees regarding what knowledge and technology was used to solve the case and how all this experience can be used in similar cases.

Disappearances occurred in different contexts throughout the world's history, some related with common crime, and some of the most remarkable contexts are dictatorships, armed conflicts, and drug trafficking. These illegal actions have been used as a spreading-terror strategy in several countries and regions around the world such as Sri Lanka, Syria, and Mexico, countries where enforced disappearances have become a serious problem not only for the victim himself/herself and governments but also for his/her close relatives.

But what happened with common disappearances related to common crimes, and is law enforcement prepared to search for these victims? In Chile, the statistics say that police receive 10,000 missing reports per year. In 9,000 of them, the victim came back to the family by himself/herself or via the police, but 1,000 cases per year remain open. Chile has more than 15,000 missing persons cases opened with no clues.[1-4]

This case review is about one of the Chile's most well-known missing cases. Fernanda Maciel was 21 years old and 7 months pregnant. On February 10, 2018, at 5:00 p.m. she left her house and disappeared. After 16 months of searching, we found her buried in the backyard of a house used as a tent warehouse, located just 100 yards from her house.

The body of the victim was processed using a different kind of technology, as was her clothing, which found crucial evidence to reconstruct the events related to her murder. The forensic analysis of this evidence was processed for several months, and with the results, we were able to put all the pieces together in a forensic report.

The main objective of this presentation is share the experience with attendees and what was used to solve the crime, so if someone has a similar case, this can be a helpful experience to find the victim and the necessary evidence to guide the investigation.

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Reconstruction; Missing Persons; Chile

F142   Trailside Murder: A Random, Vicious Attack

Kelsie Bryand*, Sam Houston State University, Huntsville, TX; Andra Lewis-Krick, Texas Tech University, Huntsville, TX

Learning Overview: After attending this presentation, attendees will have a better understanding of the challenges associated with outdoor crime scene management. The collection of evidence and the establishment of perimeters are extremely important components in processing large outdoor crime scenes.

Impact Statement: This presentation will impact the forensic science community by helping attendees gain greater awareness of the challenges of large outdoor crime scenes. This information will also demonstrate how witness testimony and the location of the DNA evidence can impact an investigation. This case demonstrates the challenges of working a large crime scene with numerous entry points in a public outdoor area frequented by hundreds of people. This case is also illustrative of allowing the evidence to direct an investigation and to not let biases sway the direction of an investigation.

Case Information: The female victim was an executive secretary and during her lunch hour, she would often walk on a popular walking canal trail near her workplace and talk on her cellphone. On May 13, 2003, while on the walking trail and talking on her cellphone with her husband who was in Europe on a business trip, she was attacked. He heard a gasp, and the phone line went dead. After repeated attempts to reach her, he called her coworkers, who went to look for her to no avail. The police were contacted and after searching for some time, a motorcycle officer located what appeared to be a trail of blood on the walking trail. He followed the apparent trail of blood down an embankment into a wooded area where the female victim was located alive and in a pool of blood. She died from her injuries enroute to the hospital.

Evidence: The murder weapon was a piece of metal fence post covered in blood; however, no fingerprints were found. The only other evidence present within the immediate crime scene were hairs, a shoe, partially torn clothing, and pools of blood. Located along the chain link fence surrounding the canal were a pair of women's sunglasses. No cellphone was collected from the crime scene. A large perimeter had been established, resulting in the collection of hundreds of items of evidence, including cigarette butts and numerous items from homeless camps.

Investigation: The victim had been sexually assaulted and, after testing individuals who frequented the area, the DNA profile did not match any profiles in the statewide DNA database. The investigation produced two separate witnesses who provided enough information to develop a police sketch that resulted in an identification from a police lineup. A K-9 bloodhound was brought to the scene in an effort to track the suspect. The K-9 led to a home about a mile from the crime scene; however, prior to serving the search warrant, the family was notified that their son, who had a history of mental illness and had several police contacts along the canal trail, had committed suicide by jumping off the Golden Gate bridge. The DNA of the victim was checked against this individual and was determined not to be a match. Nine days after the murder, a witness came forward who recognized the police sketch. The day of the murder, the witness stated he had a conversation and smoked a cigarette with an individual who looked like the police sketch. This witness, along with detectives, returned to the crime scene and collected the cigarette butts, which were over 500 feet from the initial scene. DNA was recovered from the cigarette butts that matched the DNA found on the victim. Eventually, the DNA was linked to an individual being held on robbery and assault charges in Indiana. He was charged and convicted of murder and was sentenced to death.

Crime Scene Investigation; Evidence; Death Investigation

F143 Hanged in the Window of His House: An Accidental Hanging Clarified Only After a Late Scene Examination

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Learning Overview: After attending this presentation, attendees will recognize the importance of discussing the manner of death, namely the difference between suicide, homicide, and accidental deaths, and how difficult it can be. It is an unusual case of mechanical asphyxia that serves as a prime example of how things may sometimes take an unexpected turn.

Impact Statement: This presentation will impact the forensic science community by emphasizing the potentialities of the scene examination to clarify the manner of death, even after the autopsy. It will highlight, first and foremost, the importance of knowing the exact position of the body at the moment of death to understand the signs and lesions observed at autopsy.

Death by fatal pressure to the neck is usually associated with one of the following: strangulation, mugging, hanging, and sexual activity-related asphyxia. Hanging is usually a result of suspension by the neck, using a rope, or rope-like functioning objects such as cables, sheets, and such. But every now and then, we can find typical hanging marks on the neck with no object of such a nature having been used, forcing pathologists to stop and think: what actually happened?

We report the case of a 48-year-old male found dead at home, below a broken window, whose autopsy was ordered by the prosecutor, after the gravedigger found a suspicious ligature mark. No scene examination was conducted.

The postmortem examination revealed a large, parchment-like abrasion with 10cm x 7cm and a submandibular hanging mark with a inferior border of 10cm in length and superior border of 8cm in length. This rectangular abrasion corresponded to the shape of a piece of broken glass that came with the corpse, whose origin was unknown. We also found more superficial abrasions on the posterior thoracic wall and both arms. Internally, there was an extensive neck subcutaneous tissue condensation corresponding to the abrasive area found externally and cricoid cartilage dislocation, as well as left carotid artery laceration. Toxicological examination revealed a blood alcohol level of 2.35g/L.

As the strange ligature mark wasn't compatible with a typical hanging, but with the glass described above, the authors felt the necessity to know more about the circumstances of death to get the full picture of the case, namely the manner of death. To try and understand the conditions the victim was found in and to recreate the event, pathologists involved in the case have been taken to the victims' house and tried to recreate it—a kind of scene examination after the autopsy.

Drops of blood and glass fragments found below the window suggested that the victim should have entered at home through the window. The man was not particularly tall and, apparently, would have been able to cross this window. Proving this hypothesis, two of the authors also entered the house through the same window. According to the police, the man who lost the keys of his house lived alone and had a history of alcohol abuse. The authors combined all the pieces of the puzzle and debated how they fit together to and achieve both the cause of death and manner of death.

The cause of death was a fatal pressure to the neck by the border of a window that the victim without keys had broken to try to get inside, going in feet first. In the end, he got trapped by the neck, which lead to his passing. As per the manner of death, given all the information gathered, we concluded it was accidental, making it an interesting case of "accidental hanging."

In conclusion, the case presented emphasizes how the importance of the scene examination and event recreation, even after autopsy and especially when it was not clear, is useful and sometimes crucial to clarify the manner of death.

Scene Investigation; Hanging; Accident

F144   A Case Study: Parallels of Intentionally Inflicted Blunt Force Trauma and Blunt Force Trauma in Motor Vehicle Collisions (MVCs)

Emily Ditto*, Tippecanoe County Coroner's Office, Dayton, IN; Brett Bulington*, Tippecanoe County Coroner's Office, Lafayette, IN; Carrie Costello, Coroner's Office, Lafayette, IN

Learning Overview: After attending this presentation, attendees will have gained a comprehensive understanding of the shared characteristics between blunt force traumatic injuries observed in child victims of fatal beatings and those encountered in MVCs. Also discussed will be the importance of helping a jury understand those similarities for successful prosecution and eliminating other possibilities by conducting a comprehensive death investigation.

Impact Statement: This presentation will impact the forensic science community by pointing out the parallels of blunt force trauma that was intentionally inflicted and blunt force trauma due to MVCs. Distinguishing between major accidental trauma and minor accidental trauma becomes especially critical when investigators are confronted with differing explanations for the mechanisms of injury leading to death. In cases where multiple plausible explanations for injuries exist, it is imperative to gather all relevant facts and broaden the scope of possibilities to accurately differentiate between accidental and intentional trauma.

This presentation describes the case study of a 3-year-old, non-verbal autistic child who was discovered deceased under the care of his mother and her boyfriend only a month after being placed back in their custody in 2021. Upon immediate observation, investigators were confronted with severe bruising throughout the child's entire body, prompting questioning of those involved in his care. The mother and her boyfriend claimed the bruises resulted from minor falls and bumps, but the autopsy identified almost 50 distinct injuries, both internally and externally, that are commonly associated with deaths from MVCs. Injuries to this extent, especially when seen out of the context of an explainable accident, are frequently associated with child abuse cases and serve as clear indications of intentionally inflicted blunt force trauma.

The severity of the injuries found in this case, requiring significant external force, contradicted the explanations provided by the caregivers. For instance, a ground-level fall on concrete would not likely cause multiple broken ribs and internal organ lacerations in a healthy 3-year-old. Similarly, an accidental head bump on a table would not account for the numerous contusions and hematomas observed on the child's head, face, and neck at various stages of healing. Through a comprehensive death investigation, postmortem examination, X-rays, and interviews with potential suspects, investigators conclusively determined this to be a homicide.

The child's mother and her boyfriend were both convicted of his murder and received sentences of 55 and 65 years, respectively. The successful attainment of a guilty verdict in this case heavily relied on effectively conveying the severity of these injuries and the striking similarities between them.

Autopsy; Blunt Force; Child Abuse

F145   The Occurrence of Natural Deaths in Motor Vehicle Collisions: A Case Study

Emily Ditto*, Tippecanoe County Coroner's Office, Dayton, IN; Carrie Costello, Coroner's Office, Lafayette, IN

Learning Overview: After attending this presentation, attendees will understand the importance of conducting a complete forensic autopsy on all deaths that appear to be linked to Motor Vehicle Collisions (MVCs).

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of forensic autopsies on all deaths believed to have occurred due to MVCs. This case study presents the compelling investigation of a fatal MVC where initial on-scene observation led investigators to assume the death was a result of blunt force trauma from the MVC; however, a comprehensive investigation subsequently determined that the cause of death was, in fact, attributable to natural causes.

During the death investigation of fatal MVCs, it is crucial to ascertain whether the collision directly caused the demise or if the demise was the triggering factor for the accident. Investigators should be cognizant of the potential occurrence of cardiac death while an individual is operating a vehicle, causing loss of control. Drawing a clear distinction between these circumstances is necessary for ascertaining the definitive cause and manner of death in these instances.

Investigators in Tippecanoe County, IN, were called to the scene of a 43-year-old male found deceased next to his wrecked moped on the side of the roadway. It was reported that the moped was traveling at a high rate of speed when it took a wide turn and hit the curb, causing the decedent to be ejected. Although external trauma was evident on the body, postmortem examination findings conclusively negated its role in the demise. Instead, the autopsy results pointed to pre-existing cardiac conditions as the probable cause of death, with the collision being an outcome of the decedent experiencing a fatal cardiac arrhythmia, leading to a loss of control over the moped.

Fatal MVCs often involve many natural deaths, thus emphasizing the critical need for a comprehensive death investigation to ensure accurate classification. Should investigators presume each fatality in MVCs to be a consequence of blunt force traumatic injuries before substantiating such findings through a comprehensive forensic autopsy, there is a risk of overlooking key evidence that could significantly alter the case's outcome.

Autopsy; Motor Vehicle; Cardiac Death

F146   Visualizing Trends in Subway-Related Fatalities in New York City, NY: 2008–2021

Justin Z. Goldstein*, New York City Office of Chief Medical Examiner, New York, NY; Jeremy A. Simmons, New York City Office of Chief Medical Examiner, Brooklyn, NY; Bradley J. Adams, New York City Office of Chief Medical Examiner, New York, NY

Learning Overview: After attending this presentation, attendees will have a greater understanding of the spatial distribution of subway-related fatalities in New York City (NYC) from 2008–2021.

Impact Statement: This presentation will impact the forensic science community by providing context for understanding subway-related fatalities in NYC over a 14-year period. The study explores temporospatial trends using ArcGIS® and considers decedent demographics and manner of death, among other variables.

Train-related fatalities represent a public health concern, particularly in a large urban region such as NYC. The NYC subway system is the largest subway system in the United States and one of the largest public transportation systems in the world. Previous research has analyzed trends in NYC subway-related fatalities between 1990–2003 and 2003–2007.[1,2] Both studies quantitatively assessed trends in decedent demographics and manner of death over time, but neither applied geospatial analyses. This research expands on previous research by analyzing subway-related fatalities between 2008–021 through not only quantitative methods, but also utilizing ArcGIS® Pro software to visualize temporospatial trends.

All train-related fatalities were queried from the NYC Office of Chief Medical Examiner case management system using key word searches. Case documents were then manually reviewed to determine the subway line and subway station location for each case. Cases were also reviewed to ensure that only NYC subway-related fatalities were included in the research, as opposed to other train lines operating in the city (e.g., Long Island Rail Road [LIRR], Amtrak®). Cases were excluded if deaths occurred on a subway or in a subway station but were not related to the subway system itself (e.g., cardiac event occurring in a station). Once the data were vetted, cases were imported into ArcGIS® Pro and geocoded. The points were split by year, date, and time of death, season of death, manner of death, and decedent demographics. Age cohorts were summarized for less than 20, 20–40, 40–60, 60–80, and over 80 years of age. Kernel density functions were applied for each point layer and normalized to assess relative hotspots. Ordinary Least Squares (OLS) regressions were also performed

In total, 838 subway-related fatalities were assessed within the designated study period. Subway-related deaths consistently increase over time between 2008 (n=46) and 2021 (n=77). Kernel density maps indicate that overall subway-related fatalities are strongly clustered in Manhattan (n=369), particularly in mid to lower Manhattan. Fatalities in Brooklyn (n=225), Queens (n=138), and the Bronx (n=106) appear relatively dispersed, consistent with the subway structure in these boroughs. Suicide was found to be the most common manner (n=409), followed by accident (n=277), undetermined (n=143), and homicide (n=9). Decedents were more commonly male (n=706, 84.2%) than female (n=132, 15.8%). These percentages appear relatively consistent across all manners of death. Age trends largely followed the overall data distribution, with suicide being the most common manner of death among all cohorts. Most deaths occurred in the 20–40 cohort (n=347, 41.4%), followed by 40–60 (n=313, 37.4%) and 60–80 (n=122, 14.6%). Density distributions by reported ancestry indicate that suicide was the most common manner of death across all reported groups, except for Hispanic, which had equal numbers of accidents and suicides.

Looking at seasonal data, suicides were consistently the most common manner of death regardless of the time of year. Overall, subway fatalities were highest during the winter months (n=223, 26.6%), followed closely by summer (n=211, 25.2%) and spring (n=206, 24.6%). Interestingly, during the winter the rate of accidents increased to equal suicides (n=90 for both accident and suicide). Looking at weekday data, the most deaths occurred on Fridays over time (n=139, 16.6%). However, suicides occurred more frequently on Mondays (n=71, 17.4%), Tuesdays (n=73, 17.8%) and Fridays (n=64, 15.6%), whereas accidents occurred more frequently on Fridays (n=51, 18.4%), Saturdays (n=57, 20.6%), and Sundays (n=47, 17.0%).

Although OLS regression models indicate no statistically significant geospatial predictors for manner of death, kernel density maps indicate practically significant trends. Understanding the patterns highlighted based on manner of death, subway station location, season, and day of the week may be informative when assessing risk factors associated with transit-related fatalities.

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Subway; Suicides; Accidents

F147 The Secret Life of Dorothy

Melanie Thomas Armstrong*, ThroughLine Consultants, Vienna, VA

Learning Overview: After attending this presentation, attendees will better understand how a combination of DNA with traditional genealogical research can uncover secret lives from the past and investigate, reassess, and corroborate true stories and true identities.

Impact Statement: This presentation will impact the forensic science community by demonstrating the multitude of often unpredictable, improbable, and novel identifications of family members and relationships that genetic genealogy can unearth—and some of those family members will meet for the first time at this conference.

The techniques and approaches used in forensic genetic genealogy—genetic genealogy in a legal context—are the same as those used in genetic genealogy outside a legal context, for example, when performed to solve an adoption or identify the parent of a Non-Paternity Event (NPE). The only differences are the specific limited databases that law enforcement are permitted to use and the limited number of people who have "opted in" to share their data with law enforcement.

The unique case study presented in this session will show how genetic genealogy was used to solve an adoption case and, in the process, expose multiple NPEs and secret adoptions within the same family. While the findings in this case have rattled the siblings' understanding of their own family, they have also substantiated a variety of family rumors, stories, and comments that didn't make sense at the time. To date, the ensuing genetic genealogy work in this case has identified five NPEs—all previously thought to be full siblings but now proven to be half siblings with a shared mother and five different fathers. Additionally, three previously unknown children within the family have appeared in the DNA matches.

Using a combination of DNA, oral history, and documentation to correlate evidence as well as resolve conflicting evidence, we are able to establish a new narrative about Leo and Dorothy that provides a much more complete picture of their lives.

This case highlights the critical components of any case involving genetic genealogy, be it a cold case investigation and subsequent presentation in a courtroom, or for a family member simply looking for answers. In this case, the DNA results from more than six people were accessed to find answers. In a law enforcement case, this luxury is not possible, making it much more difficult to determine when the DNA does not match the records. This is yet another reason—beyond the limited databases—that renders cold cases much more difficult to solve than adoptions. The science of DNA is a powerful investigative tool in the hands of genetic genealogists.

Genetics; Genealogy; DNA

F148   1 . . . 2 . . . 3 . . . Baby Deaths: A Case Involving One Common Denominator

Anita Marie Roman*, Charleston County Coroner's Office, North Charleston, SC

Learning Overview: After attending this presentation, attendees will understand the purpose of a thorough medicolegal death investigation. When investigating an infant's death, there are numerous steps to take throughout the entire investigation. This presentation will discuss the various agencies to contact, the proper way to interview, and what to look for while at the scene and performing a doll reenactment. This presentation will also discuss various red flags, the importance of obtaining a medical and social history, and the need to conduct a child death review.

Impact Statement: This presentation will impact the forensic science community by providing various tools to utilize during a child death investigation from the very beginning to case closure. Medicolegal death investigators will have a better understanding of how to recognize the red flags in all aspects of the investigation and the importance of maintaining communication with all agencies involved, including the Department of Social Services. It is also recommended to have another medicolegal death investigator assist with the investigation, whether they respond to the incident location while another photographs the body and conducts the interviews or work side-by-side throughout the entire investigation.

When a child is pronounced deceased at a separate location from where the incident took place, it is vital that the investigator responds to the incident location following the examination of the infant and interviews. First and foremost, upon notification of the demise, immediately contact the law enforcement agency involved and make sure an officer secures the incident location and removes all parties from the dwelling in order to prevent any further evidence contamination. Upon arrival, make sure to photograph and scan the scene, looking for anything that may appear suspicious and/or contributory to the death, such as melatonin near a baby bottle, type of bedding, and closely examine the reported location(s) where and how the baby was placed and also found. How to perform a doll reenactment and who participates will also be discussed in this presentation.

The interview process with the caretaker(s) will be discussed as well as recognizing signs of deception to include body language. It is suggested that the interview be recorded by any means available at that time. If it is inside of a motor vehicle, utilize a portable recording device such as your work cellphone or hand-held recorder. If the interview is held in an interview room at a police department, make sure it is being recorded, then obtain a copy. The purpose of recording is that you as the death investigator can pay close attention to body language, emotions, or any change in behavior when certain questions are asked rather than continuing to look down as you take notes. It is also suggested that the interviews be conducted alongside the detective(s) so the interviewee will not have to repeat themselves and be re-traumatized.

Obtaining medical records on the decedent and the mother is crucial in a child death investigation. When warranted, medical records may also be requested of other children in the home. It is suggested that the investigator be aware of any testing performed at birth, which may include newborn genetic testing. If there is more than one infant death history with the mother, consider genetic testing if there is no other evidence to suggest the cause for the death.

The author will present a case of a 2-month-old infant who was pronounced at the scene. This was the third infant belonging to the mother who had died under her care. The importance of notifying the Department of Social Services is crucial in the beginning; this case will provide an example as to why. The similarities among the cases will be shared as well as the cause and manner of death. Remember, as a medicolegal death investigator and forensic professional, speaking for the dead is our duty and responsibility to not only bring justice for the decedent but Justice for All.

Infant Death; Medicolegal Death Investigation; Scene Investigation

F149   Suicidal Decapitation: Bizarre Ingenuity

Stacey L. Chepren*, United States Air Force, Tucson, AZ

Learning Overview: Attendees of this presentation will learn about trends found at the scenes of deaths involving suicidal decapitation. Attendees will also learn the patterns of wound morphology in these cases.

Impact Statement: This presentation will impact the forensic science community by providing trends identified at the scene and in the background of decedents who self-decapitate. By being informed on this topic, practitioners in the field will be able to confidently conclude the manner of death in cases involving this method of suicide.

Cases involving decapitation are rare in forensic practice. Most cases involving decapitation are the result of an accident or a homicide. There is a very small percentage of suicides involving unassisted, complete, external decapitation. A complete external decapitation is total severing of the head from the body to include all neck organs and structures.

By reviewing various cases in literature, clear trends emerge. Males are more likely than females to complete suicide by involving complete external decapitation. In the rare event a female utilizes this method, it is most often by laying on railroad tracks in front of a train. There are usually no witnesses to the event where death occurs, meaning a thorough scene investigation is paramount.

Considerations should be made to examine the scene for devices that may be constructed to enable the mechanism used to cause the complete external decapitation without the help of another individual being present. Some scenes appear complex at first; a reconstruction can provide a valuable demonstration of how the action of the decedent was, or was not, possible. In cases that involve vehicles that are not running when found, fuel levels and key position in the ignition should be noted. The gear the vehicle is in should also be documented. These factors can support or refute the presence of another individual at the scene prior to discovery.

The mental health history of a decedent should be analyzed. Toxicology can also be very helpful by providing evidence if a decedent is not taking a therapeutic medication prescribed to them. Investigation into the field of work of the decedent is important. Some decedents have been found to have fabricated parts for the device they constructed for use to decapitate themselves at their place of employment. Their type of work may contribute to the motive to use a particular method.

The head of the decedent in a suicidal decapitation is always found in close proximity to the body. An exception to this would be animal activity. In homicidal cases, the head is only sometimes located with the body. A decapitated body without the head in the immediate vicinity should be cause for suspicion of a manner of death other than suicide. Another key consideration is the lack of injuries to the rest of the body in cases of suicidal decapitation. A homicidal decapitation is more likely to occur lower in the cervical spine. Suicidal decapitation is more likely to occur in the mid to superior cervical spine.

With the rise of books and internet websites detailing various suicide methods, suicidal decapitation is beginning to surface more in forensic practice. It is important for those in the field to have the knowledge and tools available to them so that a thorough medicolegal investigation can be conducted and a confident determination of manner of death can be made.

Suicide; Medicolegal Death Investigation; Decapitation

F150   Beware of People with Pens in Shirt Pockets and Walking with Canes: Regarding Two Medicolegal Autopsies

Fabrice Dedouit*, Hopital Rangueil, Toulouse, Midi-Pyrenees, France; Agathe Bascou, MD, Chu Toulouse, Toulouse, Midi-Pyrenees, France; Frederic Savall, MD, PhD, Chu Toulouse-Rangueil, Toulouse, Midi-Pyrenees, France; Ryan Toutin, MD, Chu Toulouse, Toulouse, Midi-Pyrenees, France; Anthony Ramirez, MD, Chu Toulouse-Rangueil, Toulouse, Midi-Pyrenees, France; Manuelo Turkiewicz, MD, Chu Toulouse-Rangueil, Toulouse, Midi-Pyrenees, France; Norbert Telmon, MD, PhD, Chu Toulouse-Rangueil, Toulouse, Midi-Pyrenees, France; Fatima Zohra Mokrane, MD, PhD, Chu Toulouse-Rangueil,, Toulouse, Midi-Pyrenees, France

Learning Overview: The goal of this presentation is to illustrate the interests and potentialities of radiography in ballistic trauma secondary to the use of "unusual" guns. The first case presents the use of a pen gun and the second involves a cane gun.

Impact Statement: This presentation will impact the forensic community by providing examples of ballistic trauma with unusual weapons.

Introduction: The authors present two cases of deaths caused by the use of "unusual" weapons (a pen gun and a cane gun).

Case 1: A 66-year-old woman was found dead at home. A letter with suicidal intentions was found. The body was found one month after the last sign of life. A medicolegal autopsy was ordered by the public prosecutor. The external examination revealed massive putrefactive changes. The body was found with a pen in the right hand. Radiographs revealed the presence of a single radiopaque foreign body in the head. A metallic pen was visible near the right hand. A ballistic trauma with a rifled gun and a single projectile was therefore suspected. The autopsy of the head revealed a bone defect of the clivus, consistent with a bony entry wound secondary to a gunshot. The brain was liquefied and contained a metallic foreign body interpreted as a bullet. A careful examination of the pen revealed that it was 13cm long and that it was a pen gun. It looked like a handmade product, with a "rack and pinion" model. The forensic pathologists concluded that the death was due to cranio-encephalic ballistic trauma secondary to the use of a pen gun.

Case 2: A 68-year-old man was found dead at home. A cane was found near the body in the living room. Several pellet holes were found in a wall in the living room. A medicolegal autopsy was ordered by the public prosecutor's office. According to police investigations, the weapon used in this case was possibly a cane gun. The external examination revealed a right temporal skin defect. A black soot deposit and an area of tattooing were visible. Abnormal mobility of the right facial bones and right part of the skull were noted. Radiographs of the head revealed the presence of multiple radiopaque foreign bodies in the right part of the head. Ballistic trauma with a shotgun was therefore suspected. A careful examination of the cane confirmed that it was a cane gun. The cane was a French product. The size of the lead pellets ranged from 9 (1.27mm diameter) to 12 (2.3mm diameter). Autopsy revealed a massive dilaceration of the frontal and temporal parts of the brain, with multiple fractures. Many pellets were found, particularly on the right temporal bone. All of these aspects were consistent with ballistic trauma from a cane rifle and shotgun pellets.

Discussion: The pen gun: Known since the 1940s, a pen gun is a firearm that resembles an ink pen. They are generally small caliber (e.g. .22 LR, .25 ACP, .32 ACP, .38 caliber, etc.). Compared to conventional handguns, pen guns may have similar energy levels to small caliber sporting pistols and therefore have a similar potential for injury. Some historical examples of use and production of pen guns will be described by the authors.

The cane rifle: A cane gun is a walking stick with a concealed pistol built into it. Almost all surviving examples of cartridge-fired cane guns (late 1850) are rimfire weapons built for small calibers, but there were a limited number of shotgun versions produced with center-fire cartridges. Percussion and rimfire versions were made with smooth and rifled barrels, but of course the shotgun models were all smooth bore. Some historical examples of use and production of cane guns will be described by the authors.

It is important for the forensic pathologist to be aware of the existence of such "camouflaged" firearms and their operation in order to guide the forensic investigations.

Shotgun; Pens; Cane

F151   A Story With a Not-So-Ferret-Ale Ending: What to Do When OBTI Won't Work

Amanda Fitch*, ASPCA, Newberry, FL

Learning Overview: After attending this presentation, attendees will be aware of a criminal case study in which Hexagon OBTI was not usable due to the species of the victim. Included in the discussion will be the details of the incident, how the forensic testing was subsequently handled, and the final outcome of the case.

Impact Statement: This presentation will impact the forensic science community by introducing an alternative option to OBTI testing when the presence of Mustelidae blood is of concern.

Most crime scene investigators are familiar with the product Bluestar® OBTI and its use as a presumptive field test for human blood. Most crime scene investigators are also familiar with the warning of a false positive result in the presence of non-human primate blood or weasel blood.[1] In cases of animal cruelty, OBTI testing is commonly used to test blood evidence for the same purpose as human cases—to determine whether blood samples of an unknown origin are human or non-human. The greatest difference is that in cruelty cases, it is expected that the OBTI test will show a negative result, and speciation is then determined through DNA analysis. But what happens when the presence of "weasel" blood is actually a concern?

The American Society for the Prevention of Cruelty to Animals (ASPCA) supports a Veterinary Forensic Science Center (AVFSC) in Gainesville, FL. This facility is a resource for law enforcement agencies nationwide that are in need of forensic assistance with animal cruelty cases. This presentation will discuss a case of criminal animal cruelty in which blood evidence was submitted to the AVFSC by the Anne Arundel County Police Department/Animal Care and Control. In this case, there was concern about utilizing the OBTI test due to the victims being members of the Mustelidae family. Therefore, the Rapid Stain Identification of Human Blood (RSID-Blood) test was utilized instead because the test is advertised as the first commercially available confirmatory test for human blood and the only similar test known that does not cross-react with any other non-human animal blood.[2] As a case study, this presentation will discuss the suspect and actions leading up to the incident, the acts of cruelty committed against the victims, the evidence submitted to the AVFSC for analysis and the forensic testing performed, and finally the outcome of the case.

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1.   Bluestar® OBTI: Immunochromatographic Test for Confirming the Presence of Human Blood Traces for Forensic Use; September 2021.
2.   Rapid Stain Identification of Human Blood (RSID-Blood): Technical Information and Protocol Sheet for Universal Buffer, cat# 0330; April 2011.

Animal Abuse; Bloodstains; Evidence

F152   Equestrian Death Investigation Best Practices: An Effort to Support Fatal Rotational Fall Analysis

Stacey L. Chepren*, United States Air Force, Tucson, AZ

Learning Overview: Attendees of this presentation will learn about two cases in which horseback riders each experienced a rotational fall during a competition that resulted in fatalities. Attendees will also learn the most common fatal injuries that occur in this type of fall involving horse and rider. Best practices in death investigation of equestrian fatalities will be highlighted in an effort to assist increased data gathering and analysis of fatal rotational falls involving equestrians. The gathering of this data will allow engineers and forensic analysts to reconstruct the fatal mishap and further test prototype safety equipment and allow officiating personnel to adjust equestrian competition conditions to reduce fatal rotational falls.

Impact Statement: This presentation will impact the forensic science community by providing education on fatal injuries involving equestrians experiencing rotational falls and best practices in the death investigation of fatal equestrian incidents, which will lead to more accurate analysis in reconstruction to reduce future fatalities.

A fatal rotational fall involves a horse dragging, hitting, or catching a knee or foot over an obstacle that is intended to be jumped. The horse dragging, hitting, or catching a knee or foot on or in the jump causes the horse to rotate its entire body over the jump. Once the horse has rotated over the jump, it lands on its rider, killing them. Fatal rotational falls are on the rise in the Cross-Country phase of the equestrian sport known as Eventing. Two case reports will be reviewed involving two riders that experienced fatal rotational falls during competition. Both riders died from blunt force head injuries.

New safety equipment has been designed for both horse and rider to help prevent fatalities in these types of falls; however, the new safety equipment is inadequate due to the type of fatal injuries in rotational falls. This new equipment would not have prevented the fatal injuries in either of the presented case reports. A large focus is put on what is known as an air vest, which protects the thorax of the rider. However, a review of autopsy reports from these types of cases shows that almost all fatal injuries are blunt force head injuries.

There is no mandated process for the gathering of information in an equestrian death investigation. To better document these incidents and assist in data gathering and analysis of these types of fatal incidents, some best practices have been identified. Written or photographic documentation of environmental factors such as weather and terrain are helpful to analyze conditions at the time of the fall. These conditions can significantly impact a horse's ability to clear a jump. The height and width of the jump, and if the jump is solid or frangible, is important to assess the efficacy of newly developed frangible fence technology. A description of the jump itself is helpful to determine if the horse misread the jump. The safety equipment (helmet, air vest, non-air vest) the rider was wearing is extremely important to analyze if the equipment was effective in any attempt to minimize injury.

An effort should be made to interview witnesses to collect additional photographic and video evidence. There is almost always a professional photographer and/or videographer onsite at these events. Annotation of available medical services onsite should be made.

The documentation of this information will allow engineers and forensic analysts to reconstruct the fatal mishap and further test prototype safety equipment. The documentation of this information will also assist in the education of the governing bodies of equestrian sports and those involved in officiating those sports and allow necessary adjustments to reduce fatal equestrian rotational falls.

Medicolegal Death Investigation; Accident; Equestrian

F153   Does the Delivery Method Determine the Success Rate in an Introduction to Forensic Science Course?

Claire E. Shepard, Louisiana Delta Community College, Monroe, LA*

Learning Overview: After attending this presentation, attendees will better understand whether face-to-face, traditional online, or publisher-based online introductory forensic science courses are most likely to result in student success.

Impact Statement: This presentation will impact the forensic science community by assisting forensic science professors with determining the most optimal delivery method (face-to-face, traditional online, publisher-based online) to ensure student success in introductory forensic science courses. In addition, this information will assist forensic science students in determining the modality of introductory courses in which they are most likely to be successful, based on data.

When forensic science courses began being taught in college forensic science programs, the primary, and often only, method of delivery available was traditional face-to-face classroom instruction. As the culture of education has evolved and the needs of students have shifted to a more flexible learning environment, many courses and programs have shifted to online instruction, especially traditional lecture courses such as an introductory forensic science course.

However, while online learning is beneficial to many students for various reasons, other students may be more successful with traditional face-to-face instruction. In addition, some courses may be better suited to more traditional methods of instruction. For example, an Introduction to Forensic Science lecture course is often offered online, due to the nature of the course. But are students more likely to be successful in a traditional classroom-based course? Furthermore, are the students more successful in a conventional online course with instructor-created resources or in a more modern online course with publisher-created resources and integrated platforms.

By determining which modality students are most likely to succeed in, students will have additional information when choosing the type of courses they register for when they have a choice between various modalities of instruction. Furthermore, this information can assist professors in determining which type of courses to offer in their forensic science programs.

In addition, while traditional college students may have a lifestyle allowing them to enroll in face-to-face classes, non-traditional college students often need the flexibility of online learning. If that is their only option, are traditional online courses better or are publisher-based online courses more likely to ensure successful outcomes?

This presentation will compare ten years of data in face-to-face, traditional online, and publisher-based online instruction of an Introduction to Forensic Science course to determine which delivery method is most successful for students.

Education; Forensic Science; Evidence

F154   Restructuring the University Forensic Science Seminar: Lessons for New, Current, and Future Instructors

Joe C. Trevino III*, Pace University, Sunnyside, NY; Amanda A. Murray*, Pace University, New York, NY

Learning Overview: After attending this presentation, attendees will be informed about how two professors at Pace University (Forensic Science Program) restructured the undergraduate and graduate forensic science seminar courses to address contemporary professional and technical issues not often covered in the standard forensic science curriculum. A background on previous iterations of the courses, the thought process(es) behind the restructure, how instruction time constraints played a role, student feedback before and after the semester, and how both instructors maintained a flexible but unified front will be addressed.

Impact Statement: This presentation will impact the forensic science community by providing important information about navigating the post-secondary forensic science education landscape in a way that adequately prepares students for employment in what seems like an uncertain future. The forensic science community will have another tool in the toolbox for responding to evolving instructional needs for both students and faculty.

The forensic science seminar is often an opportunity for students to enhance their skills, develop new interests, explore current interests, and apply the concepts they learned from the traditional curriculum. The forensic science seminar is often an opportunity for instructors to enhance the skills of their students, facilitate the development of new interests, provide the space to explore students' current interests, and make their students apply the concepts their students learned from the traditional curriculum. The course content of the seminar is usually crafted to fit well within a forensic science program and changes only as the program changes. What happens when the curriculum stabilizes but employment expectations and requirements change? What do two newly assigned instructors do with full autonomy and clear expectations over course design? What do two newly assigned instructors, both working industry professionals, do with full autonomy, clear expectations, and a responsibility to present a true and unbiased view of the field their students will soon enter?

An undergraduate and graduate forensic science seminar course were restructured from the ground up and with a coordinated effort to continue the success of previous semesters but with fresh ideas that keep students up to date and ready for life after school. The instructors chose a multi-method approach to discussing contemporary concepts and ideas in forensic science through standard lecture, group and individual discussion, weekly knowledge assessments, skills assignments, and guest presentations from other working professionals. This was the result of pre-semester communication and planning of goals, ideas, and personal perspectives. Each instructor structured the semester as they saw fit and accounted for the difference of undergraduate and graduate student calendars, but the overall mission was to stay in tune with each other as the semester progressed. After only one iteration of the course, both instructors have come away with more efficient ways to deliver the desired lessons as well as introduce topics and concepts discovered as the semester was underway.

Education; Forensic Science; Research

F155   Active Learning in Forensic Biology

Kristi Bugajski*, Valparaiso University, Valparaiso, IN

Learning Overview: The goal of this presentation is to cover an active learning exercise used in a forensic biology course for undergraduate education. Attendees will learn how to incorporate the activities into their own forensic science courses.

Impact Statement: Education is an important part of forensic science. Allowing students to have real-world situations as part of their education experience enhances their engagement and learning. This presentation will impact the forensic science community by discussing one such example from a forensic biology course.

Educating future forensic scientists is an important job for a lot of forensic scientists in academia. This presentation will discuss a multiple-week assignment used in forensic biology that meets many Student Learning Outcomes (SLO's) for undergraduate students. At Valparaiso University, forensic biology is an upper-level elective course for undergraduate biology majors. The course is required for all forensic science minors. The course has a lecture and laboratory component. The keystone assignment for forensic biology is a realistic mock trial in which the students play the role of the expert witness. The students are divided into pairs, and are presented with a set of forensic evidence, and they are responsible for examining the evidence and drawing conclusions based on those examinations. They are required to follow proper evidence-handling procedures, including the security of the evidence, and they provide a written report that we use in court. They experience the entire process of being a forensic scientist, which culminates in a mock trial. We use one of the mock trial rooms at our former law school to make the experience realistic. The students want to impress their peers (who make up the jury), and me (the cross-examining attorney), so they put a lot of effort into the trial. Another component to the trial is to make a CV that would give them the credentials to testify as an expert witness in course. This exercise forces them to think about what level of education and expertise is appropriate for their assigned area in forensic science.

This project spans four weeks in the laboratory and gives the students control of the outcome of their trial. The students really rise to the challenge and continue to impress me with their professionalism and eagerness. The presentation will go into detail on how the assignment is assigned, executed, and assessed. Attendees will be able to take the lessons learned from this presentation and apply them to their own courses.

Education; Expert Testimony; Forensic Science

F156 **The Use of Instructional Strategies in Forensics Education: Results From a National Survey of Post-Secondary Forensic Science Educators**

Tamra Legron-Rodriguez*, University of Central Florida, Orlando, FL

Learning Overview: After attending this presentation, attendees will have gained insights into how instructors implement a variety of instructional strategies in undergraduate forensic science courses. The use of instructional strategies will be discussed within the context of other criteria such as course modality, perceived autonomy, and faculty rank.

Impact Statement: This presentation will impact the forensic science community by informing forensic science educators about the use of various instructional strategies used in post-secondary, undergraduate courses in the United States.

Research has shown that the use of student-centered, active learning instructional strategies in Science, Technology, Engineering, and Math (STEM) courses increases student performance. Nevertheless, since the publications of Freeman and Stains, the STEM education community has been slow to implement these instructional strategies.[1,2] Student-centered strategies that promote cognitive engagement and student interactions have been shown in the education literature to improve performance in STEM courses, yet the predominant instructional choice for STEM faculty is still lecturing during class time. This study seeks to investigate the adoption of instructional strategies in postsecondary forensic science courses across the United States. Because student-centered, active learning instructional strategies have been shown to improve student success, it is important to uncover if these strategies are being adopted in forensic science courses and bring these results to the forensic science education community.[1,2]

Data on instructional strategies used in forensic science courses was collected using a national survey of instructors at United States institutions that teach undergraduate forensic science courses. The survey was adapted from a previously published instrument for the audience of forensic science educators.[3] The survey was also used to collect data that will allow the research team to correlate instructional strategies to course type, institution type, and faculty appointment type. This presentation will provide a better understanding of the landscape of undergraduate forensic science education in the United States regarding methods of instruction.

Preliminary analysis of the survey indicates that most of the participants (forensic science educators) lecture during every class meeting, ask and answer student questions during class time, and engage in real-time writing as part of their instruction. Additionally, most participants indicated they never provide follow-up feedback after activities. Regarding the types of assessments used, participants identified a variety of assessments, including homework, quizzes, surveys, writing assignments, and examinations. Results from this study can be used to inform educators and administrators about future course reform efforts for undergraduate forensic science courses. Adopting student-centered, active learning instructional approaches based on best practices from research can improve student performance and overall course success.

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Education; Forensic Science; Survey

F157   **Courtroom Testimony Training for Professional and Expert Witnesses Through the Lens of a Laboratory Director—Optional or Mandatory: The Saint Lucia Experience**

Fernanda Henry*, Forensic Science Services, Saint Lucia Forensic Science Laboratory, Dennery, Dennery, Saint Lucia; Alfredo Eugene Walker, University of Ottawa, Ottawa, ON, Canada

Learning Overview: This presentation will share the Saint Lucian context and experience of expert witness training. After attending this presentation, attendees will understand the key areas of focus for expert witness testimony evaluation. Attendees will also understand the benefits of, and how to implement, expert witness testimony training in a regular and cost-effective way at a forensic laboratory.

Impact Statement: This presentation will impact the forensic science community by highlighting the benefits of expert witness testimony training that is executed consistently. This will motivate laboratory managers to ensure analysts are exposed to such training through innovative methods employed by a small island forensic laboratory that was able to attain International Organization for Standardization (ISO) 17025:2017 accreditation.

Who wants to be an expert witness? Those of us trained in the various specialties of forensic science are assigned this role by default, but are we really ready? Are we ready to play our part in the theatre of the courtroom? Because that is just what it is, a world completely different from the scientific world we are so comfortable in and accustomed to. An isolated world where scientific literature rules the day, and we can easily find "evidence" to corroborate or refute a scientific theory. However, in the courtroom, it is a battle of the minds, a battle of words. Who is the most articulate? Who is able to dissect procedure and quality in a manner that is easily understood, so that the trier of fact can get a glimpse of what transpired in the laboratory or the autopsy suite?

Forensic professionals are carefully and expertly trained in procedure and execute it to best quality practice. We are certain we did our work the right way and without bias. Yet, when we are confronted by a learned defense attorney or by the mere thought of having to testify under oath in a court of law, we become petrified. Courtroom testimony training then becomes the key to remaining calm and composed under immense pressure.

Generally, medical undergraduate medical students, interns, and post-internal doctors receive no courtroom testimony training. Undergraduate or graduate forensics students sometimes get an opportunity to practice with a case and an associated mock trial that is well executed in a real courtroom with a sitting judge or magistrate and the assistance of law students as "attorneys," but the theoretical training component is lacking. Unfortunately, courtroom testimony training is by far the exception instead of the norm. In the real world, most forensic experts are unprepared and seldom have pre-trial conferences with the prosecution. The anxiety and mental anguish run so high that the most intelligent medical or forensic professional can appear incompetent and incoherent, especially by aggressive lawyers.

The "expert" must be armed through appropriate training to recognize the tactics commonly employed by defense attorneys in order to be ready to respond. This presentation seeks to share the Caribbean experience of courtroom testimony training. It will highlight key areas of focus for evaluating expert witness testimony and incorporate how we adapted to a virtual mode of training due to the constraints of the COVID 19 pandemic.

Expert Testimony; Accreditation; Evaluation

F158   Investigating Potential Factors That Can Impact Students' Professional Identities in Forensic Science

Abigail Castillo*, University of Central Florida, Burke, VA; Cameron Bechard, University of Central Florida, Orlando, FL; Tamra Legron-Rodriguez, University of Central Florida, Orlando, FL; Nicole Lapeyrouse, University of Central Florida, Orlando, FL

Learning Overview: The goal of this presentation is investigating and identifying potential factors that may impact a student's professional identification within forensic science.

Impact Statement: By investigating student identity within their desired professional field, this presentation will impact the forensic science community by seeking to aid and increase retention rates in Science, Technology, Engineering, and Math (STEM), focusing on forensic science.

There has been an increased interest in researching students' professional identities as a potential factor that can improve student retention. Professional identity is how an individual defines themselves within their profession. There is a gap in the literature investigating forensic students' professional identities. In 2015, Mancini et al. validated and published the *Professional Identity Status Questionnaire (PISQ-5D)*.[1] This survey measures five social constructs to discern how an individual may identify within their chosen field of profession and was administered to undergraduate and master students within the field of psychology.[2-4] However, in recent years, the survey has been modified and adapted for use in other professions such as nursing, social work, and STEM.

For this study, the survey language was modified to include the phrase "professional in your field" as well as the addition of open-ended questions. The open-ended questions were added to the survey to gather more in-depth knowledge on how students were identifying as professionals in the field of forensic science. Undergraduate forensic science majors in an introductory forensic science course and senior-level course were given this modified survey to expand the understanding of how students relate to their future forensic science profession. Thematic analysis was used to analyze the open-ended responses.

This presentation will focus on the analysis of the open-ended questions, which will allow for further insight into what may impact a student's professional identity in forensic science. This study has identified several themes among students. One such theme was that some of the students felt prepared and confident to identify as a professional in forensics, whereas there were still a high number of students who held a large degree of uncertainty within the students' confidence in their professional identity due to multiple factors such as job insecurity and excessive workloads. Furthermore, additional students mentioned finding themselves questioning a lifetime commitment to forensic science. The importance of this research will aid in identifying key components that could help students' connections within the field of forensics which would result in increased rates of student interaction through searching out new opportunities and relationships with others and self-connection within the field of forensic science.

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Identity; Education; Qualitative

F159   Bridging the Gap Between Academia and Police Casework

Sofia Goia*, The University of Warwick, Coventry, England

WITHDRAWN

F160   International Forensics Summer School (IFOSS): Fostering Interdisciplinary Excellence in Forensic Sciences, Including Multimedia Forensics and Biometrics, in Its Second Edition

Sebastiano Battiato*, University of Catania, Catania, Sicilia, Italy; Donatella Curtotti, University of Foggia, Foggia, Puglia, Italy; Giovanni Ziccardi, University of Milan, Milano, Lombardia, Italy

Learning Overview: After attending this presentation, attendees will have gained valuable insights into the IFOSS and its role in fostering excellence in forensic sciences. The overview will highlight the significance of continuous interdisciplinary training in addressing the ever-evolving challenges of modern investigations. Attendees will be informed about the diverse disciplines covered in IFOSS, such as Multimedia Forensics and Biometrics, and how they equip professionals to combat image and video manipulation, face and fingerprint recognition, and signal falsifications.

Impact Statement: This presentation will impact the forensic science community in the following ways

Skill Advancement: Equipping attendees with advanced knowledge and skills in various forensic disciplines, including Multimedia Forensics and Biometrics

Interdisciplinary Collaboration: Fostering collaboration among academics, practitioners, law enforcement, and young researchers, promoting the exchange of ideas and best practices

Regulatory Understanding: Exploring international regulations, such as the European (EU) Artificial Intelligence (AI) Act, to navigate legal and ethical aspects of incorporating AI technologies in investigations

Enhanced Law Enforcement Tools: Showcasing specialized tools and techniques from police forces, ensuring agencies stay at the forefront of forensic investigations

Real-World Case Studies: Sharing authentic case studies for practical understanding of forensic challenges and solutions

Networking Opportunities: Connecting professionals from diverse backgrounds to facilitate ongoing collaborations and knowledge sharing

Inspired Future Professionals: Empowering and inspiring the next generation of forensic experts to contribute to the field's development and innovation.

The IFOSS held in Sicily in July 2023 remains steadfast in its commitment to advancing excellence in forensic sciences through continuous training and interdisciplinary knowledge.[1] In the second edition, IFOSS elevated its curriculum, addressing critical issues in the field, including the proliferation of deepfake technologies and the far-reaching implications of AI, underscored by international regulations such as the EU AI ACT.[2]

Distinguished by its inclusivity, the IFOSS successfully brought together academics, practitioners, law enforcement, and young researchers, fostering a collaborative learning environment where ideas and expertise converged. Participants had the unique opportunity to explore cutting-edge topics like Multimedia Forensics, delving into image and video manipulation detection and Biometrics, focusing on face and fingerprint recognition, and combating signal falsifications. One of the highlights of the second edition was the active involvement of various police forces, who showcased their specialized tools and techniques, addressing the pressing challenges posed by technological advancements. Experts from law enforcement agencies shared valuable insights into their experiences and provided real-world case studies, enriching the learning experience for all participants.

Throughout the two editions, nearly 90 enthusiastic students attended the school, concluding the course with dedicated homework assignments that further solidified their understanding of the material covered. These young minds left the IFOSS inspired and empowered, ready to apply their newfound knowledge and skills to contribute positively to the field of forensic sciences.

The IFOSS's impact reaches far beyond the classroom, as its alumni have gone on to make meaningful contributions to forensic research, investigations, and the development of new forensic technologies. The summer school's unique blend of theoretical and practical education has proven to be instrumental in nurturing a community of professionals who are adept at tackling emerging challenges in the ever-evolving landscape of forensic sciences. By continuing to address critical issues, embracing innovation, and promoting international cooperation, the IFOSS stands as a beacon of excellence in forensic training, empowering individuals to uphold the highest standards of justice and integrity in their pursuit of truth and resolution. As the field of forensic sciences continues to expand, the IFOSS remains committed to providing a platform for continual learning, collaboration, and growth, shaping the future of forensic investigations and ensuring a safer and more just society for all.

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Education; Forensic Science; Multimedia

F161   The African Forensic Sciences Academy (AFSA)—Transforming the Forensic Science Landscape in Africa

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Learning Overview: The goal of this presentation is to provide insight into the establishment of the AFSA. Its purpose and role in forensic sciences in Africa and how it will support and serve forensic science practitioners in Africa will be outlined. Its role in the international forensic sciences community will be indicated.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the AFSA has the potential to transform the forensic sciences landscape in Africa. In the past, the practice of forensic sciences in Africa was fragmented. The AFSA can change that paradigm as the first formally registered body that represented the interests of all forensic science practitioners in Africa.

Prior to the founding of the AFSA, Africa had no formal, officially registered, and recognized representative body for its forensic science sector. This hampered several efforts on the continent, including the establishment of a robust network to support forensic science practitioners from all fields in forensic science.

The AFSA was formally registered in March 2023 as an International Non-Governmental Organization (INGO) in Rwanda, where it is officially hosted by the Rwanda Forensic Institute (RFI) in Kigali.[1] Its INGO status was conferred by the Rwanda Governance Board (RGB), which also provides independent oversight.

At the outset, it was decided to adopt appropriate best practice from around the world. To this end, definitions such as "African" were adopted from the African Union (AU) and "forensic science" was defined as outlined in the Sydney Declaration.[2] Specific forensic science categories that are recognized by the AFSA was adopted from the Organization of Scientific Area Committees (OSAC), but adapted to suit our continental needs and reality.[3] To this end, categories for Forensic Pathology, Clinical Forensic Medicine, Forensic Psychiatry, and Forensic Imaging were added to the Medicine category fields recognized by the AFSA. The AFSA is considering adding Forensic Art to this list. In addition, the biology category was divided into Human Forensic Biology and Non-human Forensic Biology. The AFSA recognizes the 55 member states of the AU.[4]

The AFSA experience in unique in the global forensic sciences landscape, especially when working across 55 different countries, thus jurisdictions, with over 2,000 living languages. Prior to its founding, this aspect was strategically considered and discussed at its founding meeting. It was evident to the Founding Members that with the AFSA, the opportunities far outweighed the challenges, although the challenges are significant. Pragmatic decisions had to be made in terms of the diversity within the continent at many levels (e.g., adopting English and French as the main languages within the AFSA, with English being its operational language).

The AFSA exists to support forensic science practitioners throughout Africa. Among other roles for the AFSA, it aims to make the forensic science work conducted in Africa visible to the global community in our field. In time, it will also provide a credible referral system in terms of expertise and experience across Africa. In the first four months of its existence, the AFSA has recruited members (ongoing), started to put organizational structures in place, built its network across the globe, and is starting to serve as a "first point of contact" for forensic sciences in Africa.

Forensic sciences are often not at the forefront when conflict, justice, science, or society is discussed in Africa. The AFSA aims to change that by highlighting the role that forensic science is playing in these areas throughout the continent. An effective AFSA can deliver high impact via forensic sciences across the continent in order to deliver justice for all in Africa.

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International; Forensic Science; Geographical

F162   An Evaluation of Italian Medical Students' Attitudes and Knowledge Regarding Forensic Medicine: Could This Course Guide the Student in Choosing the Residency Program?

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Learning Overview: After attending this presentation, attendees will understand the knowledge about, and attitudes toward, the medicolegal relevance of forensic medicine concepts, their opinions regarding the course, and the impact of the course on students' knowledge and attitudes in choosing a medical residency.

Impact Statement: This presentation will have an impact on the forensic science community as it could serve as a key to understanding what the strengths and weaknesses in forensic science courses during undergraduate courses are and to implement the awareness of "future doctors" regarding the forensic medicine.

Introduction: Forensic medicine is the branch of medicine that deals with the application of medical knowledge to legal questions. In Italy, forensic medicine does not only consist of medicolegal autopsies and forensic pathology, but, as a medical science, can be implied in many different fields such as: ,(1) cases of work-related accidents, due to fortuitous, violent and external events, that produce traumatic injuries; (2) road traffic accidents, events in which vehicles, human beings, or animals are involved and which result in injury to individuals; and (3) medical liability, when, during the provision of medical services, a medical error (diagnosis or treatment) causes harm to a patient. In Italy, the forensic physician determines the percentage of damage (biological damage) suffered by a patient as a result of erroneous medical treatment. For all these reasons, it is understandable why the role of the forensic physician is often misunderstood. This study aims to assess students' knowledge of forensic medicine concepts and their opinions regarding the course taught during the degree course in Medicine and Surgery in an Italian university. Consequently, the study aims to assess the impact of the course on the students' knowledge and attitude in choosing a medical residency.

Materials and Methods: A cross-sectional study was conducted through an anonymous web-based survey of medical students at the Università Cattolica del Sacro Cuore. Descriptive and univariate analysis were conducted.

Results: In all, 1,166 students completed the survey. The forensic medicine course appears to be extremely important in providing training in the fundamental concepts of forensic medicine, especially professional liability, defensive medicine, and forensic pathology.[1] Attending autopsies is important during the course, and there should be more than ten attended. Additionally, univariate analysis demonstrates that students' awareness of forensic medicine topics, such as the function of the forensic physicians, professional liability, and defensive medicine, improves in the fifth or sixth year of the course. After the course, more students would choose to pursue a non-clinical and non-surgical specialty, and forensic medicine specifically, for their residency.

Conclusions: The course appears well placed in the first semester of the fifth year where it is able to guide the choices of future medical practitioners toward the specific medical field and specialization. Autopsies, and forensic pathology in general, appear to be an extremely well-known and relevant component of medicolegal professionalism, preceded only by knowledge of the risk of medicolegal litigation. For the students of medicine and surgery, attending autopsies is important during the course, and there should be up to ten autopsies attended. However, few students felt that the number of autopsies they witnessed was adequate. For these reasons, within the medical and surgical course, it would be necessary to implement the number of internship hours devoted to autopsies. For these reasons, within the medical and surgical course, it would be necessary to implement the number of internship hours devoted to autopsies to better define the objectives of autopsy and, through this, implement their clinical knowledge.[2] Thus, the forensic medicine course has the task of defining the duties and responsibilities of forensic physicians, providing indispensable tools for future medical practitioners, regardless of their future specialization, and limiting the increasing use of defensive medicine. In conclusion, we recommend further multicenter studies to evaluate the role and direct effect of undergraduate forensic medicine courses on students and on the quality of practice.

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Forensic Medicine; Medical Education; Residency Programs

F163 A Comparative Forensic Education Via a Study-Abroad Approach: Pedagogical Experiences in the United Kingdom, Scotland, and Canada

John Z. Wang*, California State University-Long Beach, Artesia, CA

Learning Overview: After attending this presentation, attendees will know how to conduct a comparative forensic course via a study-abroad approach. The following six main topics will be discussed: (1) selection of a targeting country, (2) initial agreement, (3) budget details, (4) pedagogical contents, (5) comparative focuses, and (6) end results.

Impact Statement: This presentation will impact the forensic science community by providing a comparative method on forensic education via a study-abroad approach so that our future work force will have a broader understanding, knowledge base, and skill set and better abilities to address more forensic challenges. Attendees will learn the rationales, steps, coordination, and potential issues in conducting a study-abroad course.

One of the critical issues that the forensic science community is facing is to examine evidence from transnational crimes, such as rapid determinations of white powders in drug trafficking, latent fingerprints on metals in weapon smuggling, and difficult interpretations of DNA electropherograms. In many situations, a successful criminal investigation and conviction in both countries requires a mutual understanding of standards of the legal systems, technical methods of forensic examinations, and procedures of a cross examination in a targeting country. For this purpose, there is a strong need to conduct a comparative forensic course so that our students can go to a selected country and learn the necessary legal principles, practical examination skills, and prepare themselves for their future job requirements.

Based on the author's 13 years of experiences in the United Kingdom, Scotland, and Canada, several benefits can be summarized. First, from a comparative perspective, a study-abroad course allows the students to process mock crime scenes, visit crime labs, conduct evidence examinations in university labs, testify in mock trials, and observe criminal trials under a different system. Second, a study-abroad course may enrich our students' knowledge on probative value of certain types of evidence, technical standards on different types of evidence, and strengths and weaknesses of each criminal justice system. Next, from lectures by both university professors and practitioners from local law enforcement agencies, many confusing concepts on evidence examinations can be compared and contrasted (e.g., sensitivity vs. specificity, magnification vs. resolution, validity vs. reliability, false positive vs. false negative, and positive control vs. negative control). Further, certain technical issues can also be explored and discussed, such as peak height imbalances, spikes/blobs, inhibitions as well genealogical calculations in the DNA examinations. Finally, such an opportunity allows our students to be evaluated by crime scene technicians, lab examiners, detectives, prosecutors, and defense attorneys in the hosting country. The end results include a *voir dire* resume, a testimonial check list, an international certificate, and a better understanding of comparative forensic work in the field, in the lab, and during the investigations in both countries.

Education; Forensic Analysis; Crime Scene Investigation

F164   Unveiling Forensic Science Topics for Enhanced Knowledge Discovery

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Learning Overview: The goal of this presentation is to provide attendees with a comprehensive understanding of the application of topic modeling in forensic science research. By identifying key forensic science topics and uncovering emerging trends, attendees will gain insights that can support evidence analysis, facilitate interdisciplinary collaborations, and contribute to the advancement of forensic science practices.

Impact Statement: The findings from this research have significant implications for the forensic science community. By leveraging topic modeling, researchers and practitioners can efficiently identify key topics, discover emerging areas of research, and enhance interdisciplinary collaborations. This knowledge discovery process aids in the effective utilization of forensic science in solving complex cases, promoting justice, and advancing the field as a whole.

The field of forensic science plays a critical role in the criminal justice system, and understanding the key topics within this domain is essential for advancements in the field. In this study, we employ the Latent Dirichlet Allocation, a powerful topic modeling technique, to analyze a corpus of 3,689 forensic science abstracts from the *Journal of Forensic Sciences* published between 2009 and 2022, covering various forensic categories, including anthropology, criminalistics, digital and multimedia sciences, engineering and applied sciences, pathology/biology, psychiatry and behavioral science, jurisprudence, questioned documents, toxicology, and more.[1]

Through the Latent Dirichlet Allocation algorithm, we discovered 122 distinct topics as an optimal number of topics that capture the diverse and intricate facets of forensic science research. Our comprehensive analysis effectively captured the meaningful structure of forensic data and aligned with the *Journal's* specified disciplines. Diagnostic topics reveal characteristic words for each category.[2] To illustrate, we limited our study to find the most diagnostic topics for each forensic category among the abstracts in the year 2020. We provided diagnostic topics with corresponding terms and compared the defined categories. Our findings reveal certain topics as reasonable diagnostics for Questioned Documents, Odontology, and Jurisprudence.

Furthermore, by averaging the topic distribution across years, we aim to identify dynamically shifting topics that showcase evolving scientific interests. This helps us identify "Hot" and "Cold" subjects, referring to topics that have shown a significant increase or decrease in popularity over time.[2] The model found seven topics, of which three topics are identified as the hottest, closely related to Toxicology, Criminalistics, and Jurisprudence. Conversely, the coldest topics are closely related to Biology, Pathology, Odontology, and Anthropology.

Overall, our study provides valuable insights into the content, trends, and dynamics of forensic science research, offering opportunities for evidence-based decision-making, interdisciplinary collaborations, and advancing the field through enhanced knowledge discovery. The application of Latent Dirichlet Allocation allowed us to uncover hidden themes, understand the significance of diagnostic topics, and explore the changing landscape of forensic science research over the years. Our research contributes to the advancement of the forensic science field by providing a deeper understanding of its key topics and their evolution over time.

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Forensic Science; Machine Learning; Statistics

F165 Macro, Meso, Micro: Core Concepts in the Development of Forensic Science

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Learning Overview: After attending this presentation, attendees will better understand several core concepts established over the course of forensic science's history and development that relate to its scale, scope, and domain as a science.

Impact Statement: This presentation will impact the forensic science community by helping attendees develop a better appreciation for and understanding of the development of forensic science and how it relates to other sciences as a separate discipline.

Forensic science has seen several watershed concepts that have led to its current operational state. The first was in 529, when Emperor Justinian approved the *Codex Constitutionum*, an extensive reconfiguration of old and new Roman law. In it, doctors were acknowledged as witnesses who could provide judgments based on this expertise and not only on eyewitness testimony; today, this privilege is called being an expert witness.[1] The second was in 1883, when Hans Gross published arguably the first forensic textbook, *Handbuch für Untersuchungsrichter als System der Kriminalistik* (*Handbook for Examining Magistrates as a System of Criminalistics*). Gross recognized that it was the crime scene and not the body that was of paramount importance in a criminal investigation. His book designated the crime scene "as a distinct analytical space, bounded conceptually and operationally by explicit rules of practice, and recognized as such by forensic investigators and the broader public alike."[2] The third was Alphonse Bertillon's creation of the first forensic database used in his anthropometric technique in the mid-1890s. Anthropometry allowed for comparison to the database of measurements, but only if the person of interest was present to be measured. The fourth discussed in this presentation is the use of proxy data, like trace evidence and fingerprints, that allowed for the comparison of evidence that was left behind to a set of records without the person needing to be present. Although Faulds and Henry considered the potential of fingerprinting in criminal investigations, it was Edmund Locard who realized Gross' dream of making the crime scene and its contents the focus of forensic activities. With his access to the criminal files that contained fingerprints of arrestees, Locard could now identify individuals who had been at a crime scene without them having to be present. Additionally, this allowed for the recognition of a fingerprint left at a scene or on an object to be the goal of a search in the files (the database) with no person of interest identified.[3] These core concepts delineate the scale of forensic science from the macro (doctors and the deceased body) to meso (the scene) to micro (trace evidence and fingerprints) across its development.

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Expert Testimony; Crime Scene Investigation; Fingerprint

F166 Turn RIGHT: A Roadmap to Improving Research and Technology Transition in Forensic Science

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Learning Overview: After attending this presentation, attendees will better understand the challenges that limit transition of forensic research and technologies into operational laboratories and steps they may take to foster a culture of Research, Development, Testing, and Evaluation (RDT&E) strategies.

Impact Statement: This presentation will impact the forensic science community by shedding light on the perspectives of various forensic science community members to explain why the barrier to implementing forensic research and new technologies in operational laboratories is high. This presentation will also help attendees consider realistic and actionable approaches to improving transition success.

The development, validation, and subsequent adoption of forensic technology are critical steps to continuous improvement in the forensic community. Successful technology implementation stems from productive research and development to address practitioner needs, testing and evaluation to improve the technology, validation of the technology, and finally broad adoption across multiple Forensic Science Service Providers (FSSPs). However, realities of the forensic science community can often lead to siloed efforts and limit the productive partnerships that drive the creation of value-adding technologies. As a result, innovative technologies do not make the transition from research product to implementation and end up in one of many "valleys of death" that limit broad adoption, despite strong forensic community motivation toward RDT&E.

The Forensic Laboratory Needs Technology Working Group (FLN-TWG), formed by the National Institute of Justice (NIJ) in partnership with its Forensic Technology Center of Excellence (FTCOE) at RTI International, leveraged perspectives across the forensic community to identify issues limiting forensic research transition and propose a path forward to bridge these "valleys of death." The roadmap recognizes and details actionable steps that various community players can take to move toward improvement of research and technology implementation and promote strategies of "evidence to action" in the forensic sciences.

The pathway to bridge "valleys of death" to improve research and technology transition requires cultural change and engagement across forensic community members, including FSSP leadership, FSSP research scientists and practitioners, academic institutions, industry-based technology developers, and conveners/enablers (e.g., professional organizations, governmental agencies, early adopters). This presentation will summarize critical action areas that may drive transition of technology into casework, including: (1) enhancing FSSP and academic leadership buy-in to create a culture that prioritizes RDT&E; (2) equipping FSSPs with the resources, policies, and processes to streamline RDT&E processes; (3) sharing knowledge to improve alignment of community RDT&E efforts to operational needs; and (4) developing partnerships that lay the ground work for productive FSSP engagement with academia, industry, and other laboratories.

Technology Transition; Research; Valleys of Death

F167 Detecting Illicit Use of AI and Other Cheating in Forensic Student Submissions: Ensuring Justice for All Student Assignments

Stewart Walker, Flinders University, Adelaide, South Australia, Australia*

Learning Overview: After attending this presentation, attendees will gain a better understanding of challenges facing forensic science educators with the options for students to use a number of new ways of cheating, including search engines, report writing and editing services, and Artificial Intelligent (AI) sites. Attendees will also be aware that the same resources that are available for students to cheat are also useful for academics to detect cheating.

Impact Statement: This presentation will impact the forensic science community by demonstrating the importance of being aware of potential for cheating using modern technology and also the ways in which academics can use modern technology to detect and catch cheaters and ensure justice for all students—so the cheaters do not gain an advantage over the non-cheating students

Cheating is nothing new, but the way in which students can attempt to obtain an advantage over other students—and the system—by using new methods of gaining assistance with preparation of assignments has changed recently. Copying from other students and engaging assistance to write and edit have always been available but have been restricted to those in the immediate vicinity. Now with widespread internet, fast download speeds, remote writing and editing service providers, and AI report writing, the options to cheat and get away with it have increased.

Those of us who remember library document retrieval where requests for documents held in other repositories were made, actioned, and followed up by posting copies of the document realize that in order to request a document, you had to know it existed and where it existed in the first place. The ability to type a few words into a search engine and receive—almost immediately—information of what and where in the world material related to the topic can be accessed is a great step forward. Like all technology it can be used for good or for evil. Globalization of material with microsecond retrieval times may be an advantage to academics preparing material for classes or publications but also opens opportunities for an unprecedented level of access to remote sites and material to students to use or misuse. Part of the process of using this technology is to be able to identify the good material from the chaff, and this is something that gives the academic an advantage over a student who is not well-versed in the topic and cannot distinguish between "good info" and "dodgy info." The internet provides advantages to cheat but also advantages to catch cheaters.

This presentation will give examples, from the presenter's experience, of use and misuse of modern technology, including search engines, report writing and editing services, and AI-derived materials. Some examples are: (1) a student who requested "help" from a web-based tutor and the tutor who the request went to happened to be a graduate who recognized the questions as being one of my Honors assignments; (2) a student who answered a question worth 5% with four pages of random information—the answer was in there somewhere; and (3) a student in June 2023 who included the keywords and discussion they had had with the AI site in their submission; and the two answers submitted for the one question were both wrong anyway.

From this presentation, attendees will have an understanding of the ways in which such opportunities can be used by students and ways in which academics can detect such use.

Education; Student; Artificial Intelligence

F168   The National Institute of Justice Research and Development Program

Lucas W. Zarwell, Office of Investigative and Forensic Sciences, Washington, DC*

Learning Overview: After attending this presentation, attendees will be informed about the National Institute of Justice's (NIJ's) research and development programs and how to get involved. Attendees will learn about existing resources, current activities, recent research awards, and future plans from the NIJ.

Impact Statement: This presentation will impact the forensic science community by informing attendees of a program designed to support a culture of research across the forensic science community by familiarizing everyone with NIJ's resources and programs. The mission of the NIJ's Office of Investigative and Forensic Science (OIFS) is to improve the quality and practice of forensic science through innovative solutions that support research and development, testing and evaluation, technology, and information exchange for the criminal justice community.

The NIJ is the research, development, and evaluation agency of the United States Department of Justice, dedicated to improving knowledge and understanding of crime and justice issues through science. As the lead federal agency for forensic science research and development, the NIJ, through the OIFS, sponsors extramural research projects across the spectrum of forensic science disciplines, coordinates with the stakeholder community to identify needs and develop resources, and facilitates technology transfer and implementation of emerging technologies into forensic practice. This presentation will discuss the NIJ program updates, including an overview of the Research and Development (R&D) portfolio, and communicate how forensic practitioners can get involved in research initiatives to improve their laboratories. The goal of the NIJ is to better inform laboratory management about research trends, technologies, partnership opportunities, and federal efforts in hopes that the knowledge leads to increased forensic laboratory collaboration and capabilities.

The NIJ will discuss significant projects developed by the Forensic Technology Center of Excellence (FTCOE), the Research Forensic Library, the National Center on Forensics (NCF), and working groups such as the Forensic Laboratory Needs Technology Working Group (FLN-TWG) and the Research and Development Technology Working Group (FSRD-TWG). In addition, the NIJ will provide updates about upcoming projects, OIFS collaborations with the Center for Disease Control (CDC), the National Science Foundation (NSF), and other components within the Office of Justice Programs (OJP).

Finally, this presentation will demonstrate how the NIJ is seeking to advance a culture of research to advance forensic science. The mission of the NIJ OIFS is to improve the quality and practice of forensic science through innovative solutions that support research and development, testing and evaluation, technology, and information exchange for the criminal justice community. The NIJ invites feedback from the American Academy of Forensic Sciences (AAFS) community on ways our agency and partners can further strengthen the current and future practice of the forensic sciences.

Grants; Research; Forensic Science

F169   Mass Fatality Response and Disaster Infrastructure Among the Nation's Medical Examiner and Coroner Offices, Data From the 2018 (CMEC)

Micaela A. Ascolese*, RTI International, Cary, NC; Kelly Keyes, RTI, International, Yorba Linda, CA; Hope M. Smiley-McDonald, RTI International, Research Triangle Park, NC; Jeri D. Ropero-Miller, RTI International, Creedmoor, NC; Sean Wire, RTI International, Baltimore, MD

Learning Overview: After attending this presentation, attendees will be aware of results from the 2018 Census of Medical Examiner and Coroner Offices (CMEC) as it pertains to mass fatality and disaster planning trainings and resources. Attendees will be given background information on mass fatality response and disaster infrastructure and response among the nation's Medical Examiner and Coroner (MEC) offices, gain insight into the findings of the study, and see recommendations for next steps.

Impact Statement: Because of the rise in mass fatality incidents, it is important to bring awareness to the United States' current mass fatality response and disaster infrastructure. The forensic science community, specifically those involved in mass fatality and disaster response, such as law enforcement, medical examiners, coroners, death investigators, and other professionals, will be impacted by and can benefit from data found in this study.

With the rise of Mass Fatality Incidents (MFIs) from natural disasters, mass shootings, and epidemics, it is important to review the mass fatality and disaster response resources available to MECs in the United States. This study provides a timely update regarding the scope of these resources since the Bureau of Justice Statistics' (BJS') 2007 seminal report and analyzes data from the 2018 CMEC.[1] Data included questions to respondents regarding access to mass fatality and disaster planning trainings and resources, either directly or through a partner. MECs were also asked whether their office participated in county/statewide emergency response drills.

Findings included a high percentage of respondents having access to mass fatality and disaster planning trainings and resources. Although the numbers regarding this access was higher than anticipated, they align with Gershon et al.'s study reporting that 95% of agencies had an MFI plan in place.[2] In addition, respondents in the Midwest and South as well as those with smaller populations, had less access to resources. Last, agencies with larger budgets and more full-time staff had more access to resources. Overall, it should be noted that all of these factors (population, budget, and employment) are likely correlated with larger, busier, and better-funded agencies having more resources. It is difficult to single out one contributing variable for MEC offices possessing more mass fatality and disaster response resources than others.

Although this analysis discusses potential contributing factors for these results, the data only begins to address gaps in mass fatality response and disaster infrastructure for MECs, and further research should be conducted. Acknowledging any limitations, more research should be conducted on mass fatality and disaster infrastructure as new types of challenges and incident types arise. Overall, this analysis seeks to give an overview of MEC's mass fatality response in the United States, offer recommendations for future CMEC surveys, and identify future advancements for responder policies and practices.

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Emergency; Census of Medical Examiner and Coroner Offices; Data

F170 Forensic Science Standards: A Look at Conformity Assessment and Implementation Models in the United States and Abroad

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Learning Overview: After attending this presentation, attendees will have insight about the forensic quality standards framework in the United States and other countries and how these standards fit into conformity assessment. Specifically, attendees will know about the opportunities and challenges of standards implementation in the United States model of self-declaration and the resources available to enhance it.

Impact Statement: This presentation will impact the forensic science community by informing attendees that from the crime scene to the courtroom, having valid and reliable forensic science is essential. Standards help to enhance the validity and reliability of results—but they are only beneficial if they are used. Over 150 forensic science standards are available on the Organization of Scientific Area Committees (OSAC) Registry. Implementing these standards can help to improve consistency within and across forensic science disciplines and ensure confidence in the accuracy, reliability, and reproducibility of results.

Standards and conformity assessment are important to strengthening the science and quality of forensic science. Accreditation is one type of conformity assessment activity. Traditional forensic laboratories are often accredited to the **International Organization for Standardization**/International Electrotechnical Commission (ISO/IEC) 17025 standard. In addition, ISO/IEC 17020 can be used for scene examination with examples noted in Europe and the United States. Internationally, there is a supplemental guidance document to ISO/IEC 17025 and 17020, issued by the International Laboratory of Accreditation Cooperation (ILAC) G-19:2022. Many accrediting bodies have developed their own supplemental requirements in addition to ISO/IEC 17025 and 17020 due to the lack of availability of documentary standards in this sector. This creates an environment in which different standards and requirements are being used for accreditation.

While most other nations have a single accrediting body, the United States has many. In the United States, third-party accreditation is privatized and decentralized, like its standards development system. The United States does not regulate forensic science standards and accreditation at the federal level except for DNA laboratories that produce profiles eligible for the Federal Bureau of Investigation's (FBI's) Combined DNA Index System (CODIS). For other forensic science disciplines, the standards implementation model is based on self-adoption and self-declaration.

To address the standardization gap, the United States' National Institute of Standards and Technology (NIST) administers the Organization of Scientific Area Committees (OSAC) for Forensic Science, which is a collection of over 800 volunteers with diverse subject matter expertise, divided into 20+ forensic science discipline-specific subcommittees. OSAC provides the nationwide infrastructure to facilitate the development and promote the use of high-quality, discipline-specific forensic science standards. These standards are designed to be more prescriptive than ISO/IEC 17025 and ISO/IEC 17020 to further standardize laboratory activities.

Forensic science is not regulated in the United States, which makes standards implementation a voluntary, not required, activity. Voluntary implementation of standards by forensic laboratories is a challenge, given their extensive caseloads. Training, tools, and technology investments are being made to lower the barriers to the implementation of these important standards. There are over 150 forensic science standards on the OSAC Registry and forensic science service providers are encouraged to use them in their everyday practice. Over 120 forensic laboratories in the United States (and internationally) have voluntarily implemented standards from the OSAC Registry, helping to improve the forensic science field through standardization.

Standards; Implementation; Conformity Assessment

F171 First "One Stop" Sexual Assault Center in Turkey

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Learning Overview: After attending this presentation, attendees will understand that considering that a thoughtfully planned and well-executed design with the cooperation of forensic science experts and architects can significantly impact the efficiency, accuracy, and credibility of forensic investigations, we designed and put in function the first "One Stop" Sexual Assault Crisis Center in Istanbul, Turkey. Medical, physical, legal, and psychological care is provided together with all required legal procedures for all vulnerable sexually assaulted victims and survivors.

Impact Statement: This presentation will impact the forensic science community by informing attendees of the details about the first interdisciplinary "One Stop" Sexual Assault Crisis Center established in Istanbul, Turkey, by the close collaboration of forensic science experts and architects to form a best example of such a facility.

Sexual violence and assault have a wide range of negative consequences. The treatment of the victims requires special organization and care. Evidence-based crime combat and prevention is the key element for public safety. Therefore, proper evidence collection with minimum harm to the victim is essential.[1,2] Although special Sexual Assault Crisis Centers are established to provide care for the traumatized individuals in most countries all over the world, no such a facility has been organized in Turkey, and the victims have to visit various institutions one after the other to provide evidence.

The Forensics Department of Istanbul Yeni Yüzyıl University undertook the responsibility to organize the first "One Step" Center in Istanbul, the most crowded city in Turkey with more than 16 million people. The main objective of the center is to achieve a single application center and save the victims from re-victimization due to several visits to the police, hospitals, and the prosecution office.[3-5] Vulnerable groups, such as children and mentally disabled people, together with all sexually assaulted or victimized citizens can apply for care. The facility is organized in an interdisciplinary approach providing medical, legal, and psychological care for the victims in a specially designed complex according to the latest international standards.

Architectural design in such facilities is even more critical when it comes to accommodating vulnerable people such as victims, witnesses, or suspects who may require special considerations due to their physical, emotional, or mental conditions. Moreover, a well-designed layout can ensure that the flow of evidence through different stages of analysis is smooth, logical, and efficient. This helps minimize contamination risks and ensures that evidence is processed in a timely manner. In this respect, with the collaboration of forensic scientist, psychologists, social workers, medical doctors and lawyers, the workflow optimization, contamination control, security and access control, safety considerations, ergonomics and efficiency, regulatory compliance, including International Organization for Standardization/International Electrotechnical Commission (ISO IEC) 17025 accreditation standards, controlled access points, air filtration systems, and separate workspaces for different types of analyses to reduce the risk of cross-contamination have been taken into consideration by the architects.

As clinical support will also be the main part of the provided services, collaboration between different forensic disciplines is essential for comprehensive investigations. Our design encourages communication and interaction among scientists from various fields to enhance the overall effectiveness of the laboratory. Additionally, such a well-designed, state-of-the-art facility can instill confidence in the public and legal community regarding the accuracy and reliability of forensic evidence; therefore, we paid attention to this too. We expect that this center will also serve as a discouraging effort for the perpetrator once it is focused on disclosing the various rape cases by the use of correct evidence collection and evidence analysis.

The aforementioned establishment is the first in Turkey, and we hope that it can form an architectural as well as workflow-based model for further initiatives all over the country so that the re-victimization of the assaulted will be diminished and crimes will be solved.[6]

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Sexual Crimes; Architectural Design; Istanbul, Turkey

F172 Challenges and Solutions for the Implementation of Standards in the Non-Traditional Forensic Setting

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Learning Overview: After attending this presentation, attendees will understand the many challenges associated with the implementation of standards in the non-traditional forensic setting. Once this topic is introduced, various strategies supported by real-world success stories will be provided to demonstrate that this process is not as difficult as it appears, and implementation has various benefits.

Impact Statement: This presentation will impact the forensic science community by informing attendees that approximately more than 10,000 forensic units are operating outside of the roughly 400 traditional forensic laboratories often cited in various reports. The implementation of standards in these units would have a major impact with respect to ensuring that high-quality work is being performed and consistent results are being obtained with the overall goal of ensuring justice for all.

Concerns raised by the 2009 National Academy of Sciences' (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward*, included insufficient scientific rigor and a lack of standardization among many disciplines.[1] The Report opened the eyes of the law enforcement and criminal justice communities to revisit the forensic sciences and seek solutions that would mitigate many of the issues raised.

In 2014, the National Institute of Standards and Technology (NIST), in conjunction with the National Institute of Justice (NIJ), initiated the Organization of Scientific Area Committees (OSAC) for Forensic Science. The goal of this organization was the formalization of a coherent system of forensic science working groups to replace the patchwork of Scientific and Technical Working Groups (SWGs and TWGs) that had previously worked, primarily on a volunteer basis, with some funding from the NIJ and the Federal Bureau of Investigation, to diligently produce forensic standards across multiple disciplines.

The benefits of this approach included, but were not limited to: (1) a more consistent source of funding for the development of forensic standards, (2) the formation and implementation of an oversight body to manage processes and ensure high-quality work product, and (3) a cross-cutting structure through which common areas of interest between disciplines (e.g., terminology, quality assurance/quality control, education and training, and approaches for analytical method validation) could be harmonized.

Initially, the primary entities targeted for better application of standards were the approximately 400 traditional Forensic Science Service Providers (FSSPs) identified by the NIJ Office of Justice Programs. Although important, this focus overlooked the thousands of "non-traditional" FSSPs (e.g., identification units and digital forensic laboratories) that support the efforts and investigations of law enforcement and the judicial system on a daily basis who can also benefit significantly from the development and implementation of scientifically sound standards. It is the latter of the two (the "non-traditional" FSSPs) that will be the focus of this presentation.

After a brief introduction and review of OSAC processes and the OSAC Registry of Standards, this presentation will articulate the benefits of implementation and will cite discipline-specific examples of standards from the "non-traditional" perspective. Additionally, the presentation will address common concerns with implementation in this sector. Many non-traditional FSSPs have engaged in the implementation process and some of these "success stories" will also be shared. Finally, the presentation will share how the OSAC Program Office can help provide tools, guidance, and support for those non-traditional FSSPs that are interested in pursuing implementation.

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Standards; OSAC; Forensic Analysis

F173   Reporting on Ethics Committee Approval in Cadaveric-Based Research Publications: An Update of a Five-Year Study

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Learning Overview: After attending this presentation, attendees will be updated on the current status of reporting of ethics approval in research publications. In addition, attendees will be updated about ethical guidelines, laws, and legislation applicable in India that was pursued with reference to the rights of the dead and cadaveric research.

Impact Statement: Who advocates for the rights of the dead in research? Research on cadaveric and skeletal remains helps medicolegal forensic anthropological investigations. This presentation will impact the forensic science community by informing attendees that learning from the mistakes and updates about the reporting status provides important suggestions to mitigate mistakes and help in developing the humanitarian approach while conducting/publishing the research on dead/skeletal materials.

Introduction: Cadaveric research plays a vital role in biomedical breakthroughs such as understanding disease and its risk factors and developing novel surgical techniques.[1,2] In advanced forensics, molecular-level and protein-level research on cadavers were used for vital information.[3] Human cadavers and cadaveric tissue are used for histological changes and isotope analysis [4, 5, 6] in the forensic context.[4-6] The ethical rights of the deceased should never be maltreated, and research involving the dead should also be subject to an ethical review.[7] All invasive investigations and procedures, when performed on living beings, are regulated by either the universal law or the local cultural norms. However, research on cadavers is controversial as regulations are either not found or found violated, if available.[8]

Objective: The present study was undertaken with two objectives: (1) to investigate cadaver-based research papers published globally and determine their status of ethics approval in research publications; and (2) to analyze the available ethical guidelines and laws in India about cadaveric research.

Materials and Methods: Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline, 490 articles out of 2,484 articles were collected from PubMed®, Medline®, Google® Scholar, and the snowball technique.[9] The research publication was retrieved using keywords mentioning Cadaver/Postmortem examinations/Autopsy/Postmortem/Skeletal remains with the Boolean terms "and" based study/research/analysis. Only original research publications published between 2015 and 2021 were considered for the study. Following PRISMA guidelines, all 490 research publication articles were thoroughly reviewed regarding the mention of the ethics committee or institutional review board approval, place of approval, and approval number, if available, and other important attributes. In the Indian context, the Indian Council of Medical Research (ICMR) ethical guidelines 2017, law governed by the Indian Penal Code (1860), and the Constitution of India were pursued in the context of rights of the dead and research on cadaveric/dead.[10-12]

Results: The status of Ethics Committee (EC) approval was found to be very poor in research publications based on cadavers. Less than 50% of cadaveric-based publications mentioned ethics committee approvals. Among the mentioned EC approvals, 60% of the publications failed and did not mention any EC approval reference number. A negative correlation was with the number of authors with mentioned EC approval.

Indian constitution and legislation provide fundamental rights for the dead, but there was no clause that mentioned or monitored research on the dead in India. Body donation for academic and research purposes comes under state-specific anatomy acts, but there are no guiding principles for conducting research on the dead in any anatomy acts. ICMR ethics guideline 2017 mentioned that forensic authority is considered as granting permission for the research. But in reality, investigating officers are in charge of dead bodies in India. Details regarding the research on cadaveric fragments/parts were not extensively covered in ICMR ethical guidelines 2017.

Conclusion: Failed to mention the EC approval and related details raise the question on humanitarian grounds. There is an urgent need to sensitize scholars using cadaveric samples to follow ethical norms in a robust manner. The International Committee of Medical Journal Editors (ICMJE), while scrutinizing research based on cadaveric samples, must thoroughly investigate ethics approvals, reference numbers, consent, and statements about the proper disposal of the samples after the research is over.

In the Indian context, there is an urgent need to develop a specific national ethical guideline exclusively for researchers while obtaining samples from cadavers, the recently dead, or from the stored anatomical collection. In addition, in countries where no specific guidelines are available in this context, researchers, publishers, and reviewers may follow recommendations for research on the deceased developed by the International Federation of Associations of Anatomists (IFAA) and Anatomy Acts very strictly.[13]

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Ethics; Cadaver; Research

F174   Current Hiring Requirements and Expectations in Forensic Science: Considerations for FEPAC, OSAC, ASTM, and Employers

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Learning Overview: After attending this presentation, attendees will be knowledgeable about forensic science service provider hiring expectations and requirements of entry-level applicants, how they engage with current education standards structures, and what metrics they emphasize or prioritize during the hiring process. Attendees will also learn how current research can be used to inform standards development organizations during their development cycles.

Impact Statement: This presentation will impact the forensic science community by contextualizing the general education-level preferences in the hiring of entry-level forensics professionals by providing much more discrete "building blocks" to inform better policies. Published research mostly relies on overall degree level/type and program type. What happens when applicants all have the same preferred degree? What gets prioritized? What happens when no applicants have the preferred degree type? What do employers consider then? This presentation provides a bit of clarity into what forensic science service providers are looking for.

Trends in forensic science education have been identified and documented before and after major milestones in the development of our field.[1-4] Central to those trends and what has now become a standard metric of measurement in applicants is education preference, either as the type of degree or type of program. This is valuable information that signals where possible changes in hiring trends and requirements will happen, but it does not take a nimble metric to adjust to changes in coursework or specific course requirements from forensic science service providers. Research has touched on specific coursework, but it is not a permanent accompanying metric with the rest of what are now traditional education preferences.[4] This limits our ability as a field to inform all stakeholders in the education standards process: Standards Development Organizations (SDOs), forensic science service providers, post-secondary forensic science programs, educators, parents, and students. Assuming all applicants possess the preferred degree type for a job at a particular forensic science service provider, what differentiates each applicant? How are hiring decisions made? What do those involved in the hiring process emphasize or prioritize when evaluating applicants? Moreover, what role do current forensic science education standards play in the hiring process? Are all employers aware of existing education standards?

A self-report questionnaire survey was designed to address education preferences in the hiring process for entry-level crime scene investigators and entry-level forensic laboratory personnel. Degree preference was examined as well as coursework amount and preference, familiarity with current forensic science programs accredited by the Forensic Education Programs Accreditation Commission (FEPAC), the weight that carries in an application, and other common metrics (internships, research, involvement with professional organizations). The survey was created in Qualtrics and distributed via personal and professional networks on LinkedIn®, the International Association for Identification (IAI) website, the American Society of Crime Laboratory Directors (ASCLD) website, the weekly American Academy of Forensic Sciences (AAFS) newsletter, and Association of Forensic Quality Assurance Managers (AFQAM) members forum post. Results indicated some familiarity with FEPAC structure and standards and also revealed that some employers do not know what FEPAC is, nor do they take FEPAC education standards into account when making hiring decisions. Results also indicated a revision in hiring practices due to some students not having all courses required by the Federal Bureau of Investigation (FBI) Quality Assurance Standards (QAS) for DNA analysts.

These details underscore the need for regular and directed conversations with forensic science service providers about hiring expectations and requirements so that those involved in the standards development process know what outreach needs to be done, what questions need to be asked of employers, and how to engage post-secondary institutions offering the forensic science programs being shaped by the standards being developed.

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Education; Standards; Forensic Science

F175 There Are 16,000 Predatory Journals: A Practical Guide to Identifying and Avoiding Deceptive Publications

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Learning Overview: This presentation will provide a brief overview of predatory journals (and predatory conferences) and the very real dangers they pose to scientific research, employment and career advancement, professional reputation, and, not least, one's credit card. After attending this presentation, attendees will understand the risks of publishing in, citing, and even reading predatory journals. Attendees will also learn various methods to identify and, hopefully, avoid these journals

Impact Statement: This presentation raises awareness within the forensic science community about the significant threat posed by predatory journals to the integrity of scientific research, impacting both authors and readers. By understanding the red flags and strategies of predatory journals, researchers can make informed decisions about where to publish their work, safeguarding their academic careers and scientific progress, and readers will learn to evaluate article sources so fraudulent research can be avoided.

The proliferation of predatory journals in the scientific publishing landscape has raised serious concerns about the integrity and reliability of scholarly research. Predatory journals exploit the open access publishing model, preying on researchers seeking to disseminate their work quickly and broadly. This presentation will provide a brief history of how predatory journals came to exist, the measures that the scientific and academic community have taken to try and educate researchers, the risks for researchers in associating with predatory journals, and, finally, ways in which the journals can be identified and, hopefully, avoided.[1]

Predatory journals exploit the open access publishing model by masquerading as legitimate journals, relying on professional-looking websites, false information, and clever manipulation. These journals charge fees to publish articles, often without providing adequate peer review or editing. As a result, articles published in predatory journals may be of poor, or even fraudulent, quality.[2] Early-career scholars are particularly vulnerable to the allure of these journals due to the pressure to publish as a means to career advancement. Publishing in predatory journals, however, undermines the credibility and impact of their research, jeopardizing academic careers and scientific progress.

Equipping researchers with knowledge about the perils of predatory journals is paramount to making informed decisions about where to submit their work.[3] By understanding the red flags and strategies employed by predatory journals, researchers can identify legitimate publishers that prioritize rigorous peer review, editorial quality, and adherence to ethical publishing practices. There are resources available to help authors identify legitimate journals, and academic and professional institutions can play a pivotal role in fostering a culture of responsible publishing by establishing clear publishing guidelines, educating researchers about predatory publishing practices, and providing resources for identifying reputable journals.

Researchers should also be aware of the risks of predatory conferences.[4] These conferences are often advertised as opportunities to present research, but they often function simply as a way to collect registration fees.

In conclusion, learning about the risks of predatory journals is crucial for researchers and readers in the forensic science community. By understanding the deceptive practices employed by predatory journals and by promoting awareness, stakeholders can protect both themselves and the integrity of scientific research.

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Predatory Journal; Peer Review; Professional Responsibility

F176 Artificial Intelligence, the Law, and Forensic Science: New Scenarios and Ethical Implications

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Learning Overview: After attending this presentation, attendees will understand how ethical competence is substantial in the use of innovative technologies, specifically Artificial Intelligence (AI), and how reflecting on this competence can be an increasingly authoritative means of making sense of experience, verifying the validity of what has been learned, including with regard to the congruence and effectiveness of actions taken in compliance with ethical principles.

Impact Statement: This presentation will impact the forensic science community by presenting a reflection on how the possession of ethical skills is fundamental to the use of innovative technologies, AI in particular, and how the possession of ethical skills can affect the responsibility the decision-making process of the professional working in forensic science.

Forensic sciences have accustomed us to a constantly transformative reading of the meaning ascribable to this discipline, the latter being in constant evolution, when the boundaries dictated by the use of innovative technologies, of AI, are constantly being redrawn, as they are, influenced by a very rapid technological evolution. AI can be defined as a discipline as well as a branch of computer science that deals with the design of hardware systems and software program systems capable of providing the electronic processor with performance that, to a common observer, would appear to be the exclusive domain of human intelligence. AI is now a component that characterizes many moments of our existence from daily and nearly "domestic" use through apps, to even more articulated use through drones at different levels of use.

Here we can configure different scenarios and correlations existing between AI and the law or especially between AI itself and criminal law. AI could make a significant contribution to forensic sciences, law enforcement activities, and criminal law, especially with regard to four specific areas: the use of decision-making algorithms for the purpose of resolving criminal disputes, predictive policing, law enforcement, and prevention of a criminal event, assessment of criminal dangerousness entrusted to predictive algorithms, to allow the profiling of an individual and identify possible behaviors also relevant under a criminal profile, evaluation of the possible hypotheses of involvement as a tool, as a perpetrator or as a victim, of a system equipped with AI for the commission of a crime.[1,2] There are numerous implications arising from the use of artificial intelligence and on its implication in the legal sphere. A number of complicated situations also appear to be emerging, attributable both to the possibility of considering AI-equipped entities as perpetrators of crimes or whether deserving instead of criminal protection and being considered victims.[3] There are still many issues to be addressed in such a complex and constantly evolving field as that of innovative technologies with implications in the ethical sphere, in criminal law, in privacy matters, and in the fundamental rights of human beings.[4]

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Artificial Intelligence; Ethics; 3D Technology

F177   Suicides in Prison: Fatalities or Predicted Tragedies?

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Learning Overview: The goal of this presentation is to, through the retrospective study of the cases of forensic medicine in Palermo, identify possible red flags in prisoners at high suicidal risk.

Impact Statement: By sharing this case, we aim to impact the forensic community and improve our understanding of red flags in prisoners at high suicidal risk

The risk of suicide in prison and in cells is still very high both in developed countries and even more so in developing ones. It represents a social problem of great impact.

In Italy, according to article 27 of our constitution, detention has the main purpose of re-education for the reintegration of the convict into society. Being able to understand in a timely manner what could be signs of a high risk of non-self-preservative actions in prisoners is an essential objective for correct re-education. According to the latest data published by the World Health Organization (WHO), the suicide rate in Italy in 2019 was 0.67 cases per 10,000 people. In the same year, the suicide rate in prison was 8.7 per 10,000 inmates on average.[1-6]

This report describes characteristics, circumstances, and histopathological and toxicological findings of three cases of suicide in prison in Palermo.

Circumstances: Case one is a 45-year-old male, first-time offender with a history of cannabinoid cocaine addiction and family abuse. At the interview with the prison's psychologist, he reported no previous acts of self-harm, but he showed anxiety, difficulty falling asleep, and difficulties with attention and concentration. He showed mood deflection and was being treated with anxiolytics and antidepressants. He was found hanged in the cell bathroom window by a belt. Period in prison was less than three mouths.

Case two is a 26-year-old male, celibate without children; his father and uncle were also in prison. He was a first-time offender with a history of cannabinoid and alcohol addiction. At the interview with the prison's psychologist, he reported previous acts of self-harm, important difficulty of social integration with the other prisoners, anxiety, difficulty falling asleep, and frequent visits to the nursing home for diffuse and ill-defined somatic pain. He was being treated with anxiolytics and antidepressants. He was found hanged in the cell's bed by a sheet. Period in prison was less than three mouths.

Case three is 30-year-old male, a second-time offender with a history of family abuse. At the interview with the prison's psychologist, he reported no previous acts of self-harm, but he showed anxiety and difficulty falling asleep. He was being treated with anxiolytics and antidepressants. He was found hanged in the cell window by suit trousers. Period in prison was less than three mouths.

Histopathological and Toxicological Findings: Case one: The cadaveric external examination showed mild cranio-cephalic congestion and a soft hanging mark on the neck. The autopsy showed classical signs of hanging with a soft hanging mark and pulmonary emphysema. Toxicological investigations confirmed the use of benzodiazepines and antidepressants.

Case two: The cadaveric external examination showed important cranio-cephalic congestion, a hanging mark on the neck, and distal hypostases. The autopsy showed classical signs of pulmonary emphysema and thin intimal laceration of the left carotid artery "Amussat's sign." Toxicological investigations confirmed the use of benzodiazepines and antidepressants.

Case three: The cadaveric external examination showed mild cranio-cephalic congestion and a hanging mark on the neck. The autopsy showed classical signs of pulmonary emphysema. Toxicological investigations confirmed the use of antidepressants.

The Cause of Death: Cardio-respiratory arrest secondary to acute mechanical asphyxia.

In conclusion, the family anamnesis, age, male sex, the complaints of the prisoners, anxiety, and insomnia associated with characteristic circumstantial data such as the psychiatric therapies set and the period in prison that are similar in the three cases reported, in our opinion, constitute alarm bells that should have pushed the authorities to give different attention to these detainees. The autopsy data demonstrate how the anti-conservative gesture took place in a very short time and this explains why, in our opinion, these prisoners should have been kept under protected surveillance 24 hours a day.

References:

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4. World Health Organization, *Suicide Worldwide in 2019, Global Health Estimate for the year 2019*, https://www.quotidianosanita.it/allegati/allegato1377340.pdf.
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6. Bryce E. Stoliker & Frances P. Abderhalden. Suicidal Research - Pages 231-245 | People in Custody With a Suicidal History: An Ideation-to-Action Perspective Involving Individuals Incarcerated in Two U.S. Jails- Published online: 28 Sep 2021, *PubMed*.

Forensic Science; Hanging; Prison Death

G1   The Use of Forensic Science Standards in the Legal Process: An Update

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Learning Overview: After attending this presentation, attendees will be informed about new case law since the last American Academy of Forensic Sciences' (AAFS') meeting dealing with the application of forensic standards, particularly those on the Organization of Scientific Area Committees (OSAC) Registry. Attendees will also learn about the National Institute of Standards and Technology's (NIST's) efforts to improve legal engagement with standards on the OSAC Registry.

Impact Statement: The federal government is investing resources to facilitate the development of forensic science standards for use in the criminal justice system, and there are active efforts to encourage forensic science service providers to implement these standards in their operations. These standards are also being used by lawyers and judges in legal proceedings and legal work supporting those proceedings. Understanding when and how these standards are currently being used or referenced during the legal process will impact the forensic science community by helping prepare forensic scientists and attorneys for this engagement. Likewise, this presentation will educate the community about NIST's efforts at supporting standards use.

Since its official inception in 2014, the NIST-administered OSAC for Forensic Science has engaged in strengthening the practice of forensic science by facilitating the development of technically sound, science-based standards and promoting their implementation by Forensic Science Service Providers (FSSPs). OSAC reviews and elevates specific standards to the OSAC Registry and promotes the forensic science community's use of these standards.

In 2022–2023, NIST engaged in an information-gathering effort involving legal research and qualitative interviews with lawyers and other stakeholders engaged in forensics to better understand if and how standards are being used in legal proceedings. This work also examined what obstacles existed to higher levels of legal engagement with forensic science standards. The initial results of this project were presented at the AAFS annual conference in 2023. This research revealed that while some standards had obtained a degree of purchase in the legal system, many lawyers were not using standards and did not understand them. Obstacles to higher engagement included general lack of knowledge and perceptions about the quality and utility of standards.

Drawing on this work, NIST has continued to monitor developments regarding the use of standards in legal proceedings. These important developments will be explored during this presentation, and attendees will learn how courts have treated standards in the past year with particular attention to any trends in judicial treatment. The presentation will examine why some standards may have received more legal attention than others in the past year and beyond. Likewise, NIST has sought to address some of the obstacles identified during the survey phase through various efforts aimed at encouraging better legal engagement with standards. This presentation will cover some of those efforts. Attendees will learn about how NIST is seeking to support a better understanding of and use of the standards by stakeholders in the legal system and how to access these resources.

Standards; Legal Research; Quality Assurance

G2   Standards on the OSAC Registry—Improving Justice for All

Mark D. Stolorow*, National Institute of Standards and Technology, Special Programs Office, Frederick, MD; Vincent Desiderio, Jr.*, National Institute of Standards and Technology, Gaithersburg, MD; Allison Getz, National Institute of Standards and Technology, Monrovia, MD; John Paul Jones II, National Institute of Standards and Technology, Gaithersburg, MD; Steven L. Johnson, Ideal Innovations, Inc., Arlington, VA

Learning Overview: The implementation of forensic science standards on the Registry of the Organization of Scientific Advisory Committees for Forensic Science (OSAC) has a direct connection to the overall improvement of "Justice for All" in the United States. Attendees of this presentation will learn how the development and implementation of forensic science standards are manifested in the improvement of achieving justice in criminal cases. Attendees will learn details of five impactful ways that nationally recognized standards improve the administration of justice.

Impact Statement: Implementing the nationally recognized forensic science standards on the OSAC Registry favorably impacts achieving Justice for All by minimizing bias, improving the reliability of testing, reporting, and testifying in courts of law, thereby increasing the likelihood of exonerating the innocent and convicting the guilty.

Published in 2009, the National Research Council (NRC) Report, *Strengthening Forensic Science in the United States - A Path Forward*, criticized the practice of forensic science in America for (among other things) its failure to have in place a network of nationally recognized, consensus-based standards with scientific merit[1]. In 2014, the National Institute of Standards and Technology (NIST) and the United States Department of Justice (US DOJ) responded by creating the Organization of Scientific Area Committees (OSAC) for Forensic Science to facilitate the development of high-quality standards and encourage the implementation of them by the forensic science community.

Implementation of standards on the OSAC Registry has a direct connection to the overall improvement of justice for all in the United States. So, how are the development and implementation of forensic science standards manifested in improving justice? Here are five impactful ways that standards improve justice.

Harmonize Forensic Practice across Jurisdictions: The level of scientific rigor applied to evidence should not depend on the jurisdiction in which the evidence is collected. Nationally recognized standards ensure that a minimum level of consensus-built scientific rigor is applied to evidence in any jurisdiction, ensure that courts are empowered to adhere to the same quality of scientific inquiry, and the admissibility of expert forensic testimony can be applied more uniformly nationwide.

Improve the Reliability of Forensic Analysis and Interpretation: Using standards related to best practices for minimum qualifications and education of practitioners, applying scientifically based procedures, and limiting the results and interpretation to conclusions that are not overstated nor exaggerated will lead to more objective, reliable, and reproducible reports and expert testimony.

Minimize Bias: Bias is real and a part of human nature. It can affect evidence collection, analysis, interpretation, report writing, and expert testimony. Using high-quality standards that contain processes to minimize bias can be built into the standard operating procedures implemented by forensic scientists. Examples include ethics-based training and guidelines for effective technical and administrative review of casework. Such measures reduce errors and provide a framework for consensus building to resolve disagreements in data interpretation among qualified forensic scientists.

Improve Reviews through the Use of Scientific and Technical Reviews (STRs): OSAC introduced the STR process in 2020 to provide subject matter expert and peer review to all relevant proposed standards during development. STRs provide an independent technical review of drafted standards going through the OSAC Registry approval process. The positive impact is that the review process for standards is more rigorous in ensuring scientific validity by taking note of potential uncertainty and providing limitations where appropriate. The STR process also reduces possible bias and improves objectivity, reliability, and reproducibility of results.

Reflect New Changes to Federal Rules of Evidence 702 (FRE 702): In early 2023, the Federal government updated FRE 702 to ensure that expert witnesses do not exaggerate their opinions[2]. Specifically, the amended FRE 702 states, "The expert's opinion reflects a reliable application of the principles and methods to the facts of the case." The official notes accompanying this change reflect the intention to limit the testimony to that which is scientifically supportable. Adherence to FRE 702 and the implementation of standards on the OSAC Registry can positively impact improving justice for all.

This presentation will cover each of the five areas mentioned above in detail and provide real-world examples to illustrate the impact of each.

References:
1.   National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. https://doi.org/10.17226/12589. https://doi.org/10.17226/12589.
2.   *28 USC App Red R Evid Rule 702*: Testimony by Experts (https://uscode.house.gov/view.xhtml?req=granuleid:USC-1999-title28a-node246-article7-rule702&num=0&edition=1999).

Standards; Justice for All; OSAC

G3   The Benefits of a Coherent System of Forensic Standards: From the Classroom to the Courtroom

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Learning Overview: After attending this presentation, attendees will know about the history of forensic standards, the benefits of standardization throughout the forensic sciences, and how the support of various forensic entities factor into the development of a coherent system of standards. A discussion will be provided regarding the current status of standardization within the field and where we should be looking to go.

Impact Statement: This presentation will demonstrate that the entire forensic community is required for developing and implementing a harmonized system of standards that is in place. Such a system will be integral with respect to ensuring that forensic practice is consistently applied nationwide.

Pure scientific practice, at its root, is a haphazard process through which scientists probe the limits at the edges of human knowledge. New techniques and approaches are typically employed to collect the data necessary to prove or disprove new hypotheses. Sometimes this works and new discoveries are made, and sometimes these paths lead to dead ends. Either way, there are few dire consequences, and something is always learned. As one proceeds further back from these edges, the body of scientific knowledge grows more concrete, the inquiries become more applied, and the techniques employed become relatively routine.

As opposed to pure scientific endeavors, where the greatest consequences are typically the loss of monies spent on the process, applied sciences may have significant consequences when failures occur. Take for example a submersible vessel that is not engineered properly, not built to standard specifications, and not properly tested before embarking on a journey with passengers two miles deep into the ocean. Ideally, applied sciences adhere to various sets of standards to manufacture consistent products, ensure safe operations, and attain reliable results (e.g., various disciplines within engineering, materials science, and the environmental sciences).

Forensic science is an applied science at its core. It is highly beneficial for the forensic sciences to incorporate scientifically sound and thoroughly vetted standards into its practices. Failures in this realm could have dire consequences as innocent people could be incarcerated, dangerous individuals could be overlooked, and people's lives could be unnecessarily endangered.

The need for standardization in the forensic sciences is not a novel idea. Through various activities, numerous forensic practitioners and researchers have valiantly dedicated their time and efforts to the creation of forensic standards since at least 1970 when the American Society for Testing and Materials (ASTM) International's Committee E30 on Forensic Science was first formed. These efforts were amplified by the formation of various scientific and technical working groups (SWGs and TWGs) beginning in 1988 and continuing mostly through 2014 with some SWGs still producing standards to this day. In 2009, the National Academy of Sciences' National Research Council (NRC) issued its seminal Report, *Strengthening Forensic Science in the United States: A Path Forward*.[1] Among the many recommendations put forth was the call for higher levels of standardization within the forensic sciences. Following these recommendations, the National Institute of Standards and Technology (NIST), in conjunction with the United States Department of Justice (US DOJ), launched the Organization of Scientific Area Committees (OSAC) for Forensic Science to better meet the standardization needs of the field. A great deal of progress has been made because of these efforts; however, the road to a coherent system of standards within the forensic sciences remains long and arduous with many gaps between the classroom and the courtroom.

This presentation will provide a brief history of forensic science standards development, discuss the benefits of standardization, and propose a future in which there is greater harmonization both within and between the body of standards being created across multiple forensic disciplines. This standardization superhighway must be several lanes wide, begin in the classroom, proceed through training, wind its way from the crime scene through the laboratory, and ultimately end in the courtroom, where scientific results and opinions are presented.

Although not a panacea, a coherent system of carefully created, thoroughly vetted, and meticulously validated standards implemented at a national level would go a long way toward ensuring a high level of quality is applied across forensic laboratories throughout the United States in the most consistent manner possible.

Reference:

1. National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. https://doi.org/10.17226/12589.

Standards; OSAC; Harmonization

G4 Cross-Examining Experts on Standards and Other Authoritative References

Terry-Dawn Hewitt*, McKenna Hewitt, Denver, CO

Learning Overview: After attending this presentation, attendees will understand legal issues to be addressed before an attorney is permitted to cross-examine a forensic expert witness using standards and other authoritative references at admissibility hearings (e.g., *Daubert* hearings) or trial. When the court permits such cross-examination, attendees will learn strategies that can be employed by attorneys to structure the cross-examination or by experts to respond to questions arising from authoritative references.

Impact Statement: This presentation will impact the forensic science community by first reviewing the legal rules governing the use of authoritative treatises to cross-examine experts, then by delivering practical recommendations to assist attorneys and expert witnesses to develop strategies for dealing with standards and other authoritative references in the context of cross-examination.

Cross-examination can be a mighty sword. That sword can be even more deadly in the hands of an attorney who is intimately familiar with standards and other authoritative references governing an expert witness' discipline. Conversely, a knowledgeable expert can use authoritative publications as a shield to deflect or defeat these lines of questions.

The first step in planning how to use authoritative publications as either a sword or a shield begins with understanding the "learned treatise" rule in the jurisdiction where a case will be tried. "Learned treatise" is the legal term generally used to describe reliable authorities that experts in a given discipline reasonably rely upon. These authoritative references include industry standards. Simply stated, the learned treatise rule is a rule of evidence governing how learned treatises (authoritative publications) can be used to cross-examine experts.

This presentation is equally directed to both attorneys and experts in either criminal or civil litigation. Respecting attorneys, we acknowledge that it is incumbent upon them to know the rules of evidence in the jurisdictions where they practice, including the learned treatise rule. However, there is a big difference between knowing the law relevant to the learned treatise rule and knowing how to use it. Knowing the law is seldom enough to empower attorneys to make effective use of learned treatises in cross-examining an expert. Therefore, we will raise some issues attorneys can explore on their own or by consulting with their experts when planning a cross-examination using authoritative publications.

As for experts, becoming conversant with how cross-examination can be structured using authoritative publications is a matter of self-preservation. This is particularly so with the continued growth of admissibility challenges under *Daubert*, *Frye*, or state admissibility tests. Not only should experts understand how the learned treatise rule operates in jurisdictions where they may testify, experts may need to assist attorneys with planning how to challenge opposing experts using authoritative publications.

There can be much complex strategizing to plan an effective cross-examination of an expert witness. Experts who understand the basics of the learned treatise rule can be immensely helpful to the attorneys with whom they are working. Moreover, an expert should be prepared to deal with cross-examination questions opposing counsel asks about authoritative publications. To this end, experts will need to anticipate such questions in order to elicit an attorney's advice about how to deal them.

This presentation will start with an overview of the learned treatise rule. Since most of the states have rules that are the same or similar to the one in the Federal Rules of Evidence, we begin with the federal rule.[1] Where states have different rules, we summarize how their rules differ from the federal rule.[2]

Next, we will identify some of the practical issues that may arise when a court is evaluating whether a particular publication is authoritative. For example, the situation where an expert denies having read the proffered text, or where the expert tries to avoid being cross-examined on a publication by stating that the publication proffered by the opposing attorney is "not authoritative."[3] We offer suggestions to overcome these issues and more.

Finally, we provide some strategies for structuring a cross-examination of an expert witness using authoritative publications, including standards, reference texts, and scientific articles. We draw our examples from publications in the fire investigation discipline.[4] Attendees from other forensic disciplines can apply similar techniques using the standards and other authoritative references from their field.

References:
1. Fed. R. Evid. 803(18).
2. See, e.g., Wharton's Criminal Evidence, Part Two, Chapter 27 Rule 803(18)—Learned Treatises (Hearsay Exception) - State Rules, (2023).
3. See, e.g., Theodore F. Roberts, Medical Literature in the Courtroom: Learned Treatise Trial Practice and Procedure, 45 Am. J. Trial Advoc. 297 (2022).
4. See, e.g., National Fire Protection Ass'n Technical Committee on Fire Investigations, NFPA 921 Guide for Fire and Explosion Investigations (2021 ed.), Nat'l Fire Prot. Ass'n Technical Comm. on Fire Investigator Professional Qualifications, NFPA 1033 Standard for Professional Qualifications for Fire Investigator [hereinafter NFPA 1033] (2022 ed.).

Expert Testimony; Cross-Examination; Standards

G5   **"Fairness for All": A Discussion of the Evolution of Legal Principles and Practical Systems Designed to Ensure Fairness in Forensic Testimony and in the Criminal Justice System as a Whole**

John Haried*, United States Department of Justice, Washington, DC

Learning Overview: The goal of this presentation is to discuss recent developments designed to ensure fairness in the criminal justice system through court procedural rules governing expert forensic testimony and the rules of evidence and discovery, through the business practices that regulate what evidence and information federal prosecutors must disclose to criminal defendants, and through new guidance for prosecutors and defense attorneys grappling with new forms of digital evidence.

Impact Statement: This presentation will impact the forensic science community by helping attendees understand the interplay of legal principles and practical systems that determine what "fairness" looks like in individual cases and how rules governing their forensic testimony advance the goal of fairness.

"Fairness for All" is the central goal of the criminal legal system because it promotes public confidence in the reliability of jury verdicts and plea agreements. "Fairness" extends to defendants and to victims, witnesses, and critical public interests, like privacy, privileged information, and national security. The federal criminal justice system sees 110,000 individual defendants in 65,000 new cases each year, which encompasses a huge variety of cases, whether it is the January 6 Capital Riot prosecutions, the Boston Marathon bombing, the Elizabeth Holmes blood testing fraud case, the El Chapo drug ring, or a garden variety homicide.

So, what does "fairness" mean for each defendant's case, and how can we breathe life into the legal principles that undergird "fairness" through people, practices, technology, and governance structures like rules and collaboration between prosecutors, defense attorneys, and judges? The principles and practices that govern forensic evidence also govern many aspects of the criminal justice system.

The criminal justice system is constantly under the microscope, as it should be. If the public loses confidence in the reliability of who is convicted and what sentences they receive, then the core fabric of our democracy is at risk. We saw in the George Floyd case in Minneapolis in 2021 and countless other criminal cases how the public's perception of fairness and questions about the overall reliability of the criminal justice system go to the heart of citizens' acceptance of democratic government and its institutions. Many people are constantly striving to improve the criminal justice system—including professional associations like the American Academy of Forensic Sciences (AAFS), federal prosecutors, federal public defenders, judges, and others. Their collaborative efforts are focused on practical solutions that promote the legal standards designed to ensure fairness and, at the same time, meet the practical needs of victims, witnesses, defendants, prosecutors, defense attorneys, and judges. This presentation will describe new rules and practices that that give life to "fairness" in the vast federal criminal justice system, and the strategies behind them.[1-4]

References:
1. Federal Rules of Evidence 702 (Expert Testimony) and 902 (Authentication).
2. Federal Rules of Criminal Procedure 16 and 16.1 (Criminal Discovery).
3. The Criminal Digital Discovery Protocol.
4. The Guidance on Providing Electronic Discovery to Pretrial Detainees.

Justice; Regulation; Evidence

G6   Using Forensic Science to Speak for a Victim Who Was Unjustly Silenced in Murder

Valeria N. Spencer*, Violent Crimes and Immigration Enforcement, United States Attorney's Office, District of Colorado, Denver, CO

Learning Overview: After attending this presentation, attendees will understand the value in interdisciplinary forensic investigation in proving homicide where accidental death is claimed and there are no eyewitnesses, no direct evidence, and a scene that says, "accident."

Impact Statement: This presentation will impact the forensic science community by emphasizing the forensic aspects of the case: cell phone (texts, calls, voicemail), cell tower location data, computer analysis, crime scene survey using the Federal Bureau of Investigation's (FBI's) latest technology, and crime scene reenactment to compile proof beyond a reasonable doubt in a circumstantial murder prosecution. This presentation will focus on the interdisciplinary collaborative efforts of the many forensic specialties that came together to find justice for victim Toni Henthorn and her family.

On September 28, 2012, Harold Henthorn surprised his wife, Dr. Toni Henthorn, with a surprise trip to Estes Park, CO, for their 12th anniversary. After staying the night at the historic Stanley Hotel, he drove them into Rocky Mountain National Park (RMNP) to hike. The couple hiked Deer Mountain. Around 3:00 p.m., they went off-trail to a secluded area of rocky terrain and sheer cliffs. After stopping to eat and take pictures, they scrambled down a rocky scree to get a better view from a cliff top. At the clifftop, Toni used Henthorn's phone to take photographs of him on top of a ledge. Minutes later, Dr. Toni Henthorn fell off that ledge some 120 feet to her death.

A few days later, agents from RMNP Police called the United States Attorney's Office (USAO) in Denver with concerns about the death. Partnering with the FBI, RMNP agents and USAO began an investigation that ultimately led to the prosecution and conviction of Harold Henthorn for first degree murder.

Question: How did we get from a fall to a push, from an accident to life in prison? **Answer**: Forensics.

The Scene: Members of the Operational Response Section with the FBI in Quantico, VA, performed a crime scene survey at Deer Mountain in order to precisely determine the distance of Dr. Henthorn's fall from the buttress to the trees below. The use of Total Station Mapping methods, techniques, and procedures for capturing data for use in determining trajectories and distances helped in reaching conclusions as to how the victim fell. Using GoPro® cameras, drones and other specialized 360-degree equipment, every foot from the base of Deer Mountain to the fall site was captured to bring the mountainside to the jury.

Cell Phone Analysis: Photos (including deleted photos), video, calls, texts, and voicemail were analyzed. The cell phone data placed Harold Henthorn and Dr. Toni Henthorn at the exact spot on the Deer Mountain outcropping right before her death. Timing of text messages and 911 calls were a crucial part of the timeline. Cell tower records demonstrated Harold Henthorn's premeditation as prosecutors were able to prove that Henthorn took multiple trips to RMNP to find the perfect spot on which to stage the murder.

Coroner's Report to Corroborate Prosecution Theory and Disproving Henthorn's Version of Events: The autopsy report opined that Toni Henthorn died of multiple blunt force injuries when she fell or was pushed down a cliff while hiking in RMNP. The report, diagrams, and photos were consistent with the autopsy findings that the manner of death could not be determined, but that homicide could not be excluded.

The Motive: A paper trial was painstakingly compiled that made clear that Harold Henthorn had enormous financial motive to kill his wife, Dr. Toni Henthorn. Insurance company records, annuity records, bank records, tax return records, and year end newsletters established that Harold Henthorn was postured to receive 4.5 million dollars in proceeds upon the accidental death of his wife.

Harold Henthorn's first wife died under mysterious circumstances. A reinvestigation, including detailed scene reenactment and testing, caused authorities to change their minds. The new data made it possible to use the first death as F.R.E. 404(b) in the trial of Dr. Toni Henthorn's murder trial.

Dr. Toni Henthorn died at the hands of a clever criminal who is now serving a life sentence without possibility of parole. In times past, an "accident" would likely have been declared and Harold Henthorn would have walked free. Because of advanced forensics applied in an interdisciplinary manner in a tenacious investigation and prosecution, justice for victim Dr. Toni Henthorn was achieved.

Digital Evidence; Homicide; Autopsy

G7   A Critical Re-Examination

Barbara P. Hervey*, Texas Court of Criminal Appeals, Austin, TX

Learning Overview: After attending this presentation, attendees will have learned about the importance of forensic science in the courtroom and the need for rigorous training to bridge the divide between science and the law.

Impact Statement: This presentation will impact the forensic science community by showing how forensic science inextricably links scientists and lawyers, and, consequently, scientists and lawyers need to know each other's role in the criminal justice system. For example, lawyers should know the limits of what science can support.

The National Academy of Sciences (NAS) Report was distributed far and wide in 2009.[1] The **President's Council of Advisors on Science and Technology** (PCAST) Report followed in 2016 but a critical question remains.[2] Where are we now in the advancement of science and the integration of science into the law?

Although the legal system has progressed in recognizing the everyday impact science has on the law, much more education, understanding, and appreciation is needed. Likewise, scientists need a thorough understanding and appreciation of the difficulties in navigating the law, especially when addressing these new or advanced methodologies.

Some progress can be attributed to the attention paid to "actual innocence." Some was accomplished via a critical re-examination of traditional means of solving crimes, many of which have been found to be flawed, debunked, or outright false.

Coordinating the scientific with legal advancements is often difficult and slow moving, but achievable. But some reform has already occurred though exemplary legislative action such as redesigns in eyewitness identification procedures, videotaped confessions, collection and preservation of evidence, and the availability of post-conviction DNA testing. Conviction integrity units, innocence projects, forensic science commissions, and increased scrutiny over laboratory techniques have also taken place. Many post-conviction writs of habeas corpus are remanded to trial courts for further hearings, designed to develop a bigger picture, beyond the original proceedings and the facts and circumstances originally presented.

However, challenges remain.

What do the present studies and improvements in the sciences criticized in the NAS Report reveal? If the data to support the reliability in these fields has increased, the legal community needs to know. What deliverables are available? How often are they updated? Have legislation and case law evolved to correctly apply the sciences and differentiate between old traditions and new science? What new legislation can we suggest? Are our law schools moving toward including forensics in their curriculum? Are our laboratories being accredited and given all the new training and equipment they need? Can we increase the use of blind testing? Are our attorneys being educated in forensics? Are our judges realizing their gatekeeping role? What education are we delivering to the public?

When old trial transcripts are reviewed for possible "issue spotting" while sifting through scientific testimony, who is charged with that task? Do the reviewers understand the importance of that information, the mechanics of trial procedure and the meaning of materiality? Serious dilemmas remain in areas such as "shaken baby syndrome," "false evidence," "fire science," sexual assault, and many others. Evaluation of expert testimony vs. lay testimony and the impact of such on the ultimate triers of fact remains in flux.

But as the science community strains to increase validation of its many disciplinaries, the legal community <u>MUST</u> endeavor to become better educated.

References:
1. National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. https://doi.org/10.17226/12589.
2. President's Council of Advisors on Science and Technology (PCAST). *Report to the President: Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods.* (2016). Washington, DC: Executive Office of the President of the United States.

Attorneys; Wrongful Conviction; Shaken Baby Syndrome

G8   Why Judges Won't Be Gatekeepers

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Learning Overview: This presentation will describe how and why judges have routinely failed to follow the gatekeeping requirements for the admission of forensic science evidence. After attending this presentation, attendees will understand how the legal doctrine of stare decisis and law school training conflict with the *Frye* and *Daubert* requirement for judges to assess the reliability of forensic science evidence. Suggestions are made for addressing the routine admission by judges of potential unreliable evidence.

Impact Statement: This presentation will impact the scientific and legal communities by describing reasons for improving the initial and continuing education of judges and lawyers to achieve a better understanding of the gatekeeping role of the judge regarding the admission of scientific evidence

Since the 1923 *Frye* case, judges have been charged with deciding the admissibility of forensic science testimony.[1] The 1993 *Daubert* case made judges gatekeepers of such evidence and required federal judges to determine if it was "reliable" under certain standards.[2] The reality is that in criminal cases judges have overwhelmingly failed to conduct hearings to determine the reliability of most traditional forms of forensic science. Many have abdicated their gatekeeping duty and continued to admit whatever evidence the prosecution offers.

Some suggest that judicial prosecutorial bias based on some studies indicating that prosecution evidence is admitted over objection far exceeding the rate for defense evidence.[3] Also, many judges and lawyers are simply unaware of the requirements of *Daubert*. It may also be that trial judges simply want to avoid *Daubert* or *Frye* hearings because it slows an already backlogged docket. But even when a hearing is conducted, judges continue to routinely admit forensic science evidence just because it has "always" been admitted. Some even reverse the burden of proof and require the defense to show why evidence is inadmissible.[4]

A plausible explanation is that judges are doing what they were taught in law school about stare decisis. Lawyers are taught to seek answers to issues by asking how judges in the past answered the question.[5] There are stability reasons for stare decisis, but it perpetuates the status quo, which is never the issue in science. Nothing in *Frye* or *Daubert* indicates that stare decisis should be applied to scientific evidence admissibility. *Frye* refers to "general acceptance" in the *scientific* community, not the *legal* community. *Daubert* makes no suggestion that judges should consider legal precedent and its "general acceptance" factor is also limited to the relevant *scientific* community. Nevertheless, judges choose to ignore the findings of the National Academy of Sciences (NAS) Report and the subsequent Presidents Council Report and the ensuing scientific research and continue to adhere to the status quo.[6,7]

There are possible solutions. Recent amendments to FRE 702, and possible similar amendments to State rules, reinforce the judge's duty to make a finding of reliability by a preponderance of the evidence and to place the burden of proof on the proponent.[8] Better education of lawyers and judges will ultimately be the solution.[9] Forensic science is rarely offered in law schools other than a short chapter in a broader evidence course.[10] Continuing education of judges and lawyers should specifically address the *Daubert* gatekeeping requirements.

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Judge; Admissibility; *Daubert*

G9　　Polygraph Testing and Wrongful Conviction

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Learning Overview: This presentation will explore the relationship between the use of polygraph testing and wrongful conviction. Specifically, it will demonstrate how polygraph testing lacks scientific reliability and how its use in police interrogation can result in false confession and, consequently, wrongful conviction. After attending this presentation, attendees will better understand the role this flawed forensic technique plays in criminal prosecutions and how to best challenge its use.

Impact Statement: This presentation will impact the forensic science community by showing how the flawed forensic technique of polygraph examination can contribute to wrongful convictions even when the government does not seek to introduce it as direct evidence in a case.

Polygraph testing is not a valid science because it is neither standardized nor objective. Instead, the process involves an examiner's subjective interpretation of an examinee's physiological responses to questions.[1] Moreover, many factors entirely unrelated to truthfulness can impact the results of a polygraph test, including demographic characteristics, personality types, medical and psychological conditions, expectations of the examiner and examinee, and methods of selecting questions and conducting the examination.[2] Because polygraph examination has never been demonstrated to be a valid, accurate measure of deceit, polygraph results are inadmissible in criminal cases in most jurisdictions.

However, polygraph tests continue to play a significant role in criminal investigations.[3] Police often invite suspects to "prove" their innocence by agreeing to undergo a polygraph test during an interrogation. If an examiner in their subjective opinion deems the suspect to have "failed" the test, law enforcement will often leverage that result during interrogation to try to extract a confession—confronting the person being interrogated with the result as if it were conclusive evidence of guilt. Social science research has demonstrated that when faced with this kind of supposedly indisputable evidence of guilt, an innocent suspect may falsely confess to the crime—either because they are convinced that continuing to insist in their innocence is a lost cause or, in some rare cases, because they temporarily discount their own memory and wrongfully believe that they must have committed the offense because the polygraph test "proves" that they did.

Because polygraph tests can lead to false confessions, the Innocence Project has been urging courts to recognize that confronting a suspect with "failed" polygraph results during an interrogation as if the results were incontrovertible proof of guilt is so powerfully coercive that any subsequent confession by the suspect is involuntary and, therefore, should not be admitted into evidence. Alternatively, and at a minimum, the use of polygraph results should weigh heavily in favor of finding that a suspect's confession was involuntary.

This presentation will explain why polygraph-induced confessions should be challenged as unreliable evidence in criminal cases and provide a framework for how courts should deter the coercive use of polygraph results.

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Wrongful Conviction; Admissibility; Conviction

G10   **Are Courts Convicting People in Traffic Accident Cases Based on Questionable Data From Crash Data Recorders?**

Peter Alexander*, Raymond Smith & Associates, Aurora, CO; Brendan Bourdage, RPSAVA, Williamsburg, VA; John Smith, RPSA, Parker, CO

Learning Overview: The goal of this presentation is to explore how data from Crash Data Recorders (CDRs), used in civil and criminal cases by accident reconstruction experts, in reports and trial testimony, can be unreliable and inaccurate. This presentation questions whether CDR data could pass muster under a *Daubert* review or Rule 702 scrutiny and provides examples of CDR data errors.

Impact Statement: This presentation will impact the forensic science community by exploring whether the courts are convicting individuals under the assumption that CDR data is reliable and whether courts inquiring about its applicability in some cases should be questioned?

Accident reconstruction experts often employ data downloaded from CDRs in plaintiff's and defendant's vehicles. This is done in an attempt to reconstruct the collision dynamics and to establish liability in both civil and criminal cases. The purpose of CDRs (black boxes) in cars and trucks is to deploy the vehicle's air bag(s) at the appropriate moment during a crash. Accident reconstruction experts have been making use of the CDR data to assess the collision process and assign responsibility for the collision. Data recorded by the CDR such as vehicle speed, vehicle deceleration, vehicle heading, and seat belt status have been used by experts to assess the accident. The data is often used as input to numerical crash models to produce conclusions of questionable accuracy.

Standards that the CDRs must meet are set by the government, but the standards are slanted toward the original purpose of the CDR, namely deployment of the airbags. The automobile manufacturers are given considerable latitude regarding what CDR data they must record. The CDR is tested in a frontal impact with a fixed barrier or for side impacts with a moveable barrier. Most vehicle collisions involve impacts between two or more vehicles rather than a fixed barrier. Errors in the CDR data and weaknesses in the crash models can produce incorrect results during the analysis of the collision.

It should be noted that the judge or opposing counsel may not realize that CDR data has been used in the opposition's expert's report without close examination.

Crash; Accuracy; *Daubert*

G11   Wrongful Convictions Demonstrate the Need for Governance Improvements in Forensic Science

John Morgan*, Coptech Systems, Raleigh, NC

Learning Overview: After attending this presentation, attendees will be informed about the link between wrongful convictions and governance gaps in forensic science. The presenter has completed and published in the *Journal of Forensic Sciences* comprehensive research on wrongful convictions related to forensic evidence.[1,2] This session will provide new research and details on the specific issues in oversight, quality assurance, and governance related to wrongful convictions. Attendees will learn the key aspects of forensic governance improvement that will most impact the reliability of forensic evidence and the criminal justice system.

Impact Statement: This presentation will provide unique information about governance structures in light of the experience of wrongful convictions. Forensic science improvement should be closely tied to real-world experience and root cause analysis. This presentation will impact the forensic science community by providing a grounded, research-based approach to governance improvement. It closely ties to the conference theme, Justice for All, which can be supported by the adoption of governance reforms that improve the reliability and effectiveness of forensic science and its use by the criminal justice system.

A comprehensive analysis of wrongful convictions associated with false or misleading forensic evidence demonstrates that accredited forensic science organizations have adopted new technologies and scientific standards that mitigate the risk of wrongful convictions. Nonetheless, many cases involve forensic analyses that fall outside the scope of accredited forensic science organizations or existing governance mechanisms within the forensic disciplines. Improved governance mechanisms are proposed in alignment with the experience of wrongful convictions, including state-level governance commissions and boards.

The increasing complexity and specialization of scientific knowledge may contribute to wrongful convictions. In particular, police investigators and officers of the court have made many statements in the media or during trials that demonstrate a poor understanding of forensic evidence in wrongful conviction cases, even to the extent of discounting or ignoring exculpatory results. Forensic examiners made statements in testimony and reports that exceeded the limits of science or were outside the scope of their discipline. For example, DNA and print examiners made statements about the time when latent evidence was deposited, an activity-level proposition that is outside the scope of their disciplines. These problems are associated with a wide range of root causes, including poor communication across organizational and practice boundaries, cognitive bias effects among criminal justice practitioners, and shortfalls in training or scientific standards. In addition, courts have not effectively used forensic evidence or performed adequate gatekeeping in many wrongful convictions. This issue extends to the introduction of invalid evidence or poorly vetted experts, but it also includes the underutilization of valid forensic evidence that could contribute to more reliable and trustworthy verdicts.

The development of practice and testimony standards can address these issues in the context of appropriate governance mechanisms. In the United States, national governance—such as the now-defunct National Commission on Forensic Science—has not succeeded in closing governance gaps. The distributed nature of political and legal boundaries among the states and court systems has contributed to this failure. Discipline-specific governance—such as professional certification regimes—has had limited success because courts have accepted unreliable testimony from uncertified examiners.

State-level governance mechanisms could provide an effective alternative but vary considerably with respect to authorities and effectiveness. In most states, there has been insufficient consensus-building among stakeholders, including forensic scientists, prosecutors, defense attorneys, judges, law enforcement, academics, policymakers, and the general public. Key state-level governance issues related to wrongful convictions include accreditation, professional certification, investigation of laboratory deficiencies, standards (report and testimony), training, and improving communication among forensic scientists and other criminal justice stakeholders.

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Wrongful Conviction; Standards; Expert Testimony

G12   The National Association of Forensic Science Boards: A Collaborative Effort for State-Level Forensic Oversight

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Learning Overview: After attending this presentation, attendees will learn about the newly formed National Association of Forensic Science Boards (NAFSB). The NAFSB is a collaborative effort by leaders from state-level forensic science boards and commissions across the United States to both evaluate the effectiveness of and encourage adoption of different state-level forensic science regulatory models aimed at bridging critical gaps in oversight that can ensure the integrity of forensic science in our criminal justice system.

Impact Statement: This presentation impacts the forensic science community by educating attendees on the necessity of state-level forensic science oversight and the forward-looking plans for the NAFSB to provide a forum that improves collaboration between state boards and commissions and promotes the adoption of successful state regulatory models of forensic oversight that embrace a culture of collaboration, trust, and transparency among forensic science stakeholders as core values to ensure the integrity and reliability of forensic science in our criminal justice system.

In the United States, while there are significant efforts by the federal government for the development of forensic science standards, forensic research and development, and corresponding efforts for state-level Forensic Science Service Provider (FSSP) implementation and education, there is currently no national forensic science regulator or oversight body aimed at ensuring or enforcing the ongoing integrity and reliability of forensic science. Forensic science regulation is largely left up to the states. A June 2022 National Institute of Justice Forensic Technology Center of Excellence Report on state forensic science commissions reported a total of 18 forensic science state commissions or regulatory oversight bodies are currently functioning with different levels of regulatory authority oversight aimed at improving cooperation among FSSPs and the criminal justice community as a whole, ensuring standards are implemented, coordinating funding or grant opportunities, investigating allegations of negligence and misconduct in crime laboratories, and requiring national accreditation or individual forensic analyst licensing.[1]

In early May 2023, a group of forensic science leaders and policymakers, many representing existing state boards and commissions, met to discuss the development of a collaborative board to improve coordination and communication among state-level forensic science boards and commissions and formed the NAFSB.

In November 2023, the NAFSB met for a conference for the first time in Austin, TX. Speakers discussed the governance and future of NAFSB. The group addressed topics that included: benefits of state oversight for FSSPs, the importance of scientific and legal representation on any state board or commission, how to establish an independent crime laboratory, the different models of state regulatory oversight and their successes and failures, and how state regulatory agencies can interact with national accrediting bodies like ANSI National Accreditation Board (ANAB) and the American Association of Laboratory Accreditation (A2LA).

NASB's ultimate goal is to provide a forum that improves collaboration between state boards and commissions and to promote the adoption of successful state regulatory models of forensic oversight that embrace a culture of collaboration, trust, and transparency among forensic science stakeholders as core values to ensuring the integrity and reliability of forensic science in our criminal justice system.

Reference:
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Regulatory; Forensic Oversight; Commission

G13 Preparing for Court: The Research Forensic Library as a New Resource for the Forensic Legal Community

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Learning Overview: After attending this presentation, attendees will understand how the Research Forensic Library (RFL), a first-of-its-kind publicly accessible research repository, can assist them in preparing for court cases. The library offers thousands of articles and reports in every discipline of the forensic sciences, all of which are available as open access, making it an ideal resource for attorneys who have often been confronted with paywalled material. This presentation will provide an overview of the library's resources as well as techniques for optimizing their chances to find the most relevant material they need.

Impact Statement: The lack of a centralized repository of easily searchable and accessible forensic research papers and reports has been a longstanding challenge for the forensic legal community. This gap significantly hinders attorneys' abilities to effectively prepare for trial and provide comprehensive legal services to their clients, especially when time is of the essence. This presentation will impact the forensic science community by sharing how the introduction of the RFL presents a transformative solution that has the potential to dramatically enhance the capabilities of attorneys in these critical areas.

The legal community's access to comprehensive and easily accessible forensic science research has long been a challenge. However, with the introduction of the RFL, attorneys now have a centralized, publicly accessible repository that addresses their unique information needs. The RFL, a collaborative effort between Florida International University and the National Institute of Justice, stands as a groundbreaking initiative, offering an extensive collection of research papers and reports spanning all forensic disciplines.

One of the key advantages of the RFL is its user-friendly search functionality, which enables attorneys to quickly and efficiently locate relevant materials on any specific forensic subject. The RFL provides immediate access to both current and previously published research, equipping legal professionals with the necessary resources to prepare for their cases. Moreover, the library's commitment to open access material ensures that paywalls are no longer an impediment, eliminating cost barriers and streamlining the process of obtaining vital information.

During this presentation, attendees will explore the resources available within the RFL. The session will cover various techniques for searching effectively, enabling attendees to maximize their research efficiency. Attendees will also learn how to receive daily updates on newly published material, ensuring they stay informed about the latest advancements and research findings in the field. Furthermore, the RFL's librarian will be introduced as a direct point of contact for addressing specific questions and concerns related to forensic court and legal issues.

In summary, the RFL revolutionizes access to forensic research for the legal community. Through its extensive collection of curated open access materials, the RFL provides an invaluable resource to professionals working in the forensic sciences. With its user-friendly search capabilities, daily updates, and personalized support from the librarian, the RFL empowers attorneys with the tools they need to enhance their legal practice and stay at the forefront of forensic knowledge.

Legal Research; Court; Forensic Library

G14   The Real Action at Court Has Always Been Activity

Tim Kalafut*, Sam Houston State University, Department of Forensic Science, Huntsville, TX

Learning Overview: After attending this presentation, attendees will be aware of many different ways that forensic DNA testimony has addressed the activity level of the hierarchy of propositions since the beginning of DNA testimony. Attendees will learn some of the differences between how this testimony has long been utilized and best practices for addressing activity issues.

Impact Statement: This presentation will impact the forensic science community by trying to reconcile what the court is interested in with the role of the expert witness in answering questions related to, "Is it possible that . . . "

There is a growing debate in the United States related to the evaluation of DNA evidence given activity-level propositions. Cook et al. first defined various levels of propositions related to forensic evidence near the end of the past century.[1] As the discipline of forensic DNA has matured and allowed the evaluation of DNA donors and individual contributor slots in mixtures, the sub-source and sub-sub-source levels have been defined as who might be the source of the DNA and who might be the major or minor source of the DNA.

The Sydney Declaration has defined forensic science as being interested in studying both presence and actions related to crimes.[2] DNA has been considered to be the gold standard because of the population genetics driven statistics used at the sub-source level (presence). DNA can also address the other major aspect of forensic science, actions, by evaluating DNA evidence given activity-level propositions. More and more scientific organizations are providing guidelines and best practices documents as to how DNA experts can assist the court at this level, and some are providing guidance specific to legal practitioners.[3-6]

Yet there is still much debate among and between both practitioners and the legal community as to whether there are appropriate frameworks, data, knowledge, and training of experts to allow for such testimony. Part of the confusion may be related to an expectation that the gold standard statistical analysis based on population genetics still applies to activity evaluation. In truth, activity-level evaluation is the same whether for DNA or classical trace evidence such as glass.

Nevertheless, the use of DNA to address activity level in the courtroom has been common in forensic DNA, since at least the adoption of the differential extraction used to separate DNA from sperm donors to that of non-sperm donors. The very purpose of this extraction, and the source of much of DNA testimony in alleged sexual assault cases, serves to address activity-level propositions. In fact, activity evaluation related to biological evidence has been in the courtroom since body fluid identification has been used to, for example, test for blood on a knife blade. Often, activity-level inquiries at court center around why certain items of evidence were tested or not tested and on discussions of "major" or "minor" donors. Such questions are rarely recognized as activity-level issues, and yet they should be, as they require a higher level of expertise to answer compared to sub-source-level questions.

Most activity evaluation of DNA evidence is expressed in such ambiguous terms as possible, could have, and vague statements as "not very likely." Furthermore, such testimony is usually elicited via hypothetical questions about various possible explanations for the findings, which may be nothing more than a statement of the obvious, speculative, prescriptive, or fanciful in nature.

This presentation will introduce peer-reviewed, scientifically accepted methods available to the expert to address activity-level inquiries in a direct fashion. These include Case Assessment and Interpretation, Bayesian networks, and the likelihood ratio.[7-10] This presentation will address the argument that, "We don't have the experimental data to address transfer," and discuss the need to address persistence, prevalence, and recovery in addition to transfer. Three recent appellate reversals of convictions will be discussed, indicating the courts seem to recognize that when activities are of interest, sub-source DNA or vague testimony about "possible" is not sufficient. The intersection of *Daubert* requirements, FRE 401, FRE 702, and the "transposed conditional" with activity testimony will be addressed.

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Expert Testimony; Forensic DNA; Interpretation

G15   Presumption of Innocence, Probable Cause, and Prior Probability—Bayes Meets Due Process

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Learning Overview: Bayes Theorem has three ingredients: (1) prior probability, (2) likelihood ratio, and (3) posterior probability. Each one has important scientific, legal, and philosophical implications. To make matters worse, the ingredients are often confused, conflated, and transposed. The goal of this presentation is to review the precepts of conditional probability, the notation of Bayes' Formula, and the touchpoints with presumption of innocence and probable cause.

Impact Statement: In the wake of *Daubert*, the 2009 National Research Council (NRC) Report and the 2016 **President's Council of Advisors on Science and Technology (**PCAST) Report, forensic science service providers have grappled with the best ways to move away from unqualified testimony like, "match to the exclusion of all others," "zero error rate," and "scientific certainty."[1,2] Bayes' Theorem could be a useful construct for communicating accuracy and the limits of forensic science results. However, the key concepts are often misunderstood and unsettled issues about how to use Bayes' Formula in the judicial setting remain.

For the uninitiated, this presentation will serve to review the fundamentals of Bayes' Formula. The notation, basic concepts, and principles of conditional probability will be reviewed to allow for a careful discussion of several challenges for using this formula in the judicial and forensic science service provider settings. The tension between prior probability and presumption of innocence will be explored by looking at the history of the presumption of innocence in *Coffin v. United States*, *State v. Skipper*, and *U.S. v. Shonubi*.[3-5] Does a presumption of innocence mean the prior probability must be fixed at zero? What if the suspect is part of a closed population set (e.g., one of only 100 prisoners who could have committed a crime)?[6] The notion of probable cause in *Beck v. Ohio*, and the 4th Amendment to the United States Constitution will be examined to determine if physical evidence presented to a forensic science service provider is different from a randomly selected item and, therefore, has a different prior probability of matching a crime scene sample or being an illegal substance, etc. The highly desired but often elusive posterior probability will be explained with special attention to Positive Predictive Value (PPV) and common mistakes in the literature.[7,8] Finally, the likelihood ratio will be inspected. This mathematical term requires the scientist to populate both a numerator and denominator with values. What constitutes acceptable sources for these numbers is a source of debate in the development of forensic science standards. Do they need to originate from rigorous probability distributions, or can they be estimated, or even based, on an examiner's subjective, qualitative assessment?[9] Strategies for direct and cross examination of expert witnesses will be presented that answer three questions: (1) how can the forensic science results be presented in the best light, (2) how can fair concerns about accuracy and error rates be raised, and (3) how can this material be covered without confusing judges and juries?[10-12]

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Bayes' Theorem; Prior Probability; Statistics

G16   Preventing and Correcting DNA Errors and Wrongful Convictions

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Learning Overview: DNA analysts play key roles in the justice system, from processing identity evidence to interpretation, advice, and testimony. The practice has evolved significantly over the past 30 years, but errors and outdated practices still contribute to wrongful convictions. Attendees of this presentation will learn how to avoid errors, over-extensions of findings, and bias. Topics addressed will include the fallacy of "the absence of evidence...," the myth of the "sperm fraction," the necessity of photographing all subjective tests (serology and microscopy), the vast variability of statistical measurements, the challenge of obtaining internal intimate samples, the importance of testing evidence before any reference samples, and the benefits of consulting on out-of-state cases.

Impact Statement: Through the examples of wrongful convictions that involved DNA evidence, this presentation will impact the forensic science community by informing attendees about common DNA errors and what can be done to prevent and correct wrongful convictions.

This presentation will expose examples of wrongful convictions that involved DNA evidence.[1] Wrongful convictions result from cumulative errors that include outdated and erroneous practices by scientists. The search for these errors requires continuous review, inter-laboratory comparison, and dedication. Historical phrases and terms-of-art are one route of error. For example, the use of the term "sperm fraction," without any documentation of actual sperm cells, causes jury confusion and possible wrongful convictions. Labs that do not photograph subjective tests (for example, serology and microscopy) prevent meaningful review of their data. Likewise inappropriate idioms like, "The absence of evidence is not the evidence of absence," can convey the mistaken notion that properly conducted negative tests are not evidence, even when a hypothesis has been tested using a specific reference sample. The presentation will also explain that labs that process evidence and reference samples together needlessly risk contamination in an era when single-molecule detection is common. Lab personnel will learn that professional consulting on out-of-state cases is one of the best ways to assure that the field self-regulates, and that citizens enjoy more equal access to independent forensic experts. The presentation will also discuss ways for analysts to avoid bias and resist the pressure to testify in a certain manner. Finally, labs will be encouraged to form "Conclusion Integrity Units" to look for possible wrongful conclusions that may have resulted in wrongful convictions.

The presenter has more than 20 years experience in DNA case review and has helped in more than three dozen exonerations in the United States, Europe, Asia, and Africa. He teaches graduate Forensic Biology at Boise State University (BSU), is the founder of the Idaho Innocence Project at Boise State University, Director of the Forensic Justice Project, and teaches Wrongful Convictions (University of Idaho College of Law). He is coauthor with Calvin C. Johnson, Jr., of *Exit to Freedom*, the story of Mr. Johnson's 17-year wrongful imprisonment.

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Forensic DNA; Ethics; Casework

G17   Forensic Genetic Genealogy and the Court System—Where Are We Going?

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Learning Overview: After attending this presentation, attendees will have gained a perspective on important issues related to the introduction of Forensic Genetic Genealogy (FGG) in court, with an emphasis on the requirements placed on practitioners who provide expert testimony.

Impact Statement: This presentation will impact the forensic science community through a discussion of the various technical and legal challenges that expert witnesses may face as FGG moves further into the judicial system.

FGG was recently introduced into court as an investigative lead in the trial of Patrick Leon Nicholas for the 1991 murder of high school senior Sarah Yarborough in Federal Way, WA, as a way to address privacy concerns that the defense raised related to the state constitution. This landmark case has brought to the forefront important issues concerning the relevance of introducing FGG in court. FGG can only produce investigative leads; ultimately, legal identification depends on a Short Tandem Repeat (STR) match to crime scene DNA.

Even so, an expert witness must meet ANSI National Accreditation Board (ANAB) and Organization of American Studies (OAS) standards, which brings up additional concerns over the lack of training and professional background of FGG practitioners. An expert is viewed not as a subject matter expert in a narrow sense, but as a person qualified by knowledge, skill, experience, training, or education; that is, a practitioner broadly defined as an expert by his education and relevant work experience. At present, the FGG community offers many training courses in genetic genealogy, but there are few in professional development in a larger sense, and very few, if any, covering expert witness testimony. Practitioners called to testify in the near future will have little experience preparing for testimony and no knowledge of what to expect.

This presentation briefly covers developments in the Yarborough homicide relevant to FGG, from 2011, when Y-chromosomal Short Tandem Repeat (Y-STR) FGG was first suggested as a means of discovering the offender's name, through the 2019 identification of Patrick Nicholas as a suspect using autosomal Single Nucleotide Polymorphism (SNP) testing. We discuss the reasons why FGG was introduced into the case, the defense strategy, the testimony provided by the expert witness, and the jury's decision to convict Patrick Nicholas of murder in the first degree with special circumstances and murder in the second degree. He was sentenced on May 25, 2023, to 45 years, 8 months in prison.

In summary, the presentation provides insight into the technical and legal developments on the case in the context of challenges the community faces if FGG should move further into the court system.

Expert Testimony; Court; Genealogy

G18   The Incidence of Expert Testimony Within Federal Sentences in the Criminal Justice System of Mexico

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Learning Overview: After attending this presentation, attendees will have received information regarding the use of expert evidence in judicial sentences within the criminal justice system of Mexico. The data will be categorized by the type of crime and the type of expert evidence used.

Impact Statement: This study will impact the forensic science community by analyzing the impact of changes in the criminal justice system of Mexico on the use of expert evidence during criminal proceedings. This study collected a set of sentences and analyzed the type of evidence used to reach a verdict. The empirical results indicate that the use of expert evidence has increased, but it is not the most frequently used evidence. Instead, eyewitness testimony is still the most common.

In June 2016, Mexico switched its criminal justice system from an inquisitorial model to an adversarial one, resulting in a paradigm shift in the investigation and judicial work of criminal matters. One of the significant changes brought about by this shift is the role of evidence during the process, particularly in its admission and assessment by the judge. It was expected that the inquiry into criminal offenses would rely on the scientific analysis of the facts and, as a result, the evidence should focus on expert work. This is because expert evidence is presumed to be the most reliable to configuring the legal truth sought in a trial. The question that arose was if expert evidence was being used frequently and if it was truly occurring in trials. To answer this, we integrated a collection of sentences issued in the hearing trial stage by federal courts, in the period from 2016 to 2021, that corresponds to the period that the new accusatory legal system was established all around the country. By doing this, we were able to identify patterns in the types of evidence that were presented during these trials and establish the trend of the types of evidence that have been incorporated into trials. Our results also allowed us to discuss whether Mexican judges are applying standards of weighing evidence.

In total, a sample of 580 sentences was compiled and classified by type of crime, type of evidence, and type of expert evidence. We used descriptive and inferential uni- and bi-variate statistical methods to analyze the data. The main findings show that the prevalence of expert evidence is not as high as expected with the change in Mexico's criminal justice system. Instead, eyewitness testimony is still the most common at 52.13%, followed by expert evidence at 19.62%. Additionally, photographic and criminalistic expert evidence are the most common in almost all types of crimes, and certain types of crimes are more associated with specific types of expert evidence. These results are important given that empirical studies like these are not often conducted in the legal field in Mexico, and this work could be the setting stone for promoting them.

Expert Testimony; Legal Research; Sentencing

G19   Gender Inequality and Medical Malpractice: A Sneaky Oversight

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Learning Overview: After attending this presentation, attendees will be able to better understand that gender influences each person's state of health and illness and that, in medicine, there are gender-related inequalities. This presentation will present the situation in Italy, focusing on the spread of gender in the National Health System, differences in treatment between male and female patients, and the study of gender on performance in medicine.

Impact Statement: This presentation will impact the forensic science community by showing the necessity to begin to approach forensic litigation from a gender perspective, to integrate our knowledge of the "litigation phenomenon," and to interpret and study it from a gender perspective.

The World Health Organization (WHO) defines gender medicine as the study of the influence of biological differences (defined by sex) and socio-economic and cultural differences (defined by gender) on the health and illness of each individual. Indeed, many diseases common to men and women often differ not only in incidence, symptomatology, and severity, but also in adverse reactions to treatments and drugs. Access to treatment also reveals gender-related inequalities. In 2018, Italy enacted a law for the application and dissemination of gender medicine in the National Health System (Law 3/2018), which provides for a multidisciplinary approach that takes into account differences arising from gender. Women, as a matter of fact, have been discriminated against in medical practice for centuries because they are more susceptible to medical errors, to the extent that we can speak of a "gender gap" and "gender injustice." It is documented that women have a higher risk of delayed, missed, or incorrect diagnoses due to the under-representation of women in biomedical studies and research. This "knowledge gap" explains why women's symptoms are often misinterpreted and underestimated by doctors and sometimes labelled as "psychological."

Another aspect is inherent in the study of gender on performance in medicine: international studies show that male doctors are more frequently the subject of litigation than female doctors. This bias could be partly due to the leading presence of male doctors in specialties with a higher risk of litigation, such as surgical specialties. Another possible explanation is that female doctors have been shown to be more inclined toward patient-centered approaches. On the other hand, it appears that despite this, patients' trust in female doctors is lower than in their male counterparts, especially when the rating of "knowledge" is taken into account and in those specialties that are typically "male dominated." In light of these data, it is essential to begin to study this phenomenon in a gender-specific way, integrating our knowledge of the "litigation phenomenon," and also interpreting and studying it from a gender perspective.

Gender Medicine; Gender Gap; Medicolegal Controversy

G20 A Difficult Patient to Frame: An Overview of Critical Issues in the Management of the Psychiatric Patient After the Closure of Judicial Psychiatric Hospitals in Italy

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Learning Overview: After attending this presentation, attendees will be aware of recent events in the Italian news involving assaults of not only health care workers but also civilians, by psychiatric patients, which allows the reopening of the discussion of proper management of psychiatric subjects who have become criminal offenders. In fact, despite the evolution of the issue throughout the decades, it still represents a topic in which many aspects need to be clarified.

Impact Statement: This presentation will impact the forensic science community by offering a cross-section view regarding the management of forensic psychiatric patients in Italy following the closure of the judicial psychiatric hospitals. Compared to the United States, where the provisions of judgment and treatment for forensic psychiatric patients change from one state to another, in Italy, the issue of managing such peculiar patients still remains a source of debate due to the persistence of some legislative aspects that are of difficult interpretation.[6]

The enactment of the 180/1978 law, the so-called Basaglia law, and the 81/14 law determined the overcoming of Judicial Psychiatric Hospitals in favor of Residences for the Execution of Security Measures (REMS), which are managed by the Departments of Mental Health. These laws have made Italian psychiatry the center of a revolutionary phenomenon that has sanctioned the shift from a "custodial" model to one in which the therapeutic-rehabilitative dimension should be prevalent in the management of psychiatric offenders.

However, the practical application of these laws remains a critical point that constitutes an obstacle to framing and, consequently, placing those types of patients considered "difficult" to manage.[1] The main dispute is represented by the obsolescence of the legal institution of imputability (especially in the matter of total and partial mental defect) and, above all, of social dangerousness, the assessment of which suffers from the lack of clear and unambiguous scientific criteria.[2,3] Moreover, entrusting "difficult" psychiatric patients at risk of committing violent acts to the territorial health system has led psychiatrists and public services to be in contact with all those practices, tools of assessment, and clinical management that are key components of health care and control. Nonetheless, these assessments are far from a psychiatrist's common baggage of knowledge and professional training.[4] Consequences arising from such shortcomings comprehend, on the one hand, the undertaking by public services and psychiatrists of a burden of new obligations, commitments, and responsibilities, and, on the other hand, the risk of regression to an "asylum-like" regime. This last occurrence has the potential to establish a regime in which the aspects of neutralization and control of the patient prevail to the detriment of the socio-rehabilitative one.[1,5] Hence the need for a reform of the concepts of social dangerousness and imputability, as well as the organization of facilities with a strong rehabilitative connotation for "difficult" psychiatric subjects, which goes hand-in-hand with adequate training of the personnel in charge of their evaluation and treatment.

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Psychiatric Patient; Imputability; Social Dangerousness

G21   Post-Conviction DNA Testing: Annual Summary of an Innocence Project and University Collaboration to Identify and Evaluate Post-Conviction Cases

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Learning Overview: After attending this presentation, attendees will understand the California Forensic Science Institute-Los Angeles Innocence Project (CFSI-LAIP) case review model, emphasizing case prioritization, filing and arguing motions requesting DNA testing, and strategic approaches to case resolution.

Impact Statement: This presentation will impact the forensic science community by providing attendees with a law firm-university collaboration model to tackle the logistical challenges of post-conviction DNA testing.

The CFSI within the School of Criminal Justice and Criminalistics at California State University, Los Angeles, established a novel partnership with the LAIP in 2022 to review cases and locate and test evidence related to violent felony offenses where actual innocence might be demonstrated. The CFSI-LAIP team was awarded Department of Justice (Bureau of Justice Assistance award 2022-15PBJA-22-GG-01413-POST) funding under the FY 2022 Postconviction Testing of DNA Evidence solicitation to provide critical assistance to individuals convicted of a serious felony that meets specific criteria. Collectively, the CFSI-LAIP team reviews violent felony cases to locate biological evidence for submission to an accredited private forensic laboratory for DNA testing to demonstrate their innocence or assist in their exoneration.

This presentation provides a one-year overview of the CFSI-LAIP case review model, emphasizing identifying and evaluating cases that meet the CA Penal Code §1405 criteria for post-conviction DNA testing. Our summary will include the number of DNA cases reviewed and investigated; the crimes for which clients were convicted, including those that have a sexual assault component and/or resulted in the death of the victim(s); and the number of evidence searches conducted, specifying the cases where evidence was located v. destroyed or missing. Further, we will disclose the number of items submitted for Short Tandem Repeat (STR), Y-chromosomal Short Tandem Repeat (Y-STR) analysis, and whether a viable profile was obtained in addition to the probative value of the DNA profile (supporting v. refuting the post-conviction claim or inconclusive). We will also specify the number of DNA profiles uploaded to the Combined DNA Index System (CODIS) v. the number of CODIS hits. We will conclude by highlighting successful case outcomes, describing obstacles to our project objectives during the 2022–2023 award period, and demonstrating how the CFSI-LAIP model allows forensic science graduate students to participate in a service-learning social justice law project.

Post-Conviction DNA Testing; Innocence Project-University Collaboration; Service-Learning Social Justice Law Project

G22   **Workplace Violence Against Health Care Workers: An Umbrella Review of Systematic Reviews and Meta-Analyses**

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Learning Overview: After attending this presentation, attendees will understand the real prevalence of the phenomenon of violence against health workers and the current legislation on the subject.

Impact Statement: This presentation will impact the forensic science community as it could be used to understand how to guide the construction of a common document for all professional societies, such as doctors and nurses, to make the institutions understand the real extent of this increasingly worrying phenomenon.

Background: Workplace Violence (WPV) is a concerning issue in public and occupational health. It is described as violent events that pose a threat to staff members' safety, well-being, or health while they are at work.[1,2] Health care professionals, including doctors, nurses, and other staff, are at a higher risk of experiencing workplace violence due to the nature of their work and the populations they serve. In fact, according to United States data, the health care environment accounts for almost 70% of all WPV.[3]

Objectives: The aim of this umbrella review of systematic reviews and meta-analyses was to address WPV against health care workers. Several systematic reviews exist in the literature, but the diversity of settings, population considered, and type of violence investigated make it difficult to gain insight and use the vast amount of available data to implement policies to tackle WPV. With this in mind, we conducted an umbrella review of systematic reviews and meta-analyses on WPV against health care workers to examine the global prevalence of the phenomena and its features.

Study Design and Methods: Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, PubMed®, Scopus®, and ISI® Web of Science were searched for relevant systematic reviews and meta-analyses published in English up to November 2022. Data on authors, year, country, violence type, prevalence (pooled and not), setting, population, and specific considerations were extracted.

Results: Thirty-two systematic reviews were included, 19 of which performed a meta-analysis, investigating overall, physical, and non-physical violence. Even considering the variability of the data, the COVID-19 pandemic has exacerbated the scale of the problem. From our review, we found that overall violence prevalence among health care workers was reported to be as high as 78.95%, with nurses working in psychiatric wards being the profession most impacted.

Discussion: The consequences of WPV have several dimensions. Physical consequences may include injuries from assaults, while emotional and mental health effects can include anxiety, depression, Post-Traumatic Stress Disorder (PTSD), and other mental health conditions.[4,5] In addition, the effects of WPV can also extend beyond the individual, affecting the work environment with a decrease in the overall quality of care provided to patients. WPV issues should also be addressed from a legislative point of view.

The World Health Organization (WHO), International Labour Organization, International Council of Nurses, and Population Services International developed the "Framework guidelines for addressing workplace violence in the health sector," and a questionnaire to investigate the effects of violence in such settings.[6] However, no shared core of regulations has been adopted at supranational level. This is also evident in United States where a few states, such as Alaska, Idaho, and Washington, had legislation adopted to punish violence against health care workers.[7] Instead, this topic received a lot of attention in Italy. In fact, in 2020, the Parliament passed a law to impose harsher punishments for acts of violence against medical professionals, particularly when there is personal injury.[8,9] A new article was established that defines the offense especially against health care employees by imposing stronger sanctions in light of the rising number of cases of WPV against health care workers through the Law on 11/3/2020.[10] In addition, through an additional amendment, any injury that is carried out against a health care provider is directly prosecutable *ex officio*. However, non-physical aggression, which is more common, has not received the same attention.

Finally, this umbrella review revealed a high prevalence of WPV among health care workers, which varies between countries, population subgroups, and detection methods. Strengthening recognition of the problem could lead to appropriate local and international strategies to address it.

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Workplace Violence; Health Care Workers; Umbrella Review

G23   The Positive Impact of Clinical Forensic Medicine Units on Profiles of Medicolegal Cases for Insight and Reviewing Strategies to Plan Against Crime

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Learning Overview: After attending this presentation, attendees will understand that profiling of Medicolegal Cases (MLCs) is an important aspect for assessing type of crime and thereby the execution of preventive steps to reduce casualties in the future need of establishing clinical forensic medicine units in every district hospital in India and other developing countries. The ultimate goal of this presentation is that clinical forensic medicine unit data assists law enforcement agencies to prevent assault, road traffic accidents, domestic violence, and/or child abuse by providing safety awareness and security checks and by maintaining strict laws by the government authorities.

Impact Statement: This presentation will impact the forensic science community by demonstrating how profiling of MLCs helps to study genuine crime rates in an area and errors were significantly reduced in medicolegal reports prepared by forensic medicine experts and medicolegally trained doctors. Incomplete and inaccurate medicolegal reports often slow down the courts' trials and lead to incorrect judgements. Identification of such mistakes and shortcomings in reports can help prevent litigation against doctors, unbiased court judgements, and injustice.

Introduction: MLCs are an essential component of medical practice and comprise most important constituent of emergencies. MLC can be defined as a case of injury or ailment, etc. in which investigation by the law enforcing agencies is essential to fix the responsibility regarding the causation of the said injury or ailment. Common MLCs include alleged cases of assault, road traffic accidents, burns, poisoning, snake bite, insect bite, industrial accidents, alcoholic intoxication, etc.

Method: A retrospective analytical content-based analysis was made on 10,497 MLCs recorded at Civil Hospital Sector 6, Panchkula, during the period of 01/04/2021 to 3/31/23. Data was taken from district Forensic Medicine Unit from HIMS, MedLEaPR portal, and then analyzed.

Results: The study revealed that out of the 10,497 cases, 8,219 were men and 2,278 were women; police custodial medical examination 37.32% (3,918 cases), road traffic accident 19.73% (2,072cases), and assault 32.85% (3,449 cases) constituted most of the MLCs. Poisoning was 05.02% (527 cases) and constituted a smaller number of MLCs. Sexual offences and domestic violence or child abuse constituted 1.63% (172 cases) and 1.06% (112 cases), respectively. The majority of victims were in the 21-40-year age group and were 61.41% (6446) of the cases. Urban victims 76.89 % (8072 cases) out-numbered rural 23.10% (2,425 cases).

Conclusion: The study concludes that the majority of MLCs were of police custodial medical fitness examinations, assault, and road traffic accidents. A significant increase in domestic violence or child abuse cases had been observed in a secondary care hospital in North India. A large number of cases brought by police medical for fitness examinations revealed that police show a quick response for arrest of accused person due to online police information by the MedLEaPR portal of the Forensic Medicine Unit. Young age groups were involved in most of the patterns of MLCs. Thus, measures should be taken to prevent assault, domestic violence, and child abuse by providing safety awareness, security checks, and by maintaining strict laws by governmental authorities. The bulk of cases, up to 10,497 in two years, show the importance of the Forensic Medicine Unit in secondary-care hospitals in densely populated areas. The trial of the Forensic Medicine Unit in District Hospital Panchkula, Haryana, helps the government as well as the Planning Commission know about the need of establishing a Forensic Medicine Unit in every district hospital of India and other developing countries.[1-6]

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Accident; Accuracy; 3D Technology

G24 The Impact of Post-Traumatic Stress Disorder (PTSD) on Those Seeking Asylum

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Learning Overview: This presentation will consider the impact of PTSD on victims of torture who are seeking asylum and how the Istanbul Protocol can assist with the effective identification, documentation, and assessment of medical evidence within refugee status determination. It will consider the diagnostic definitions of PTSD, including its prevalence rates among asylum seekers and its wide-ranging effects and symptoms. After attending this presentation, attendees will appreciate the importance of a medicolegal report that meets the Istanbul Protocol's standards as an integral part of the assessment of refugee status.

Impact Statement: This presentation will impact the forensic science community by demonstrating the important role of clinicians in documenting and evaluating not only the physical signs of torture in asylum seekers, but also the psychological signs and symptoms such as PTSD. This presentation will highlight the importance of the Istanbul Protocol as an effective tool within refugee status determination and promote the training of clinicians in the Istanbul Protocol's standards. This will help to enable asylum seekers with access to fair justice through an asylum decision-making process, which includes the right to a medical examination and evaluation to support their asylum claim and safeguard their overall health and wellbeing.

For refugees and asylum seekers, the prevalence of trauma is pervasive and often distinct from the experiences of other traumatised populations as a result of not only experiencing events such as war, torture and persecution prior to migrating but also due to the myriad of harsh conditions faced post-migration, including separation from family, immigration detention, financial hardship, and the challenge of adapting to a new environment both culturally and linguistically.[1,2] These experiences lead to refugees and asylum seekers falling vulnerable to a constellation of mental health issues encompassing depression, anxiety adjustment disorders, and trauma-based illnesses.[3] While trauma-based illnesses include a range of disorders, such as acute stress disorder and dissociative disorders, studies conducted on the impact of conflict on mental health have confirmed that PTSD is one of the most common psychological complications among trauma victims, and for refugees, in particular, all studies have demonstrated that the greater the number of trauma items suffered, the more intense the symptoms of PTSD present.[4,5] The Istanbul Protocol, a set of international guidelines for the assessment of persons who allege torture and ill-treatment, further confirms that the most commonly diagnosed disorders amont trauma and torture survivors are depressive disorders and PTSD.[6]

A systematic review on the prevalence of mental illness in both refugees and asylum seekers, undertaken in 2020 by Blackmore and colleagues, found the prevalence rate of PTSD within this population to be 31.46%.[7] For women in particular, this rate increased slightly to 34.02% due to the increased risk of sexual violence faced by women during conflict, known to present a high risk for developing PTSD, but also other risks associated with migration trauma, including the risk of trafficking and exploitation.[8,9]

This presentation defines PTSD according to its diagnostic definitions, its prevalence, symptoms, and effects on those who are seeking asylum, and how this translates to refugee status determination. This presentation considers the importance of the Istanbul Protocol for the effective identification, documentation, and assessment of medical evidence within the asylum decision-making process. Given the high prevalence rates of PTSD among those seeking asylum, this presentation advocates for medical examinations and evidence documenting the psychological symptoms of acts of torture and ill-treatment, as per the standards set out within the Istanbul Protocol, to be considered as an integral part of the refugee status determination process.

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Mental Health; Post-Traumatic Stress Disorder; Asylum

G25 Legal Tools to Advocate for Forensic Standards in Missing Persons' Cases

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Learning Overview: The goal of this presentation is to equip attendees with the legal insight and language needed to explain the different rights at risk of being violated under international human rights law when forensic standards are not complied with in missing persons' investigations, including cases of unidentified human remains. To this end, the argument will be put forward that best forensic practices into the investigation of missing persons' cases can be reasonably accepted as giving rise to an international legal obligation.

Impact Statement: American Academy of Forensic Sciences (AAFS) members who are interested in pursuing humanitarian or human rights work may encounter a perception among interlocutors or even colleagues that the application of high forensic standards is a luxury or an option. This is so especially in contexts with no established protocols for the application of forensic sciences to missing persons' investigations. This presentation will impact the forensic science community by providing attendees with the legal tools they need to advocate for and promote best forensic practices. Attendees will thus be better prepared for the challenges they will encounter in this type of work.

This presentation will demonstrate the degree to which forensic standards are expected to be applied in humanitarian investigations into missing persons' cases and are not merely a recommended practice according to human rights courts.

Forensic practitioners understand the significance of always applying best practices to their work. What they often lack is legal backing for what they know to be scientifically necessary, especially when human remains are not managed specifically in the context of (contemplated) criminal proceedings. However, when examining the jurisprudence of human rights mechanisms around the world regarding different components of missing persons' investigations, it can be seen that international courts are willing to find human rights violations due to non-compliance with internationally accepted forensic standards. International jurisprudence discussing the collection, documentation, preservation, and analysis of forensic evidence and finding human rights violations due to defects in the forensic aspects of an investigation reveals that the application of what can be summarized as best forensic practices, including practices relating to the collection and handling of human remains, is in fact expected by human rights courts.[1-7] Courts have also recognized a rigorous obligation requiring states to investigate suspicious disappearances, indicating that the standards required of investigations are not triggered only once human remains are found.[8]

In combination with the redress mechanisms available to rights-holders, human rights jurisprudence thus rebuts the perception that there is no accountability for not applying high forensic standards to missing persons' investigations and sends the message that the accurate and timely identification and humanitarian management of the deceased is a form of justice that can be achieved for individuals who are often the most vulnerable members of society in domestic, transnational, and international contexts. Drawing the above elements together, this presentation will address the relevant international legal framework as well as individual components of investigations, specifically focusing on the search for missing persons and on the recovery, handling, examination, and disposal of human remains.

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Missing Persons; Standards; Human Rights

G26 Guidelines for the Judicial Assessment of Expert Evidence in the Mexican Legal System

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Learning Overview: After attending this presentation, attendees will be informed about information related to the scientific and technical standards applicable to the development of expert evidence in various forensic areas and its usefulness for the assessment by judges within the criminal justice system in Mexico. In addition, attendees will have learned about the methodology applied in the preparation of the guides and their scope as a forensic tool.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the Guidelines for the Judicial Evaluation of Expert Evidence represent a pioneering effort in Mexico to establish the minimum scientific and technical standards that expert evidence should contain in order to assist judges in the judicial assessment. In this way, they seek to raise the degree of certainty within the judicial processes and reinforce the importance of expert evidence as a tool for the resolution of criminal conflicts in Mexico.

One of the most important objectives of the Mexican criminal justice system is that scientific and technical evidence has a broad and correct application. The parties have the responsibility of providing it correctly; the expert, by working under accepted scientific and technical principles; and the judge, by admitting and evaluating the evidence correctly.

For the development of the objectives of the criminal justice system, the National University of Mexico (UNAM), through the National School of Forensic Sciences, prepared the Guidelines for the Judicial Assessment of Expert Evidence, Volumes I and II; in collaboration with the Federal Judiciary Council, through the Unit for the Consolidation of the New Criminal Justice System; the Supreme Court of Justice of Mexico City; the American Embassy, through the International Office for the Development of Justice Systems (OPDAT) and the International Criminal Investigation Training Program-Mexico (ICITAP); the General Attorney's Office, through the General Coordination of Expert Services of the Criminal Investigation Agency; and the National Conference of Justice Procurators.

The guides are made up of four sub-guides describing the general criteria that must be considered for the evidence assessment, as well as the errors that may occur in the expert evidence and that must be considered for its evaluation in addition to the minimum criteria, that is, the degrees of permissible tolerance associated with each stage through which the evidence passes and that are reflected in frequent failures or circumstances. This information was obtained from international and national manuals and protocols accepted by the forensic science community. The guides include the following forensic science areas: genetics, toxicology, voice analysis, lophoscopy, human identification, psychology, forensic medicine, odontology, digital video, ballistics, and criminalistics.

It is important to point out that the guides are not expert protocols; they are documents addressed to judges to help them understand the scientific and technical aspects they may face in their legal work. They are intended to contribute to improve the scientific and technical standard of expert evidence, to assist judges in their work of assessment and to provide elements that increase the level of specialization in interrogations and cross-examinations during the oral trial (principle of contradiction).

Thus, the Guidelines for the Judicial Evaluation of Expert Evidence represent a pioneering effort in Mexico to establish the minimum scientific and technical standards that expert evidence should contain. The guidelines are available at this link: https://www.cjf.gob.mx/PJD/guias/Default.aspx.

Decision Making; Judge; Evidence

G27 Exploring the Contours of Bridging the Gap Between Forensic Science and Law in India

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Learning Overview: After attending this presentation, attendees will be able to better understand the challenges faced in the realm of intersection between forensic science and law, in as much as it is being experienced at various forums that professionals possessing separate specific knowledge in both the above stated fields are not able to provide a holistic solution in the administration of justice. Undoubtedly, the ultimate objective of both these fields is to apprehend and adequately punish the real culprits.

Impact Statement: This presentation will impact the forensic science community in multi-dimensional ways because a forensic scientist must possess a basic understanding of the legal ecosystem. Conversely, a lawyer intending to practice in the robust field of forensic science must be technically sound so as to effectively communicate complex scientific theories.

It has been observed from the public domain that the criminal investigation and presentation of the evidence in the courts in India result in various people coming together from different walks of life. In court, there are lawyers, attorneys, police officers and forensic experts from different segments to present their findings in the due course of investigation. It has been observed that even the lawyers and judges require specialized training to be well conversant with scientific evidence. They must develop a bent of mind that is appropriate for the analysis of scientific evidence in criminal cases. Forensic experts, lawyers, and judges must be equipped with expertise in the interlinking areas to present the case in a proficient manner before the court. The courts must have a fool-proof review system by which they may reach at a logical conclusion in the light of the scientific evidence produced before them. In this way, forensic science will be duly represented, and its legal impact will be given due recognition by the court.

It is an irony that there is a communication gap between the forensic experts and judicial authorities as the forensic experts are bereft of specialized legal knowledge and lawyers and judges are unaware about the technicalities of forensic science. Hence, it becomes incumbent for both the disciplines to possess common knowledge so as to become well conversant in both the disciplines, which would certainly result in a better criminal justice delivery system. Authorities from both these disciplines will thus better understand the other's perspective in achieving and promoting justice.

In light of the above discussion, there is a paramount need for launching integrated curriculum in law courses at undergraduate and post-graduate levels where the law students interested in practicing criminal law are taught the subject of forensic science in detail so they will be in a position to better assist in the delivery of justice at a later stage. Apart from this, another recommendation is that there should be government-sponsored or institutionalized refresher programs for practicing lawyers and forensic experts to provide them multidisciplinary and technical education. As a result, it would aid in sharpening their skills and technical knowledge.

During this presentation, all the pros and cons of the topic under discussion will be thoroughly analyzed and more possibilities of bridging the gap between the two disciplines (i.e., forensic science and law) will be resurrected. The analysis through this study will be highly significant from the point of view of striving toward a better justice delivery system, proper investigation, and presentation of evidence in the courts of law in India. In order to safeguard the interests of justice seekers and to promote better administration of justice, there is a dire need to bridge the gap between the apparently watertight disciplines of forensic science and the law.

Forensic Science; Law; Justice

G28   Challenging Scientists: A Three-Tier System for Educating Lawyers in Forensic Techniques

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Learning Overview: By attending this presentation, attendees will gain an understanding of the three-tier system for educating lawyers about forensic techniques in civil and criminal cases.

Impact Statement: This presentation will have a significant impact on the legal and forensic science communities, especially the policy makers in both groups, regarding the three-tier education system through which one can learn both basic and advanced knowledge of forensic scientific techniques.

This presentation will bring together educators imparting legal education to a three-tier system of education for law students and lawyers in forensic scientific techniques. The three-tier system of education is the need of the hour since currently the majority of cases that come up for trial are entangled with various forensic techniques in which the lawyers lack basic understanding of its theory and methodology. It is evident from the archive of judicial decisions that forensic evidence was generally appreciated without adequate evaluation by the trial court.[1] Lawyers are in the dark about how science works. The defense lawyers are really afraid of attacking scientists on various aspects of the forensic techniques employed by the prosecution to link the defendant with the crimes. The defense lawyers are also not familiar with the basic aspects of scientific evidence like potential error rate, statistical report based on Random Match Probability (RMP), allele frequency database for DNA profiling, etc. Defense lawyers don't know which questions to ask in cross examination for attacking scientific evidence. The defendants will be victimized if any fault was committed by the forensic scientists in the process of generating the evidence.

To recover from this sorry state of affairs, the presenter after gaining from the recommendations made by the National Academy of Sciences in its 2009 Report, put forward a three-tier education system for law students and lawyers for acquiring the foundational knowledge in forensic scientific evidence.[2] I propose the three-tier system since the majority of the students who enrolled in law degree programs are from arts background. Therefore, at the first level, students who receive basic education in scientific disciplines that have a bearing on forensic techniques shall commence in the law program. At the second level, lawyers will study an advanced course that includes all forensic scientific techniques. At this level, more emphasis will be given to equipping lawyers for attacking various forensic techniques. Third, there is a continuing program, which focuses on advancing new scientific techniques. .

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Forensic Science; Judicial; Education

G29   An FBI Laboratory Decision Analysis "Black Box" Studies in Pattern Evidence Examinations

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Learning Overview: This presentation will describe a series of research studies to examine the accuracy, repeatability, and reproducibility of forensic pattern evidence examination decisions across several disciplines: latent prints, firearms, shoeprints, and handwriting. After attending this presentation, attendees will have a better understanding of the factors affecting the experimental design and the interpretation of results.

Impact Statement: This presentation will impact the forensic science community by providing a summary of design factors for four large-scale research studies regarding the examination of pattern evidence (latent prints, firearms, shoeprints, and handwriting) as well as a response to common critical observations regarding the experimental design and the interpretation of results.

From 2007 through the present, the Federal Bureau of Investigation (FBI) Laboratory, with assistance from various contractors, conducted a series of decision analysis studies, also known as "black-box" studies, to assess the accuracy, repeatability, and reproducibility of pattern evidence examiner decisions in forensic casework. The results of this research have been presented at more than a dozen scientific conferences and published in more than 20 scientific papers, with several more publications in progress.[1-20] The results of this research can inform researchers, casework examiners, and the judicial system as to the value and utility of forensic pattern evidence examination.

This research has also sparked considerable discussion at conferences, in the trade press, as well as commentary and responses in the scientific literature.[21-24] This presentation will focus on presenting and responding to the most commonly encountered critical observations regarding the experimental design and interpretation of results. In addition, this presentation will address the treatment of inconclusive results in pattern evidence examinations and in research.

Critical factors in project management and experimental design to be addressed include securing funding and logistics; definitions of objectives; method independence; design of experiments—open set design, randomization in samples, numbers of participants, samples, and decisions, sample collection, digital vs. physical samples, selection of close non-matches, among others; sample difficulty and introduced biases to create bounding scenarios; participant recruitment, representativeness, and human subject research protections; harmonization and imposition of potentially unfamiliar reporting scales.

Factors affecting the interpretation of results include participant drop-out and non-response; volunteer bias; Hawthorne effect; calculation of accuracy, repeatability, and reproducibility rates; analysis of covariates among metadata and participant demography; absence of quality control measures and secondary reviews.

One critical area for consideration is how inconclusive results are treated in research, as opposed to forensic casework in which ground truth is unknown and unknowable. A variety of approaches have been suggested following publication of our research results, each of which has various advantages and disadvantages.

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Pattern Evidence; Black Box Studies; Accuracy

G30 Error Rate Studies: The Good, the So-So, and the Unacceptable

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Learning Overview: After attending this presentation, attendees will understand how to interpret estimated error rates arising from black box studies. In this presentation, we will discuss some shortcomings of this type of study and will provide guidance about their potential impact.

Impact Statement: This presentation will impact the forensic science community by informing attendees that in some disciplines (e.g., pattern comparison disciplines), examiners who testify in court rely on estimated error rates from black box studies to argue that their own assessment of the evidence is accurate and reliable. While this may well be the case, the published error rates are likely to be significantly underestimating the true probability of error. Examiners have faced challenges from the court in recent years, mainly from scientists in other disciplines who have questioned the statistical analyses of black box studies. These questions can be readily resolved if users of black box studies were given access to non-identifying information about participants and about the pattern of responses and non-responses.

At present, estimates of error rates—at least in the pattern comparison disciplines—rely on data collected via black-box studies. Practitioners and researchers in forensic science rushed to conduct black-box studies in response to the 2016 President's Council of Advisors on Science and Technology (PCAST) Report, which urged forensic scientists to establish the foundational validity of pattern comparison disciplines using empirical data.[1] In the past few years, estimated error rates have been published in several disciplines, including firearm, footwear, questioned document, handprint examination, and the analysis of bloodstain patterns.

Black box studies are not easy to design or implement. This is because much of the background information on which statisticians rely when designing studies is not available in most forensic disciplines.

When we assess black box study design using widely accepted statistical criteria, many problems become evident. For example, a basic criterion met by a well-designed study is that of the *representativeness* of study participants. To obtain a random, representative sample from any population, study designers typically must be able to enumerate and characterize the population of interest. In the context of forensics, it is impractical to draw representative samples of, for example, the population of firearms examiners, because no one has defined that population and there is no national "list" that includes every person who identifies as such. Therefore, none of the black box studies that have been conducted can claim that participants represent their professional community.

Other shortcomings of the black box studies that have been published include the fact that most studies are likely to be under-powered, purposely unbalanced designs sometimes result in participants who have no opportunity to commit certain types of errors, and are rife with missing responses, all of which affect the reliability of findings. Accounting for these problems to correctly interpret the data arising from the studies requires sophisticated statistical methods.

An additional attribute that makes the analysis of black box studies tremendously challenging is the typically high proportion of inconclusive responses. An inconvenient fact is that effective black box studies that present participants with difficult, non-obvious comparisons are likely to result in large numbers of inconclusives. While in some cases there is wide agreement and most participants submit the same inconclusive response, in most instances there is enormous variability in the responses. Yet at present, all inconclusive responses are counted as correct, regardless of difficulty.

Some of the limitations outlined above are difficult to address (e.g., representativeness of participants) so it may be necessary to compromise between statistical soundness and what can be achieved in practice. Other limitations, however, can be readily resolved using statistically justified methods. Given that millions of dollars and tremendous effort by both researchers and participants have already been spent on conducting black box studies, how can we extract as much reliable, useful information from existing data? In this presentation, we refer to real studies and share insights on attributes of design, implementation, and analysis and how those attributes impact the type of conclusions that may be drawn from the results. We highlight the problems associated with the design of the studies that will be difficult to overcome and require some compromising, and also the shortcomings associated with the analysis of responses that could be addressed if researchers were willing to share some additional information.

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Error Rate; Reliability; Data

G31 Inconclusive Decisions and Error Rates in Forensic Science

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Learning Overview: After attending this presentation, attendees will be informed about how inconclusive decisions should be treated in different contexts, including measures of method performance and assessments of method conformance, and how analysts' decisions (including inconclusive decisions) should be represented to maximize the expected overall accuracy of fact-finder decisions.

Impact Statement: This presentation will impact the forensic science community by providing a perspective on how inconclusive decisions should be treated when measuring the performance of a method or assessing conformance to a method, thereby offering a path forward that we believe more effectively addresses the issue surrounding the treatment of inconclusive decisions and the implications for forensic science stakeholders (e.g., researchers, managers, and officers of the court).

In recent years, there has been controversy relating to the treatment of inconclusive decisions in forensic feature comparison disciplines (e.g., friction ridge, firearms and toolmarks, footwear, tire tracks, and handwriting). While such discourse has led to a healthy academic debate, it has also created ambiguity and disagreement within the scientific and legal communities regarding (1) how inconclusive decisions ought to be treated in comparison studies and (2) the effect that such decisions have on the validity of estimated error rates. Some commentators have suggested inconclusive decisions should not be included in error rate estimates (e.g., President's Council of Advisors on Science and Technology [PCAST]). Others, however, were critical of such an approach and offered alternative suggestions, including inconclusive decisions always be considered "correct," inconclusive decisions always be considered "incorrect," inconclusive decisions be considered "correct" in certain situations and "incorrect" in other situations, and they neither be considered as "correct" or "incorrect." The various treatments of inconclusive decisions can lead to wildly different estimates of error rates and, therefore, different representations and interpretations of the validity and reliability of forensic science methods, all with potential consequences regarding the admissibility of such evidence in criminal courts.

We offer a brief review of the various perspectives that have been recently put forth, followed by a proposal for a path forward. We argue that most prior discussions on the issue have been hampered by narrow "one-size-fits-all" views and desires to use estimates of error rates (e.g., false positive or false negative rates) as the only measure of performance. Instead, we suggest that what has been largely missing from prior recommendations is *context* around the assessment of analysts' decisions (not necessarily limited to just inconclusive decisions). Specifically, we distinguish between the contexts of *method performance* and *method conformance*. Method performance relates to measures that reflect the capacity of a method to accurately discriminate between different propositions of interest (e.g., between same source and different source comparisons). Method conformance relates to assessments of whether the outcome of a method is the result of adherence to the protocol(s) that define the method.

In the context of method performance, we argue that an inconclusive decision does not provide a conclusive assertion about the source-origin state of the compared items. For that very reason, inconclusive decisions are not, and should not, be considered accurate or "correct" decisions. For that same reason, however, inconclusive decisions are not, and should not, be considered "erroneous" decisions. Consequently, when inconclusive decisions are a possible outcome of a method, measures of error alone are neither suitable nor complete representations of method performance. Instead, inconclusive decisions should be represented as a distinct outcome of the method—in the same way as conclusive decisions (e.g., identifications and exclusions)—as they relate to the true source-origin state of an item. Doing so enables a complete and transparent description of the outcomes produced by the method and determination of the performance characteristics under specified conditions. In this way, one can assess an evidential value for any particular decision, for instance by assessing a likelihood ratio for the examiner's decision using available validation data. This approach is demonstrably capable of maximizing the expected overall accuracy of fact-finder decisions. In the context of method conformance, however, inconclusive decisions (or any decision, for that matter) can be considered *appropriate* or *inappropriate*, depending on whether the decision is the result of adhering to, or deviating from, method protocols (including established criteria for such decisions). Care must be taken to ensure measures of method performance are not confused (or conflated) with assessments of method conformance.

Forensic Science; Inconclusive; Error Rate

G32   A New Black Box Study of the Accuracy of Latent Print Examiners: What More Do We Know in a Decade?

R. Austin Hicklin*, Noblis, Reston, VA; Nicole M. Richetelli*, Noblis, Reston, VA; JoAnn Buscaglia*, FBI Laboratory, RSU, Stafford, VA; AshLee Taylor*, FBI Laboratory/Latent Print Support Unit, Quantico, VA

Learning Overview: The goal of this presentation is to help attendees understand the accuracy and reproducibility of decisions by forensic latent print examiners as assessed in black box studies designed to simulate casework using a state-of the-art Automated Fingerprint Identification System (AFIS).

Impact Statement: This presentation will impact the forensic science community by describing the accuracy and reproducibility of decisions by forensic latent print examiners when comparing fingerprints resulting from searches of a state-of the-art AFIS algorithm for candidate comparison prints.

The forensic science community is currently engaged in a variety of efforts to evaluate the accuracy and reliability of forensic conclusions in the pattern evidence disciplines. The 2016 President's Council of Advisors on Science and Technology (PCAST) Report on forensic science stated that black box evaluations are necessary to establish the validity of forensic examination methods that rely on human judgment and provided some guidance on what criteria PCAST required for such testing to be considered rigorous and suitable for court admissibility.[1]

In 2022–2023, the FBI Laboratory and Noblis conducted a new latent print examiner black box study (LatentBB22) in order to measure the accuracy and reproducibility of latent print examiners' decisions when comparing latents to known fingerprints acquired by searching the FBI Next Generation Identification (NGI) system This lecture will discuss the study design and results from LatentBB22 and compare the results of this study with those from previous studies, including the original FBI/Noblis latent print examiner black box study (which was conducted in 2008–2009), and the latent print white box and eye-tracking studies (conducted in 2012 and 2014).[2-6]

Each participant in LatentBB22 was assigned 100 latent:exemplar image pairs for examination and was asked to report a value determination, categorical conclusion, and difficulty and typicality assessments for each. The image pairs included in this study were selected to be broadly representative of {NGI} casework. Analyses are based upon over 14,000 responses submitted by 156 latent print examiners. The results of the LatentBB22 study show that the performance of latent print examiners is multi-dimensional, includes different aspects of accuracy and effectiveness, and, therefore, cannot be summarized in terms of a single discipline-wide error rate. In light of the results of LatentBB22, this presentation will also discuss the benefits and limitations of black box studies, including considerations for assessing validity and interpreting the results with respect to latent print examination overall and assessing the abilities of individual examiners.

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Latent Prints; Accuracy; Black Box Studies

G34   In a Manner of Speaking: A Medical and Legal Perspective on the Problem of Manner of Death Testimony in Criminal Trials and What We (All) Should Be Doing About It

Allison Lewis*, DNA Unit/Homicide Defense Task Force, The Legal Aid Society, New York, NY; Andrew Baker*, Hennepin County Medical Examiner's Office, Minnetonka, MN

Learning Overview: This presentation will provide an overview of the history and purpose of "manner of death" determinations, how courts have addressed the admissibility of this testimony, and why they are not fit for purpose in criminal trials from the perspective of a medical examiner and defense advocate.

Impact Statement: This presentation will impact the forensic science community by demonstrating that testimony regarding manner of death is not fit for purpose in criminal trials. After attending this presentation, attendees will understand why a growing chorus of voices, made up of both medical examiners and defense advocates, agree that manner of death has no place in criminal trials.

Manner of death determinations are made on every death certificate signed by medical examiners and/or coroners. With varying levels of certainty and subjectivity, manner of death determinations provide important public health statistical data and insight.[1] Manner of death is often utilized for other purposes, including opinion evidence in criminal trials. As medical doctors, forensic pathologists often rely on non-medical information, including scene details, the decedent's background information, the law enforcement investigation, and witness statements to supplement their autopsy findings when making a manner of death determination.[2] This information can have varying levels of reliability, having not been subject to evidentiary challenges or cross examination when received at the time of death certification. However, when a prosecution heads to a jury trial, these manner conclusions are commonly elicited as opinion evidence and proffered to the factfinder, crossing numerous important evidentiary and Constitutional lines.[3]

The legal field has struggled with this perennial problem (with varied results across the country) for many years. However, few, if any, jurisdictions have considered the voices of forensic pathologists when considering this question. This presentation will propose the amplification of these voices telling us manner of death is not intended, and potentially not fit, for consideration as evidence in a criminal trial.[4] This presentation will also examine how and why the courts have ignored these critical voices and demonstrate that it's time we started listening. This presentation will posit that many players within the forensic and legal community must play a role in preventing manner of death determinations from being improperly submitted to the factfinder in criminal trials. To continue to admit this testimony constitutes a threat to the notion of "Justice for All."

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Manner of Death; Medical Examiner; Admissibility

G35   Misleading Sexual Assault Nurse Examiner (SANE) Testimony in Child Sexual Abuse Cases

Nancy R. Downing*, Texas A&M School of Nursing, College Station, TX

Learning Overview: After attending this presentation, attendees will have heard examples from actual court testimony by SANEs in child sexual abuse cases to highlight problematic testimony that could mislead jurors about the significance of anogenital findings. Recommendations from a Texas Forensic Science Commission report to address issues surrounding forensic nurse practice and testimony will be discussed.

Impact Statement: This presentation will impact the forensic science community by informing atttendees that sexual assault and child sexual abuse have the highest rates of exonerations related to false or misleading forensic evidence. A better understanding of issues that may contribute to misleading SANE testimony regarding child sexual abuse may mitigate the risk of wrongful convictions.

Sexual assault and child sexual abuse are the two crimes with the highest rates of exonerations related to false or misleading forensic evidence.[1] SANEs are forensic nurses with specialized education in providing care for patients after sexual assault. A small body of evidence suggests exams performed by SANEs are associated with higher and more successful investigation and prosecution and higher-quality evidence collection.[2,3] SANEs have become the standard of care, and prosecutors prefer SANEs over physicians for evidence collection, documentation, and testimony.[4,5] Nevertheless, poor training, inexperience, lack of peer or expert review of exam documentation, and/or poor trial preparation can contribute to misleading testimony by SANEs.

The risk of misleading testimony may be more likely in child sexual assault cases in which consensus guidelines regarding interpretation of anogenital findings have been continuously evolving.[6] Specifically, identification and interpretation of anogenital injuries in non-acute pediatric sexual abuse and testimony about the significance of these findings have been problematic, with several high-profile exonerations illustrating the critical importance of this issue.

Mock jurors found SANEs more credible than non-SANE nurses when providing testimony in child sexual abuse cases.[7] SANE testimony increased jurors' empathy toward and credibility of victims due to SANEs' positive statements about victims.[7] Studies such as this indicate the power of SANE testimony to influence outcomes in cases of child sexual abuse. There are national guidelines for pediatric SANE education and practice and pediatric SANE certification; there are no enforceable standards regarding accountability for following guidelines, and certification is not required to perform medical forensic examinations.[8-10]

This presentation will utilize examples from actual court testimony by SANEs in child sexual abuse cases to highlight problematic testimony that could mislead jurors about the significance of anogenital findings. Recommendations from a Texas Forensic Science Commission report to address issues surrounding forensic nurse practice and testimony will be discussed. Recommendations have implications for attorneys as they review sexual assault medical forensic examination documentation and question SANEs as witnesses in child sexual abuse cases. Recommendations related to anogenital injury terminology, case review of exam documentation, documentation accuracy, SANE training, data-driven injury interpretation, and human factors' considerations will be discussed.

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Sexual Crimes; Forensic Nursing; Justice

G36 **Modeling Influences on the Understanding of Digital and Multimedia Evidence (DME) by Attorneys and Digital Forensic Examiners (DFE) Within the United States Criminal Justice System**

Joseph Levi White*, United States Army Criminal Investigation Laboratory, Forest Park, GA

Learning Overview: After attending this presentation, attendees will better recognize potential influences on the understanding of DME by attorneys and DFEs, including motivation, experience, generational influences, communication, and education/training.

Impact Statement: This presentation will impact the forensic science community by providing a framework for better understanding how attorneys and DFEs approach and understand DME. The presented data may be used to identify areas for potential increased efficiency/effectiveness in DME-involved cases within the United States criminal justice system.

This presentation is based on the published dissertation research of the author/presenter.[1] As a DFE with over 15 years of experience, the presenter has worked with attorneys on multiple cases involving Digital and Multimedia Evidence (DME). From these DFE/attorney interactions, it is believed that there is a difference between how attorneys and DFEs approach and understand DME. This research had a goal of determining potential themes that may influence the understanding of DME by attorneys and DFEs within the United States Criminal Justice System. To reach this goal, both qualitative (interviews/potential theme identification) and quantitative (questionnaires/statistical analysis) methods were utilized.

Qualitative semi-structured interviews of experienced criminal attorneys and DFEs were conducted to gather information regarding potential influences on their understanding of DME. The interview results were transcribed, and the data coded to allow for qualitative analysis. Five themes were identified from this data and are thought to play a potential role in understanding of DME by attorneys and DFEs: motivation for involvement in the criminal justice system (passion for the job, desire to work in law enforcement, monetary gain, sense of ethical obligation, and seeking justice); experience (and knowledge); generational influences (age and the CSI Effect); communication within defined roles; and education/training. A questionnaire was developed utilizing these five themes as a guide that was then distributed to attorneys and DFEs across the United States.

Low response rates to the questionnaire were observed and will be discussed. Statistical analyses were conducted on the available survey results in relation to the five previously identified themes. Attorneys and examiners agreed on many facets of each theme. The most influential motivational factor for seeking a career as an attorney or DFE is a passion for the field. One of the most influential components to understanding DME was determined to be experience. Detractors to understanding DME included increasing age and the CSI Effect. A frequency increase in communication levels between attorneys and DFEs has the potential to affect DME understanding and case efficiency. Attorneys have higher educational levels much greater than even experienced DFEs; however, technical DME training levels (which have a greater influence on overall DME understanding) are much greater for DFEs. Attorneys tend to use online research as a primary learning method, while DFEs rely primarily on technical training. Each of the identified themes shows promise for describing influences on the understanding of DME by attorneys and DFEs within the United States criminal justice system and will be further discussed within the presentation.

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Attorneys; Examiner; Digital Evidence

G37 DNA Mixture Interpretation: A History of Field and NIST Foundation Study Findings

John M. Butler*, National Institute of Standards and Technology, Gaithersburg, MD

Learning Overview: After attending this presentation, attendees will have gained an understanding of key scientific principles and foundations for DNA mixture interpretation through examining a brief history of the field and its foundational literature and information.

Impact Statement: This presentation will impact the forensic science community by advancing knowledge of DNA mixture interpretation approaches, describe issues seen in the field, and consider factors in moving forward.

Forensic DNA analysis has undergone numerous changes in the past four decades since DNA methods were first applied to criminal investigations.[1] Today, DNA testing operates in an evolving environment with an increasingly complex set of technologies, DNA tests, and statistical approaches. Often important changes and advances have been made across the community because of experiences in high-profile court cases or awareness of issues raised through participation in interlaboratory studies or collaborative exercises. An examination of the history of DNA mixture interpretation will highlight and explore several of these cases and studies along with the development of guidance documents. In a 2019 article, forensic DNA pioneer Peter Gill noted: "Interpretation of evidence continues to be the most difficult challenge that faces scientists, lawyers, and judges."[2]

A scientific foundation review, which was originally termed a "technical merit evaluation" by the National Commission on Forensic Science in 2016 [3], is a study that seeks to identify the scientific foundations that support and underpin forensic methods and practices.[3,4] Since 2018, the National Institute of Standards and Technology (NIST) has been Congressionally funded to conduct these evaluations and has recently completed reports in digital investigation techniques and bitemark analysis.[5,6] These foundation studies document and evaluate empirical evidence for the reliability of forensic methods, explore the capabilities and limitations of the method, and identify knowledge gaps and areas for future research.[7]

A draft report on the scientific foundations of DNA mixture interpretation was released for public comment in 2021 and received extensive feedback that assisted in producing a final report. This report identifies core principles, documents publicly accessible information, provides important key takeaways, and describes future efforts needed.[8] Based on these principles, a probabilistic genotyping framework offers the best available tool for DNA mixture interpretation, but its fitness for purpose in any specific casework application must be supported by validation data from known samples that are similar in complexity to those seen in casework. To assess the degree of reliability of a component or a system for any forensic method or practice, detailed empirical data are needed, such as data from validation studies, interlaboratory studies, and/or proficiency tests. When considering reliability of the entire DNA mixture interpretation process (including judgments from the analyst prior to using any software), it is important to understand and consider the assumptions around DNA transfer, persistence, prevalence, and recovery.

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DNA; DNA Mixture; Scientific Foundation

G38   Dnamix 2021: Inter-Laboratory Variation in the Interpretation of DNA Mixtures

R. Austin Hicklin*, Noblis, Reston, VA; Nicole M. Richetelli, Noblis, Reston, VA; Jonathan Davoren*, Bode Technology, Lorton, VA; Robert Bever, Bode Technology, Lorton, VA

Learning Overview: This presentation will provide detailed results from a large-scale study assessing variation among laboratories in their interpretations and statistical analyses of DNA mixtures. After attending this presentation, attendees will learn about the accuracy and inter-laboratory variability of interpretations, comparisons, and statistical analyses conducted by different laboratories on DNA mixtures.

Impact Statement: This presentation will impact the forensic science community by communicating information from a study assessing the accuracy and inter-laboratory variation in interpretations, comparisons, and statistical analysis of DNA mixtures. These results may be used by laboratory managers, standards organizations, and the legal community to assist in decision making, improving procedures and training, and highlighting areas for potential standardization.

The purpose of the presentation is to discuss the results of the Inter-laboratory Variation in Interpretation of DNA Mixtures Study (hereafter referred to as "DNAmix 2021"). DNAmix 2021 was a rigorous, large-scale study conducted to evaluate the extent of consistency and variation among forensic laboratories in interpretations and statistical analyses of DNA mixtures and to assess the effects of various potential sources of variability. The presentation will report results from DNAmix 2021 regarding laboratory policies and procedures relevant to DNA mixture interpretation, as well as the accuracy and variability of assessments of suitability, assessments of Number of Contributors (NoC), and categorical conclusions/statistical analyses.

Analyses of suitability and NoC are based on 2,272 responses by 134 participants from 67 forensic laboratories on 29 DNA mixtures. Analyses of categorical conclusions and statistical analyses are based on 765 responses by 106 participants from 52 labs, on eight distinct DNA mixtures, each of which was provided to participants either as a contributor packet (in which the Person Of Interest (POI) was in the mixture), or as a non-contributor packet (in which the POI was NOT in the mixture). All reference and mixture profiles were provided to participants as electropherograms. Mixtures were created under controlled conditions to allow for ground truth attribution and were designed and created to be broadly representative of the range of attributes encountered in casework. All DNA profiles used in this study (mixtures and references) were from real people; no profiles were simulated or contrived. DNAmix 2021 was funded by the National Institute of Justice (NIJ) (Grant 2020-R2-CX- 0049) and conducted by Noblis and Bode Technologies. DNAmix 2021 has resulted in two publications to date, with an additional manuscript in preparation at the time of abstract submission.[1,]

Note: A subset of these results was presented at AAFS 2023; this presents additional results and analyses, at a much greater level of detail.

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DNA; Mixture Analysis; Reliability

G39 **An Overview of the NIST/NIJ Expert Working Group on Human Factors in Forensic DNA Interpretation Report**

Melissa Taylor*, National Institute of Standards and Technology, Gaithersburg, MD; Nikola Osborne*, National Institute of Standards and Technology, Auckland, New Zealand

Learning Overview: After attending this presentation, attendees will have learned about the National Institute of Standards and Technology (NIST) and the National Institute of Justice (NIJ) Expert Working Group Series on Human Factors in Forensic Sciences, with a particular focus on the most recent efforts in Forensic DNA Interpretation. These research efforts will be placed in context with other recent efforts to understand and improve DNA Interpretation.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the study of human factors in forensic science is an essential element to inform our understanding of the interaction between humans and the systems they use. In understanding human factor issues, we can identify and address the potential for error and bias and improve the reliability of forensic science.

Forensic DNA interpretation is a complex and challenging task that can be prone to bias and error. In February 2020, the NIST/ NIJ Expert Working Group (EWG) on Human Factors in Forensic DNA Interpretation was formed to conduct a scientific assessment of the effects of human factors on forensic DNA analysis and interpretation.

Understanding human factors requires understanding a system. A system is any set of components that work together to produce an outcome. A systems approach examines the relationships between different elements of a system and how they influence the entire system. The study of human factors examines interactions between individuals and all other elements of a system—technology, training, products, procedures, workspaces, the overall environment, resources, institutional culture, and other internal and external factors.

Combing a human factors and systems approach, the EWG reviewed the totality of the DNA analysis and interpretation process, the institutional and organizational structures in which DNA analysis is performed, the wider criminal justice system, and the individuals and groups who produce and use DNA evidence. In doing so, they developed strategies to minimize the possibility of errors and their impact when they do occur.

This EWG comprises forensic scientists, legal practitioners, scholars, statisticians, cognitive scientists, and Forensic Science Service Provider managers. These varied perspectives were critical in applying a systems approach. This presentation will provide an overview of this EWG's efforts and recommendations and place them in context with other recent efforts to understand and improve DNA interpretation—the NOBLIS DNA Mix21 study, and the NIST Scientific Foundation of DNA Review.

This presentation is aimed at members of forensic DNA laboratories and criminal justice system partners, such as attorneys, investigators, and parent organization leadership. There will be an opportunity to ask the presenters questions and provide feedback on the EWG's efforts.

DNA; Human Factors; Reliability

G40 **Improving Forensic DNA Interpretation Protocols and Reports Through the Implementation of Human Factors Recommendations**

Britton Morin*, Union County Prosecutor's Office Forensic Laboratory, Westfield, NJ; Nikola Osborne, National Institute of Standards and Technology, Auckland, New Zealand

Learning Overview: After attending this presentation, attendees will understand how the Union County Prosecutor's Office Forensic Laboratory has implemented some of recommendations from the National Institute of Standards and Technology (NIST)/National Institute of Justice (NIJ) Expert Working Group (EWG) on Human Factors in Forensic DNA Interpretation. This presentation will include the rationale behind the changes, the steps required to implement them, and feedback from DNA analysts and laboratory managers involved in the changes.

Impact Statement: This presentation will impact the forensic science community by discussing the importance of understanding human factors to improve reliability and reduce the likelihood of error and bias in forensic DNA interpretation. It will demonstrate how to implement several of the recommendations presented in the EWG's report and provide a blueprint for how other laboratories can do the same.

The Union County Prosecutor's Office Forensic Laboratory in New Jersey analyzes physical, narcotics, and DNA evidence for criminal investigations in Union County. Its Laboratory Director, Britton Morin, is a member of the NIST/NIJ EWG on Human Factors in Forensic DNA Interpretation.

The EWG was charged with conducting a scientific assessment of the effects of human factors on forensic DNA analysis and interpretation. From this assessment, the EWG developed over 45 recommendations to minimize the possibility of errors and their impact when they do occur.

The Union County Prosecutor's Office Forensic Laboratory has already started to implement several of these recommendations. This presentation will provide an overview of the rationale for implementation, the implementation process, and the feedback from DNA analysts and laboratory managers involved in the changes, for the following recommendations.

Draft Recommendation 3.1: DNA analysts should maintain a detailed and transparent record of the reasoning, justification, and sequence of decisions not dictated by the forensic science service provider's protocols (i.e., discretionary decisions). This includes decisions regarding sampling strategy, serological testing, artifact analysis, suitability determinations, number of contributor assignments, and locus exclusions.

Draft Recommendation 5.3: DNA analysts should provide the end-user with caveats and limitations related to DNA results and comparisons. The primary caveats and limitations should make clear to end-users that:

- If any information changes, a new evaluation will be needed.

- DNA comparison results alone cannot be used to identify an individual as the source of the DNA.

- DNA comparison results alone cannot be used to determine how or when DNA was deposited.

Draft Recommendation 5.4: Forensic science service providers should provide training to the end-users of reports to help them understand the caveats and limitations of the testing such that the DNA results are properly incorporated along with the other information in the case.

The information contained in this presentation can be used as blueprint for other forensic science service providers wanting to implement recommendations from the NIST/NIJ EWG on Human Factors in Forensic DNA Interpretation Report.

Forensic DNA; Human Factors; Quality Management System

H1   A Pilot Study on Postmortem Determination of Drug Abuse on Dental Tissues

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Learning Overview: After attending this presentation, attendees will have a better understanding of teeth as an alternative matrix for forensic postmortem toxicology and of the incorporation mechanism of substances in different dental tissues.

Impact Statement: This presentation will impact the forensic science community by providing an innovative method for studying different dental tissues as possible matrices for postmortem toxicology in drug-related deaths and promising results to develop the forensic odontology application in the field of postmortem toxicology.

Postmortem toxicology constantly deals with the research of reliable alternative matrices useful for pathologists in cases in which the classic matrices for forensic toxicology are not available (e.g., carbonized or skeletonized corpses, human remains, exhumed bodies, etc.). Teeth can be applied as an alternative matrix since they are highly resistant, available, and stable after death. Dental enamel, dentine, or pulp could disclose acute or chronic drugs consumption, given the different tissues' constitutions.[1]

The previous literature does not provide information on the pharmacokinetics of substances and on the mechanism and site of incorporation of xenobiotics into dental tissues.[2,3]

This pilot research aims to investigate whether in the pulp can be detected the same substances found in the blood in drug-related death cases. Second, the study aims to disclose possible deposits of drugs in the hard tissues of the tooth (dentine and/or enamel), and the related degrees of accumulation in different dental tissue, thus contributing to reconstructing the drug abuse history (e.g., timing).

The study experimented with a novel method to separately analyze enamel, dentin, and pulp tissues, which was applied to ten teeth collected during autopsies of drug-related deaths along with blood and hair samples for classic toxicological analyses. Each tooth was prepared by the same forensic odontologist using diamond and multi-blade burs on a turbine above an amalgam aspirator coated with filter paper. Teeth were dissected into two longitudinal halves, and the pulp was extracted from the chamber and roots. The enamel was separated from the coronal dentin by pulverization. The coronal secondary dentin was separated from the primary dentin pulverizing an inner layer of approximately 1.5mm coronal and 1mm cervical thickness. The residual outer coronal dentin was separated from the roots by pulverization, then the roots were pulverized with a hammer. Each dental tissue was extracted with acid for cocaine, opiates, and metabolites and analyzed by Gas Chromatography (GC) and liquid chromatography with High-Resolution Mass Spectrometry (HRMS). The results were then compared with those obtained from blood and hair samples.

Preliminary results demonstrated that teeth are different from any other classic matrix (blood and hair), and the qualitative correlation in detecting substances between pulp/blood and dental hard tissues/hair suggests that they can be useful in postmortem evaluation for both acute and chronic consumption of drugs. The most significant result is that the mechanism of accumulation of substances in mineralized tissues seems to be influenced by the type of molecule and the method of consumption. The innovative method for dental tissues separation is absolutely promising to study the different accumulation of substances in teeth, possibly disclosing both acute and chronic consumptions in forensic cases of drugs-related cases.

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Forensic Odontology; GC/MS; UHPLC-HRMS

H2 An Overview and Evaluation of Forensic Dentistry Cases With Trauma Damage and Bone Fractures

Huseyin Afsin, Istanbul University, Istanbul, Turkey; Gulnaz T. Javan*, Alabama State University, Montgomery, AL

Learning Overview: After attending this presentation, attendees will understand the legal consequences of damage to one's teeth in Turkey.

Impact Statement: This presentation will impact the forensic community by providing information on legal approaches to forensic cases arising from trauma to teeth as a result of sports accidents, car accidents, altercations, and falls.

Evaluation of trauma damage is an integral part of forensic dentistry. Dental trauma refers to injuries to the teeth and usually occurs because of a traumatic event. These events include sports accidents, car accidents, falls, or a traumatic blow. Forensic dentists perform a thorough examination to assess the type and severity of the traumatic injury. This examination usually includes examining the physical condition of the teeth, evaluating X-rays, and observing other tissues affected by trauma (jawbone, soft tissues, etc.). Treatment of dental trauma varies depending on the type and severity of the trauma and the tissues it affects. Treatment options may include replacing the tooth, fixing the tooth, restoring a cracked or broken tooth, or replacing the missing tooth with a denture or implant. Regular follow-up and control are important to maintain the tooth's long-term health after trauma.

Legal Consequences of Trauma Cases Resulting in Tooth loss: Evaluation of each case depends on how the trauma occurred, the causes of the loss, and the extent of the damages. Legal consequences often relate to compensation for one's grievances, reimbursement of medical expenses, and compensation for loss.

Here are some of the possible legal consequences of tooth loss:

Compensation Lawsuits: If the tooth loss has occurred because of someone else's negligence, fault, or illegal act, the victim can claim compensation. The amount of compensation is determined by factors such as the causes of the loss, the severity of the damage, medical costs, and functions lost.

Health Insurance and Medical Expenses: Tooth loss can be covered by one's health insurance. Depending on the insurance policy, some or all of the medical costs for tooth loss may be covered by the insurance company.

Job Loss and Compensation: Tooth loss can affect the ability to work in some occupations. If speech or chewing functions are affected, a person's job performance and employment status may be adversely affected. In this case, job loss and income loss caused by tooth loss can be legally compensated.

Aesthetic and Psychological Damages: Tooth loss can affect the appearance of the person and cause aesthetic concerns. Aesthetic or psychological harm may also be part of a health claim. In particular, the impact of tooth loss on self-esteem and psychological stress can be legally considered. The legal consequences of tooth loss can also vary depending on local laws, insurance company policies, and the nature of the event. Your dentist will provide you with an appropriate treatment plan to monitor post-traumatic recovery and prevent potential complications.

In conclusion, the evaluation of traumatic injuries in dentistry is critical for accurate diagnosis and determination of treatment. Therefore, it is important to consult a dentist in case of any dental trauma.

Dental Trauma; Teeth; Legal Approach

H3 Justice for the Vanished but Not Missing

Elizabeth Ruth Smith*, Office of the Chief Medical Examiner, Commonwealth of Virginia, Virginia Beach, VA

Learning Overview: After attending this presentation, attendees will understand that the investigation of a death begins with a history of the involved circumstances. If a crime scene is properly investigated with systematic methodology, then it will provide the means for completing criminal investigations and resolving the case.

Impact Statement: This presentation will impact the forensic science community by exploring a perplexing category of missing person cases: those in which someone's absence is never reported.

The investigation of a death begins with a history of the involved circumstances. If a crime scene is properly investigated with systematic methodology, then it will provide the means for completing criminal investigations and resolving the case.

This is a 2015 case study of human remains of an unknown subadult of African ancestry (Black) found in the trunk of a car driven by a female pulled over by a Virginia State trooper for having expired tags. Subsequent inquiry revealed the car was not registered in Virginia, and the license plates on the vehicle were not filed with the Department of Motor Vehicles. The state trooper began impounding the vehicle and, while taking inventory, discovered human remains in a plastic double bag bound with duct tape. The female driver of the vehicle denied knowledge of the trunk findings.

The human remains were brought to the Office of the Chief Medical Examiner for dental identification. At that time, there were no known antemortem dental records. A postmortem examination was performed that included digital photographs, adult digital Full Mouth X-ray (FMX) radiographics, and a clinical examination. To narrow the search for possibilities, the University of Texas (UT) -Dental Age estimation was used to simplify and expedite the process of performing third molar age estimation. The results for a Female were 15.25 years of age with the range of +/- 2.88 years at 95% confidence and for a Male were 14.85 years of age with a range of +/- 1.86 years at 95% confidence.

That evening, antemortem dental records were located by special agents. A comparison of antemortem dental records to postmortem dental records was performed and a Positive identification was made of those unknown remains to be Quincy Jamar Davis, age 14.5 years old. Tonya Martin, his mother, had moved to Hampton Roads with her oldest son, Quincy, from Pinehurst, NC, in 1993. Two years later, she married Ronald Slaton, who was stationed in Virginia Beach with the Navy in 1996. That same school year, Quincy's mother was charged with assault and battery for injuring him. The 6-year-old's chest and back had cuts and bruises. Tonya Slaton was convicted of the misdemeanor, and a judge sentenced her to no jail time.

By seventh grade, Quincy was taking several advanced classes. Slaton and her husband separated in August 2003. Three weeks later, on September 3, Quincy was withdrawn from school, never to be seen again. In 2007, Slaton was arrested and sentenced to four years in prison on felony convictions of attempted maiming and shooting at an occupied dwelling where her boyfriend lived. It is unknown if the commonwealth's attorney knew she had children when she went to prison in 2008.

In January 2018, Tonya Slaton pleaded guilty to voluntary manslaughter in the death of her teenage son, Quincy Davis, and is receiving 8 to10 years of jail time. Quincy had not been noticed missing for 11 years. Quincy fits into a perplexing category of missing person cases: those in which someone's absence is never reported.

Dental Identification; Child Abuse; Age Estimation

H4   A Picture Reveals the Truth!

Megha Shah*, Alta Loma, CA

Learning Overview: The goal of this presentation is to help us learn that with the advanced use of Photoshop®, even when the Antemortem (AM) radiographs are missing, we can obtain a positive dental identification.

Impact Statement: This presentation will impact the forensic science community by introducing and encouraging attendees to use different tools of Photoshop® for identification.

In a homicide case in Southern California, a deceased male was found alone in a residence. He was noted to be missing for 2–3 weeks. The remains had undergone severe decomposition, and visual identification was no longer possible. As the individual lived alone, it was difficult to collect detailed information. DNA collection was also not possible due to a lack of immediate family members. Friends were not aware of his dental care, and thus dental records or dental data collection could not be completed. Eventually, photographs were located. Among them, only one photograph showed the upper anterior upper teeth from canine #6 to #11 and was used to establish an antemortem image. Postmortem radiographs of upper anterior teeth were taken and used for comparison. The deceased individual was missing an upper incisor, which made the case a little challenging. Photoshop® was used on all postmortem images and the one antemortem photograph. Different photo enhancement techniques were applied to facilitate comparison in order to attempt identification. The antemortem photograph was set as the first layer in Photoshop®. Overlapping comparison was made using imaging at different opacities (50%,75%, 90%, and 100%). Angulations were varied to determine the accuracy of comparison results. A positive identification was able to be established.

With one photograph, limited AM data, a positive ID was established. Using different moderate to advanced techniques in image modification software, we were able to conclude the case.

Software; Accuracy; Positive Identification

H5   Postmortem Histological Changes of Dental Pulp for Postmortem Interval Estimation: A Pilot Study

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Learning Overview: After attending this presentation, attendees will have a better understanding of Postmortem (PM) histological modifications of the dental pulp and their potential application to study the Postmortem Interval (PMI).

Impact Statement: This presentation will impact the forensic science community by providing an innovative method for PMI estimation based on qualitative and quantitative dental parameters available up to two weeks after death.

Teeth have proven to be one of the most stable and promising matrices for estimating the time elapsed since death from early to late PMIs.[1] Some previous studies on human teeth highlighted the occurrence of some specific histological changes in PM dental pulps due to the absence of blood circulation, prolonged ischemia, and the putrefaction process, which could be correlated with the time elapsed since death but with uneven and sometimes contradictory results.[2-4]

The study aims to verify which qualitative and quantitative PM dental pulp changes are significant for the correlation with PM interval and whether the pulp degradation could be an available medicolegal tool beyond the first week after death.

A total of 27 vital and sound teeth were extracted from healthy patients for clinical reasons (orthodontics or impacted molars). We considered the PMI as the time elapsed from the tooth avulsion, which caused the interruption of the blood supply comparable to the death of the subject. The sample size was calculated considering three tooth samples for each PMI set (group), for a total of nine groups from T0 (baseline) up to 2 weeks (T0/ 7, 12, 24, 36, 48, and 72 hours/1 and 2 weeks). All the dental samples were stored at room temperature up to the time of pulp extraction. Only the coronal pulp was removed, and it was obtained by dissecting the crown at the cementoenamel junction. All the pulp samples were fixed in 10% neutral buffered formalin, then histological preparation was completed with hematoxylin and eosin staining.

Microscopy analysis was performed to: (1) detect the cellular redistribution within the pulp tissue, (2) detect the homogenization of the collagen fibers present in the dental pulp, (3) identify the degeneration of the Extracellular Matrix (ECM) via qualitative analysis, and (4) discern the variation in cells/nuclei density of the odontoblasts, fibroblasts, immune, and endothelial cells via quantitative analysis. Two blind operators will evaluate and measure all the histological sections to verify the reliability and feasibility of the technique and the selected targets for medicolegal estimation of the PMI.

This pilot study will allow significant conclusions about the applicability of dental pulp in early and intermediate PMI evaluation (beyond the first week after death).

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Postmortem Interval; Morphological Histological Changes; Dental Pulp

H6   The Forensic Anthropological Significance of Dental Calculus Deposits as Proxy for Identity of the Host and the Oral Microbiota: A Systematic Review

Jagmahender Singh Sehrawat*, Panjab University, Dept. of Anthropology, Chandigarh, India; Niraj Rai, Birbal Sahni Institute of Palaeosciences, Lucknow, Uttar Pradesh, India

Learning Overview: After attending this presentation, attendees will be sensitized about the forensic significance of dental calculus deposits for identification of the host and the oral microbiome.

Impact Statement: This presentation will impact the forensic community by informing attendees that dental calculus is the recent hotspot in forensic odontological research work, not only for establishing the identity, but also for estimating the disease and health conditions, dietary status, and geolocation of the unidentified human remains recovered from forensic scenarios.

Dental calculus is a recent hotspot of research in different scientific disciplines, particularly in forensic anthropological investigations of unidentified skeletal remains, as it serves as a stable repository of vast bio-information about the host and the oral microbiota. It provides a safe, hospitable, and durable environment for the microbial communities and food and non-food particles as well as the host and microbial DNA. The oral microbiota acts as microbial fingerprint of the health and disease conditions of past individuals and cultures. Minute quantities of dental calculus can provide a broader antemortem profile of unknown individuals, including information on the decedent's identity, lifestyle, living conditions, dietary status, health and disease status, and possible geo-affinity.[1-5] It serves as a promising biomaterial for the identification strategies of unknown human remains recovered in forensic anthropological or bio-archaeological contexts.[6] The application of advanced genomics, proteomics, and metabolomics techniques to examine the material, cellular, and molecular inclusions of dental calculus has immensely helped in decoding the hidden bio-information contained within it for forensic purposes. Though dental calculus has been, archeologically and forensically, overlooked by the forensic community, it has recently emerged as vital evidence for forensic anthropological and osteo-archeological investigations.[6]

In this systematic review, the current status of dental calculus research for forensic anthropological purposes has been scrutinized. The scientific databases using search engines like PubMed®, ScienceDirect®, SAGE®, Springerlink®, Clinical Key®, WoS®, and Google® Scholar were searched using the terms such as "Dental calculus, Forensic identification," "Microbial forensics and oral microbiome," "Ancient DNA," "stable isotope analysis," "Occupation and dietary status from calculus," "Oral microbiota and forensic odontology," etc. A snowball technique of cross-referencing was used to identify more suitable articles, and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed to include the eligible research articles. Only full-text publications having information relevant to the scope of the present systematic review were scrutinized in depth. A total of 89 research articles were identified, out of which only 31 studies were found within the ambit of the aim and objectives of present study.

The inclusion criteria were to consider the studies related to forensic identifications based on dental calculus and those published to date. The studies not satisfying the inclusion criterion were ignored for further analyses. Systematic review revealed that dental calculus serves as a rich source of host-associated biomolecules, ingested or inhaled during routine daily life of an individual. It is a promising biological material for establishing identity, assessing health and dietary status, estimating geo-affinity, exposure to heavy metals or drugs of abuse, and other identity credentials of an unknown person.

Almost all populations have dental calculus, though its prevalence varies with the age, sex, population-affinity, systemic health, and the dietary and dental hygiene status of an individual.[7,8] Until very recently, the scientific importance of dental calculus for forensic anthropological casework was not recognized as it could not attract the desired attention of the scientific community. However, it is now being appreciated as a significant population and individualistic marker of identity due to the abundance of host-associated routinely preserved micro-particles and biomolecules embedded.[1] It is our belief that forensic anthropologists and geneticists should explore collaborative research in the field to explore the fullest potential of dental calculus as an adjuvant and non-invasive evidence for forensic human identifications. The detailed results will be presented in the textual, graphical, tabular, and diagrammatic forms in this presentation.

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Forensic Odontology; Dental Calculus Deposits; Systemic Review

H7   The Use of the Disaster Victim Identification (DVI) Method for a Mass Casualty Bus Crash in Manitoba, Canada

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Learning Overview: After attending this presentation, attendees will understand how: (1) the team from the medical examiner's office approached a mass casualty disaster in terms of victim identification; (2) the forensic odontology team collected postmortem records; and (3) the forensic odontology team utilized the DVI method for odontological reconciliation with the antemortem records.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the DVI method was important in allowing the forensic odontology team to review antemortem and postmortem records and complete reconciliation of records and identification of victims in a mass casualty incident.

On June 15, 2023, 25 senior citizens were travelling on a minibus to a nearby casino in southwestern Manitoba. When crossing a highway intersection on the Trans-Canada Highway, the minibus was struck by a semi-trailer truck. The force of the crash caused the minibus to become engulfed in flames. The Royal Canadian Mountie Police (RCMP), paramedic, and fire departments all attended the scene, with survivors being medically airlifted to urban trauma centers. A trauma unit was flown to the scene to attend surviving victims and to support medical care. In the end, there were 15 deceased victims at the scene and, subsequently, an additional individual died two weeks later due to their injuries.

Forensic odontology was required for identification due to the impact of the crash and subsequent fire of the minibus and its individuals. The Medical Examiners Office of Manitoba received 15 decedents two days after the accident. The range of age of the decedents was 58–86 years of age. The team included the RCMP officers, forensic pathologists, medical autopsy technicians, medical investigators, and forensic odontologists. The forensic pathologists in Winnipeg were able to confirm the identity of seven decedents based on surgical hardware and implants with serial numbers, radiographic, and past surgical records comparisons. The remaining eight decedents were examined by the forensic odotology team using the DVI method.

The forensic odontology team included four individuals, three dentists who had previously supported the Medical Examiners Office of Manitoba, and a fourth individual from the Canadian Forces military. In the end, seven out of eight decedents were positively identified using the DVI method and one decedent was categorized as a possible identification. The DVI method was a sound and scientific method to allow identification of the decedents and provide closure to the families. Final identification of decedents was publicly announced by the RCMP one week after the accident. The forensic odontology team played a significant role in identifying the victims in this mass casualty disaster.

Victim Identification; Mass Disaster; Dental Identification

H8   Color Change in Deciduous and Permanent Enamel and Dentin Due to Burning

Michal Peer*, National Center of Forensic Medicine; Tel Aviv University, Aurora, CO; Rachel Sarig, Tel Aviv University, Shmunis Family Anthropology Institute, Tel Aviv, HaMerkaz, Israel

Learning Overview: This presentation will discuss the impact of burning on deciduous teeth, focusing on color and enamel fragmentation, and how these changes compare to permanent teeth. Attendees will gain insights into quantitative color measurement and the influence of tooth morphology and anatomy on the observed changes.

Impact Statement: This presentation will make a significant impact on the forensic community by shedding light on the effects of burning on deciduous and permanent teeth, an area that has been severely understudied. It stands out as one of the few studies that explored burnt deciduous teeth. Furthermore, this research has the potential to inspire future investigation, improving the analysis of burnt teeth in forensic casework.

The distinct anatomical differences between deciduous and permanent teeth make understanding the effects of burning crucial for forensic investigations. However, the existing studies in this area have been extremely limited in number as well as in scope and subjective in their assessment.[1,2]

The primary objective of this research is to explore the color changes in deciduous teeth resulting from burning using a non-visual quantitative method. It also aims to compare the impacts of different burn temperatures and durations on deciduous teeth, drawing comparisons with already-observed changes in permanent teeth at equivalent temperatures. This study included 60 deciduous teeth, divided evenly into two groups (400°C and 700°C) and further subdivided into three subgroups per temperature (30 minutes, 60 minutes, 90 minutes). Additionally, data from a previously published study on permanent maxillary molars burned at 400°C or 700°C for 60 minutes was included for comparative analysis.[3]

Quantitative color analysis was performed using the SpectroShade® Micro II Dental Color Complete Tooth Analysis System spectrophotometer, assessing lightness (L*), green-red color scale (a*), and blue-yellow color scale (b*) before and after burning. The heating process was conducted in a Cole-Parmer StableTemp Box Furnace.

The results demonstrated significant differences in color measurements between unburned and burned deciduous teeth, regardless of burn time, in both temperatures. However, there were no significant differences observed between the different burn times. Significant differences were observed in enamel a* and b* colors as well as all three dentin color measurements between the two temperatures for all burn times. Comparing deciduous and permanent teeth burned at 400°C for 60 minutes, the only significant difference was found in enamel a* values. Similarly, for teeth burned at 700°C for 60 minutes, the only significant difference was in dentin b* values. Notably, 100% of permanent teeth burned at 700°C displayed severe enamel fragmentation and material loss, while 50% of deciduous teeth still exhibited that enamel was complete and partially or fully attached to the coronal dentin. These findings suggest that the unique micromorphology of deciduous teeth, such as the enamel rod diameter and density or size of interprismatic region as compared to permanent teeth, may contribute to the differences observed.[4]

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Teeth; Enamel; Anthropology

H9   **Evaluating the Relationship Between Various Oral Pathological Conditions and the Persistence of Lip Prints**

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Learning Overview: After attending this presentation, attendees will benefit by understanding the chronic changes in lip prints brought about by various pathological conditions of the oral orifice and the ability of lip wrinkles to regain their form even after severe manifestations.

Impact Statement: Various workers in the field of lip evidence analysis have claimed lip print patterns to be stable and unchangeable throughout a person's life.[1] However, barely minimal studies have been conducted to monitor the effect of various pathological conditions on the permanency of lip prints. This presentation will impact the forensic science community by addressing the general enigma related to lip prints' stability in dermatological and drug/medicine-induced changes. The findings are expected to highlight the importance of lip prints as a crucial piece of corroborative evidence.

Cheiloscopy, (human identification based on lip traces) is one of the oldest techniques used by forensic investigators around the globe since 1902 for personal identification because of its uniqueness. The skin overlays of the oral orifice are covered by a mucosal layer that bears specific elevations and depressions, forming a unique pattern for every individual. As mentioned earlier, those highly unique skin folds constitute lip prints. Authors have claimed that lip print patterns are remarkably stable and do not change during a person's life.[1] It has also been verified that lip print patterns recover even after undergoing severe alterations such as trauma, inflammation, and diseases such as herpes.[2] The disposition and the form of furrows do not vary with changing environmental factors.[1,3] However, there are specific allergies or pathological conditions of lips/mouth (like *Actinic cheilitis*, *Herpes zoster*, *Leukoplakia*, *Lupus erythematosus*, *and Lichen planus*) arising due to various reasons, including smoking, tobacco chewing and overdose of drug/medicine that tend to affect the lip print patterns.[4,5] Such chronic habitual and pathological conditions often place the permanent nature of lip print patterns under question. In the present study, an attempt has been made to study the effect of the eruption of various pathological conditions on the stability and permanency of lip prints.

A total of 50 subjects were surveyed to investigate the outcomes of oral/lip manifestations. Sampling was conducted in the outpatient department and wards of various dental hospitals and research institutes (with permission of the concerned authority). Pre-treatment and post-treatment lip print samples were collected from patients bearing pathological conditions (mostly non-carcinoma) in their oral or perioral regions. A total of 27 pathological conditions were identified and keenly observed. Follow-up samples were collected from the same patients after the lesions healed (depending upon the severity of the disease, the treatment period, and the time of healing for each lip condition). All samples were photographed, and the digital images were enhanced using Picasa software. Any subsequent changes in the patterns of wrinkles and grooves were observed and noted for further evaluation.

It was observed that after an eruption of the disease, the pustules/lesions/bullae engulf the lip print pattern abnormally (no particular growth pattern), and the print becomes challenging to classify. However, post-treatment and healing, the subsequent follow-up samples bearing minimal scars or hyper-pigmentation could be classified for comparison purposes. Furthermore, chronic lymphocytic leukaemia and necrotizing fasciitis emerged as the most stringent pathologies. Both the subjects died of the adverse effects of their respective diseases, and the lip prints were permanently lost (due to lip/face disfigurement resulting from necrosis and post-surgical debridement of soft facial tissues). Some of the manifestations healed within a few weeks; especially, the subjects bearing *Herpes labialis* got cured in almost two weeks.

The findings concluded that the non-cancerous oral diseases temporarily hamper the lip print patterns, and the pattern of wrinkles and grooves regain their form. Later, the healed lips can be used for identification purposes.

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Lip Prints; Permanence of Lip Prints; Oral Pathological Conditions

H10 The δ18O Values as Chemical Signatures of Geo-Locality of Ajnala Skeletal Remains: A Forensic Odontological Study

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Learning Overview: After attending this presentation, attendees will be informed about the significance of oxygen stable isotopes in estimating the potential geo-locality of an individual.

Impact Statement: This presentation will impact the forensic community by informing attendees that oxygen stable isotope analysis may help in the geographical provenance of unknown human remains recovered from forensic or bio-archaeological scenarios.

Stable isotope analysis of unidentified human remains has proven to be an important forensic provenance tool to reveal their geolocation, mobility patterns, dietary habits, occupations, etc. and, hence, their past geographical life trajectory.[1,2] Oxygen isotope ($\delta^{18}O$) values estimated from the bone or dental collagen are considered proxy for meteoric drinking water $\delta^{18}O$ values of the local drinking water, body water, and these isotopic abundances has a direct correlation.[3,4] It has been shown that the $\delta^{18}O$ values for bone collagen are 12.5 to 15 higher than those in drinking water, based on the isotopic offset between bone collagen and drinking water.[5]

Thousands of human skeletal remains, reportedly belonging to 282 Indian soldiers killed in 1857 were excavated non-scientifically from an abandoned well situated beneath a religious structure at Ajnala (Amritsar, India).[6-8] In the present study, the dental collagen extracted from 44 mandibular Ajnala molars were analyzed for their $\delta^{18}O$ abundances using standardized methodology and instrumentation protocols. The teeth showing very high positive $\delta^{18}O$ values were excluded from the final analysis/interpretations of the data.

The mean $\delta^{18}O$ value for the studied molar teeth was found to be 14.3 ± 7.27‰ (Vienna Standard Mean Ocean Water [VSMOW]); with values ranging from 16.77‰ to 0.40 ‰. The precipitation $\delta^{18}O$ values for the samples varied from -4.3 to 12.1‰, which changed to -1.8 to 14.6‰, after enrichment. When the obtained $\delta^{18}O$ values were comparatively analyzed with the published $\delta^{18}O$ database from India, it was found that the majority of $\delta^{18}O$ values in the studied tooth samples fall in the larger isotopic range, signifying that the individuals would have come from different geographical locations and were "non-local" to the site from where the remains were exhumed and were largely pointing to locations in the Gangetic plain (Uttar Pradesh) and coastal areas (like West Bengal and Orissa) of India. These observations corroborated the historical written accounts as well as the previous forensic anthropological and molecular findings with these remains, that the Ajnala individuals came from the Indian states mentioned in historical records.[9,10] Thus, $\delta^{18}O$ values can be potentially used as chemical signatures of geolocation of the individuals whose remains have been retrieved from forensic archaeological contexts.

There are only a very few Indian data on the bioavailable oxygen, so present study $\delta^{18}O$ abundances are expected to provide baseline data for future forensic provenance studies that will contribute to the global efforts of mapping $\delta^{18}O$ isotope variations by the isotope community. Though the forensic anthropological usage of $\delta^{18}O$ isotopes for provenance of unidentified humans is not foolproof, it will gradually get more uptake in the medicolegal/forensic domain, albeit with varying results.[11] The detailed results will be presented in textual, tabular, and graphical/diagrammatic form.

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Stable Isotope Analysis; Forensic Odontology; Geolocality

H11   Investigating the Influence of Sex and Ethnicity on Human Identification Using Palatal Scans

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Learning Overview: The goal of this presentation is to introduce attendees to the findings of this study that demonstrates the reliability of palatal scanning as a forensic identification technique, even in populations of mixed ethnicities. Nonetheless, the superimposition method may not be capable of accurately determining sex and ethnicity. Much more emphasis should be placed on palatal identification, in addition to dental identification.

Impact Statement: This presentation will impact the forensic science community by explaining the importance of using digital scans in forensic odontology. With the right legal background and sufficient digitalization, the lives of those in the field will be made easier if science is translated into practice using this level of technology.

Objective: Palatal scans present a non-invasive and reliable approach for individual identification by utilizing the distinct characteristics of palatal rugae. With advancements in digital technologies and image processing, superimposition of palatal scans has emerged as a promising method for forensic investigations. This study aims to explore the impact of sex and ethnicity on human identification using intraoral scanning and superimposition techniques. The objective is to investigate how sex and ethnicity influence the efficacy of differentiating between individuals through these methods, providing valuable insights for forensic odontology.

Method: This study included 23 participants, comprising 16 females and 7 males, aged between 23 and 35 years, representing 11 Asian and European countries. Each participant underwent three scans using an Emerald intraoral scanner. To assess the repeatability of the scans, the scan replicates of the same subjects were aligned, and the differences between scans of different individuals were evaluated. Mean Absolute Differences (MAD) were calculated and compared to determine the discrimination potential. Additionally, the study investigated the impact of sex and ethnicity on MAD by conducting multiple linear regression analysis. The distances between countries were also taken into account based on their longitude and latitude information. This research aims to explore how sex, ethnicity, and geographical variations influence the accuracy and reliability of intraoral scans for individual identification, providing insights into forensic odontology and related fields.

Results: The study revealed noticeable variations in the MAD between scans of different individuals and the repeatability of scans of the same subjects. The lowest value of between-subject MAD was 0.214mm, while the highest value of repeatability was 0.093mm. The analysis showed that sex (rP=-0.06, p=0.343) and latitude (rP=-0.12, p=0.058) did not significantly affect MAD. However, longitude had a weak negative impact on MAD (rP=-0.16, p< 0.01), suggesting that geographical variations may play a minor role in influencing the accuracy and reliability of intraoral scans for individual identification.

Conclusion: The findings of the study demonstrate the reliability of palatal scanning as a forensic identification technique, even in populations of mixed ethnicities. Nonetheless, the superimposition method may not be capable of accurately determining sex and ethnicity.

3D Technology; Human Palate; Ethnicity

H12   A Colorimetric Study of Burnt Teeth as a Diagnostic Tool to Aid in Forensic Dental Identification

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Learning Overview: After attending this presentation, attendees will have acquired foundational knowledge regarding the color modifications of teeth exposed to various temperatures and their relevance in forensic dental identification.

Impact Statement: This presentation will impact the forensic science community by informing attendees about the standard colors of burnt human teeth and how this data can be used in postmortem procedures and the collection of autoptic parameters.

Teeth are composed of the most resilient tissues in the human body, making them a valuable resource for forensic odontologists in the analysis of human remains for the identification of burnt, decomposed, and skeletonized human remains.[1-3]

The aim of this research is to analyze the colorimetric changes of the dental coronal portions subjected to high temperatures and to develop a reproducible forensic cataloging method. Sixty-three human teeth were extracted from private dental clinics and university dental schools for therapeutic reasons, following the approval of the Ethics Committee of the University, and divided into three groups. The teeth were subjected to three temperature ranges—400°C/752°F, 700°C/1,292°F, and 1,000°C/1,832°F—in a laboratory furnace for double time periods of 20 minutes and 60 minutes. Each group of teeth was analyzed with a spectrophotometer designed for dental use to extract the colorimetric data of the enamel of each burnt tooth. The coordinates found were subsequently converted into RGB values. The two predominant colors were then selected to create colorimetric averages, which show the change in color tone depending on the temperature.

The groups of teeth subjected to 20 minutes at 400°C (752°F) showed a dark gray coloration; the teeth subjected to 700°C (1,292°F) for 20 minutes showed a general increase in color brightness with beige-blue tones; the teeth subjected to 1,000°C (1,832°F) for 20 minutes showed increasingly lighter tones with pink reflections. Teeth subjected to the same temperatures for 60 minutes showed a general increase in brightness, making differentiation more complex, except for the group of teeth burned at 400°C, which displayed light blue-gray tones.

The study confirms the literature data on the possibility of linking colorimetric shifts of carbonized teeth to the maximum temperature peaks reached, providing valuable assistance to the work of forensic pathology and forensic dental identification of burnt human remains.[4,5] A further step of this research will be the development of a standardized colorimetric scale of burnt human teeth.

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Burnt Teeth; Color; Spectrophotometry

H13   A Multinational Classification of Dental Resin Composites for Forensic Purposes

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Learning Overview: After attending this presentation, attendees will have a deeper understanding of the differences in spectra and matrix distribution of resin composites used in restorative dentistry, which are brand-specific as each formula is copyrighted.

Impact Statement: This presentation will impact the forensic science community by raising awareness about the potential for geographical discrimination through the assessment of resin composites found on the teeth of unidentified human remains. This information can be valuable in the identification process.

The identification of human remains relies on the collection and comparison of primary and secondary identifiers. When teeth and jaws are present, dental data can be pivotal in the identification process, both for the initial reconstruction of the biological profile and for the definitive identification, in conjunction with fingerprints and DNA comparison.

In dental autopsies, forensic odontologists record the odontogram of unidentified human remains, describing the presence, color, and type of restorations. Dental materials can be found and, in addition to a macroscopic description, can be subjected to various analyses to identify their composition and, potentially, the manufacturer.

To achieve this goal, the spectra of unknown resin composites must be compared with the microstructural data and spectra of known dental resin composites. In 2008, a database of dental resin composites was inaugurated in the United States, creating a spectral library with storage, query, and display utilities.[1] However, no such database exists for dental resin composites manufactured in other countries.

Therefore, a multinational database was generated by collecting dental resin composites from various countries, including Australia, Brazil, China, Germany, Japan, Italy, Luxemburg, Korea, and Switzerland, using Scanning Electron Microscopy with Energy-Dispersive X-ray SPectroscopy (SEM/EDS) and X-Ray Fluorescence (XRF).[2,3] This approach obtained an archive of magnified images (2,000x, 5,000x, 10,000x), spectra, and information for each brand.

The advantage of the database is the possibility of comparing an unknown spectrum and composition of a dental resin specimen with those stored in the database with known samples from various nationalities. One of the challenging efforts in the human identification of missing and unidentified human remains is their geographical origin. Although several brands of dental materials are used worldwide and do not offer any specific information on the possible nationality, there are other dental materials that are country- or continent-specific, like the ones examined, thus allowing possible geographical localization.

The multinational-generated database is constantly updated with dental composites from other nationalities and is a library available to all forensic odontologists upon request.

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Dental Resin Composite; Human Identification; SEM/EDS

H14   A Radiographic Field Guide to Modern Dental Restorative Materials

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Learning Overview: After attending this presentation, attendees will be knowledgeable about the radiographic appearance of historical and contemporary dental materials for the purpose of comparative radiology in the identification of human dental remains.

Impact Statement: This presentation will impact the forensic science community by providing a clearer understanding of the radiographic appearance of historical and modern dental restorative materials, thereby enhancing the capacity for remote identification of dental remains, which in turn may be particularly useful in mass disaster scenarios, pandemics, and instances where collaboration with other death investigators is essential.

Introduction: The totality of an individual's dental features, which includes anatomical morphology, restorative treatment, alterations, and injuries, is considered distinct and globally accepted as a scientific form of human identification. Teeth and dental restorations are resistant to environmental extremes such as fire, desiccation, prolonged moisture, and heavy forces. Therefore, comparative dental radiology holds a sharp advantage over DNA profiling and friction ridge analysis when a lack of soft tissues renders the latter two techniques unsuitable for human identification.

Innovations in materials science are driving rapid advances in dental medicine. In the past two decades, the field has witnessed a revolution in techniques and technologies that have enhanced biocompatibility, durability, operative ease of use, and aesthetic quality. In particular, bioceramics, like biosilicate restorative agents and indirect zirconia restorations, have come to the fore. While contemporary dental materials pose myriad benefits for practitioners and patients alike, they also present new radiographic challenges for the forensic odontologist.

The traditional approach to forensic dental identification relies heavily on comparative radiology of antemortem and postmortem morphological features. This approach can be complicated by the presence of distinct dental restorative materials that mimic each other or natural tooth structure in radiopacity or radiolucency. Additional features such as preparation design can be considered as radiographic points of comparison as newer dental materials increase in popularity.

Methods/Methodology: A review of the literature was performed.

Conclusions: Recent changes in dental materials have complicated the process of human remains identification.

Results: Radiographic assessment and comparison of radiolucency, radiopacity, and preparation design can distinguish discrete dental materials from one another and from natural tooth structure, to aid in more efficient and accurate identification of human remains.

Implications: This presentation will examine the intersection of historical and modern innovations in dental restorative materials and the use of radiography to aid forensic odontologists in navigating a path toward improved identification of individuals from dental remains. Clearer understanding of present dental restorative materials will enhance the capacity for remote identification of dental remains, which in turn may be particularly useful in mass disaster scenarios, pandemics, and instances where collaboration with other death investigators is essential.

Dental Identification; Radiology; Materials

H15   Artificial Intelligence-Driven Dental Comparison for Human Identification in Mass Disasters

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Learning Overview: After attending this presentation, attendees will be informed about recent efforts to develop artificial intelligence-driven approaches to support forensic human identification based on dental digital information (odontograms and 2D/3D images). Such efforts aim to reduce subjectivity and time in dental comparison techniques.

Impact Statement: This presentation will impact the forensic science community by presenting research that is focused in two aspects. The first one is to develop and study novel dental coding systems and corresponding automatic ranking algorithms. The second is to propose innovative methods based on artificial intelligence to automatically compare and score dental images of different modality and dimensionality.

The comparison of Antemortem (AM) and Postmortem (PM) dental records remains one of the main identification methods of cadavers. The automatic comparison of AM and PM dental records and the creation of a ranking of the best possible matches has been crucial in the case of incidents involving a large number of people. The International Criminal Police Organization (INTERPOL) standard currently includes 41 dental codes, although previous studies found that four codes created a diversity of patterns similar to that of mitochondrial DNA.[1] Based on this research, it was hypothesized that dental classification software could still be effective with more simplified codes and, consequently, would have the added advantage of being easier to record and more accurate in ambiguous coding situations. In addition, combining the classification produced by different coding systems could result in a more accurate algorithm capable of automatically discovering coding errors, while maintaining the ability to individualize a larger number of codes.

These challenges have been addressed using machine learning and information fusion methods to develop different odontogram comparisons and ranking algorithms based on two different coding systems and their hybridization. The first coding system is the one proposed by Adams and summarized in seven possible codes.[2] The second system, a simplification of the INTERPOL standard codes, is reduced to 19 possible codes where only information about the whole tooth is provided. This is intended to avoid codification errors when analyzing a radiograph (2D image) to codify 3D-based codes (the five faces of each tooth). The ranking was generated first with reference to Adam's proposal, using the same characteristics extracted from odontograms comparison, based on the lexicographical order matching. In order to improve the discriminative power of this system, it was proposed to use machine learning methods, taking into account the weight of each of the features and using them in multiple aggregation methods.[3] The same methodological procedure has been applied to the second coding system. Finally, a third approach involved the use of both coding systems, as a way to combine the best features of each, reliability in the codification and a higher discriminative power, respectively.

Material: Two different data sets have been employed to test our automatic proposal: (1) 128 pairs of AM-PM dental records corresponding to real forensic cases positively solved at the National Center of Forensic Medicine (Abu Kabir) in Israel; and (2) 100 pairs of orto-pantograms acquired at different time moments in a dental clinic in Chile.

Results: The three approaches(two different coding systems and its hybridization) performed accurately. The worst results were achieved by the seven codes approach, with a mean ranking of 2.73 (i.e., on average, the positive case is within the three first solutions in the provided ranking). Percentile 75% of the ranking is 1 (i.e., in 75% of the cases, the positive match is in the first position of the ranking). Percentile 95% (i.e., in 95% of the cases), the positive match is in the ninth position (or lower) of the ranking. Results of the other two algorithms will be provided during the conference as we are still developing further experiments.

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Dental Comparison; Artificial Intelligence; Human Identification

H16   Prehistoric Therapeutic Dentistry in the Mid-Atlantic United States: The Use of Multi-Modal Imaging on Ancient Dental Specimens

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Learning Overview: After attending this presentation, attendees will be informed regarding: (1) a brief overview of Maryland's Late Woodland (AD 950–1550) palisaded Keyser villages, including the Maryland (18MO1) Hughes site; (2) the jaded archeological history of the Hughes site; (3) a description and analytic methods used for dental remains in three individuals; (4) the results of radiographic, visible, and Alternate Light Source (ALS) macro photography, and Scanning Electron Microscopy (SEM) evidence; (5) a discussion of natural processes of dental caries versus modification; and (6) the case for therapeutic dentistry as a conclusion, as well as the alternatives.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the authors believe that this type of intentional dental modification may be underreported worldwide in archeological contexts, regardless of time period, because few such examples of intentional dental intervention are known or reported. The work presented adds to a growing awareness and body of knowledge of this subject.

Late Woodland period prehistoric human remains recovered from the Hughes site (18MO1) in the 1930s were only recently submitted for bioarcheological analysis. The remains reported in this study are part of the larger Yinger collection that had been in the custody of the Natural History Society of Maryland but had not undergone previous bioarcheological examination. Among the remains are several teeth suspected of undergoing antemortem dental modification. All the examined dental cavitations were deep, and some showed excavation all the way to the pulp.

Dental remains representing three individuals (two adult females and a child) were examined to define the etiology of altered occlusal cavitations in these teeth. The findings provide evidence of prehistoric, therapeutic dentistry in the Middle Atlantic region of the eastern United States. Two mandibles and a maxillary right dental quadrant representing three individuals were analyzed utilizing dental radiographs; visible, Ultraviolet-Induced Fluorescence (UVIF), Reflective UV (RUV) and Infrared (IR) alternate light source macro photography; and scanning electron microscope operating in low vacuum mode in three modes of detection: Backscattered Electron (BSE), Secondary Electron (SE) detectors, as well as false-colored images (MIX) produced by mixing simultaneously obtained signals from the SE and BSE detectors.

All three specimens showed signs of both radial and concentric tool marks to varying degrees in both enamel and dentin borders of carious lesions. The shape of the cavitations do not follow normal pathological patterns of undisturbed occlusal decay in molar teeth, namely the formation of a cruciate pattern of cavitation, beginning with a nidus of cariogenic infection located within the anatomical fissures. Further, evidence is presented that the excavation tool motion changes from rotational drilling to radial scooping the further posterior the cavitation is located in the oral cavity. Last, it is proposed that the reason for these modifications was therapeutic dentistry instead of postmortem/taphonomic modification, ingestive behaviors/teeth-as-tools, or cultural modification for social expression.

Archaeology; Imaging; Teeth

H17   Dental Identification After an Arson Attack

Camilla George*, DentArtWork Dental Care, Lechlade, England, United Kingdom

Learning Overview: This goal of this case presentation is to go through the lead-up to before the Postmortem (PM) (including online meetings/organization of specialists/retrieval of the bodies from an unstable building). Radiology used Computed Tomography (CT) scans of two unidentified bodies and the examination of these head scans was sufficient to examine the dental restorations as a comparison to the Antemortem (AM) records.

Impact Statement: We are more aware of digital Computed Tomography (CT) scans and their use in dental identifications. This case took place under COVID-19 protocols, with an online briefing prior to meeting as a team in the mortuary. Full Personal Protective Equipment (PPE) was worn, and a Disaster Victim Identification (DVI) protocol was followed. This presentation will impact the forensic science community by providing an example of "Justice for All" as the suspect was found guilty and jailed for life.

A dental identification was needed for two individuals following an arson attack. Retrieval from a burnt building took time and meetings were online over the festive period to keep all specialists in the loop (headed by the coroner for the area), with a plan to organize the PM in the new year. Stabilizing a burning building took months of planning and finding the correct contractors. During this time, the bodies had to be protected during the winter months.

The PM took place while COVID-19 was prevalent, so full PPE was worn. Prior to entering the mortuary, a briefing was held, including an induction of Do's and Don'ts during COVID-19, where we don and doff our PPE, etc. This was classed as a DVI case, so protocols were followed to identify the individuals and retrieve all items for evidence. Two pathologists and one forensic odontologist were present along with a police force DVI team. The PM took place over two days. Both individuals were CT scanned prior to the PM and analysis of the scan enabled us to compare antemortem and postmortem information. Both individuals were correctly identified as it was uncertain who was where in the building of flats when the fire started, and neither were found in their flat.

The arsonist came forward and had stated in his diary it was his intention to cause "death" to his neighbors. This arose due to antisocial behavior by the arsonist, which neighbors had complained about on several occasions. The arsonist was found guilty on two counts of murder, two counts of grievous bodily harm, and one count of arson with intent to endanger life. It took one year for the case to go to court and the arsonist, originally from abroad and illegally in the United Kingdom, was jailed for life.

Forensic Odontology; Arson; CT Scan

H18   Developing an Animal Model for Investigating Tissue Deterioration of the Palate by Intraoral Scanner

Janos Vag*, Semmelweis University, Budapest, Hungary; Sandor Mikó, Semmelweis University, Budapest, Hungary; Arvin Irani Shahbazi, Semmelweis University, Budapest, Hungary

Learning Overview: After attending this presentation, attendees will be informed regarding the use of intraoral scanners to capture oral issues. We showed that intraoral scanners can be easily used for human identification. However, comparing antemortem to postmortem data is under debate because little information is available about the deterioration of oral tissue after death. Therefore, we are developing a cadaver animal model, and the first experience will be shared with the attendees.

Impact Statement: This presentation will impact the forensic science community by showing that the developed animal model will be suitable for studying various environmental factors, such as temperature, humidity, acidity, etc. to tissue decay. We will also reveal how tissue deterioration affects the recognition of oral structures by intraoral scanners. Intraoral scanners can become an adjunct tool for human Disaster Victim Identification (DVI) because of wide-spread usage of intraoral scanners, their ease of mobility, and the recorded reliable digital data.

Background: Previously, we demonstrated that the digital 3D scans of the human palatal vault obtained by an Intraoral Scanner (IOS) could distinguish between identical twins. Palatal rugae patterns have been suggested as a morphological structure basis for human identification. However, no data is available about postmortem destruction of palatal morphology. Due to the limited access to human cadavers, an animal model would be feasible to investigate the multiple environmental factors affecting deterioration.

Objectives: This study introduced using lamb maxilla as an animal model to investigate the effect of the palate's decomposition on recognizing, replicating, and recording the rugae pattern with an IOS. A secondary aim was to compare the washed and unwashed samples.

Methods: Ten fresh lamb maxillas were incubated for 20 days (21°C; 80% humidity). The specimens were photographed and scanned with a Planmeca Emerald® S IOS. The scans were repeated immediately three times to assess the ability to replicate. The blood and impurities were washed thoroughly in five specimens and not in the other five. One scan was made each day between days 2–20 of each of the specimens. These were compared to baseline scans from Day 1. Comparisons were made by using two scans that were aligned three-dimensionally employing an algorithm of the iterative closest point. The Mean Absolute Distance (MAD) between aligned surfaces was calculated to assess the deviation of the deteriorated scans from the fresh ones. The change in MAD values was plotted as a function of the days, and a regression equation was applied to the data. Similarly, the three scans within each day were aligned in pairs to determine the ability to replicate positioning, and one scan of each fresh specimen was aligned with the others to evaluate the inter-subject deviation. The lowest inter-subject MAD was the threshold to distinguish between identical and different specimens.

Results: The MAD constantly increased from Day 1 until Day 10, reaching a plateau. The lowest inter-subject MAD was 484μm. In washed groups, one of the five specimens was above the threshold value on Day 4. In the unwashed group, it occurred only after Day 5. Reproducibility was significantly ($p < 0.001$) worse in the unwashed group (median, 0.025mm; range 0.006–0.127mm) than in the washed group (median, 0.013mm; range 0.007–0.172). Scan reproduction did not change significantly throughout the 20 days.

Conclusions: The lamb cadaver is a feasible model to study palatal decay by IOS. The IOS could capture the decayed lamb palate over three weeks, despite the tissue deterioration and discoloration. The unwashed sample showed a similar decay curve to the washed one but with lowered ability to reproduce the image. Washing the sample before the experiment is suggested for standardization. In conclusion, the palatal scan could be used for postmortem identification by Day 4. Due to the lack of bone support compared to the human palate, the lamb palate may decay more quickly than a human one. This aspect would require further investigation beyond this study.

3D Technology; Dental Identification; Decomposition

H19   The Establishment of a Provincial Identification Unit and Its Promising Results

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Learning Overview: The goal of this presentation is introduce the establishment of an identification unit at the Laboratoire de Sciences Judiciaires et de Médecine Légale in Quebec, Canada, as well as its operations and its first promising results.

Impact Statement: This presentation will impact the forensic science community by presenting the debut of a provincial-wide identification unit, highlighting the necessary work since its inception, the plans moving forward as the unit grows, and sharing some stories surrounding a few families the unit has already impacted.

Since its foundation in 1914, the Laboratoire de Sciences Judicaires et de Médecine Légale (LSJML) in Quebec, Canada, has worked on numerous cases of unknown deceased individuals to determine the causes of death and, ultimately, restore their identities.

Over the decades, pathologists, odontologists, anthropologists, and biologists have worked together to provide a scientific identification for unknown remains. If a scientific identification wasn't possible, pertinent identification information was provided to coroners and law enforcement in an effort to help investigations discover the identity. Over the past few years, a realization of the humanitarian role of the LSJML in resolving critical issues regarding unknown and missing persons cases helped to open some doors.

After a reviewing of some of the cases in the LSJML archives dating as far back at the early 1960s, it became clear the files contained some scientific keys that could lead to potential identifications. Thus, with the support of the government of Quebec, in the spring of 2022, the Identification Unit of the LSJML was created with a forensic odontologist in charge of leading the unit.

A monumental task awaited the members of the newly founded team: revisiting and consolidating the information available in the archives of unknown and missing persons with a fresh perspective to examine the existing information, search for new leads, and utilize newer scientific methods.

Over a year has passed and with the collaboration of the Quebec main police forces and the Coroner's Office, eight identifications have been confirmed and seven other files with a high degree of correlation are waiting for further information or expertise to be completed.

As a result of the sustained efforts of passionate professionals, several families have been able to find answers to their long unanswered questions and perhaps begin the final processes of mourning their lost loved ones.

Identification; Forensic Odontology; Unsolved

H20   The Potential of Smile Photographs and a PM Intraoral Scanner for Dental Identification

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Learning Overview: After attending this presentation, attendees will be informed about a novel methodology for dental comparison involving the superimposition of Antemortem (AM) smiling photographs and Postmortem (PM) 3D intraoral scans.

Impact Statement: This presentation will impact the forensic science community by sharing a novel approach that could perform dental comparisons in complex scenarios where AM material is scarce or difficult to access (such as historical cases, cases of refugees or migrants, or disaster victim identification), given that selfie-style photographs are increasingly accessible with the widespread use of phone cameras and social media. Implementing the acquisition of an intraoral scan within the autopsy workflow could yield a considerably higher number of first links for positive identifications with a relatively low time and cost investment.

Intraoral scanners are currently gaining prevalence in dental clinics because of their state-of-the-art accuracy in the representation of dental structures. They provide exceptional detail, with resolution and accuracy in the range of microns, and require a time investment of less than five minutes for the entire process.

Within the field of forensic odontology, there have been some attempts to use photographs for comparison of teeth morphology and smile lines with the goal of identification. These approaches tend to use the comparison of incisal borders of the anterior teeth from Antemortem (AM) and Postmortem (PM) photographs or 3D models.[1-3] However, the comparison of dental images of different dimensionality has attracted little attention, and existing proposals are limited to manual superimpositions that do not properly address perspective distortion. The latter is especially problematic due to the lack of proposals tackling the replication of the optical distortion caused by the interaction of subject-camera distance and the focal length of the lens of the camera on the 3D mesh. This distortion tends to be even greater in "selfie-" style photographs because the object to be photographed, in this case the face, is close to the lens.

With the purpose of carrying out a preliminary study to test our proposed methodology and identify relevant criteria for the identification and exclusion of candidates, we tested it within a small experimental scenario with living individuals.

The materials used were a set of photographs of each of three subjects at established focal lengths; each subject was photographed smiling and showing their anterior dentition, to be used as AM material for comparison. For the PM material, intraoral scans were acquired from the same three subjects using a 3Shape TRIOS intraoral scanner. Two main challenges need to be addressed: a proper replication of the distortion and an objective, accurate, and automatic method performing the superimposition. The former is solved by modeling the problem as a camera calibration process where pose, focal length, and subject-to-camera distance are estimated based on the correspondence of pairs of features in both the photograph and the 3D model. In particular, two different approaches have been developed: the placement of a set of specific landmarks and the segmentation of the contours of all teeth. Thus, two different algorithms have been employed to automatically obtain the 2D-3D superimposition, POSEST-SFO4, and MVMO-SH5, respectively.[4,5]

Intraoral scanners will additionally change the way dentists archive digitally a patient's dental history, allowing forensic odontologists to gather dental data for the purpose of human identification. Considering that this information will be shared with patients, and the growing development of Electronic Health Records, dental data will be available for identification purposes, not only by contacting dental clinics, but also directly from the family of the missing. It is pivotal that any dental comparison for human identification purposes remains a process performed by forensic odontologists. A standard methodology needs to be developed on the basis of further research with significant sample sizes, aimed to identify the most significant features to evaluate in comparisons and whether these could potentially suffice to positively identify or serve as a first link to guide the identification process.

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Dental Identification; 3D Technology; Positive Identification

H21   The Production of Dental Remains Replicas With 3D Modeling

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Learning Overview: Mock mass disaster exercises allow forensic dental teams better preparation for true emergency responses. The traditional use of preserved human remains in these exercises presents several concerns. They are friable, difficult to transport, and present infection-control issues. The goal of this presentation is to describe the fabrication of human dental remains replicas with 3-Dimensional (3D) modeling and printing.

Impact Statement: This presentation will impact the forensic science community by providing information on how use of 3D-printed models of human remains offers the alternative of durable, reproducible, clean, easily transportable, plastic dental remains that can aid in mock mass disaster trainings for federal, state, and local forensic dental teams.

Provision of training exercises for forensic dental teams enhances the coordination of their emergency responses when needs arise. Mock mass disasters have proven to be an effective practice in forensic training.[1] The United States Air Force (USAF) provides such training within their dental residency programs. For years they had one set of preserved human dental remains that would be shipped from base to base in order for these trainings to commence. Transportation of human specimens is challenging as Federal Aviation Administration (FAA) regulations dictate that only ground shipping is allowed. Also, over the years, these one-of-a-kind specimens have succumbed to progressive deterioration. In 2017, the USAF's 59th Dental Group's Medical Computer-Aided Design/Computer-Aided Manufacturing (CAD/CAM) laboratory routinely utilized advanced 3D color printing capabilities (Stratasys Objet 260 Dental Selection). The question was raised: Could sets of human jaw remains be preserved through the production of realistic printed replicas? A two-year project proved the feasibility.

The laboratory staff performed Computed Tomography (CT) and 3D surface scans of nine individual sets of humans' remains: a total of 23 jaw fragments. Standard Tessellation Language (STL) files were created, and files were 3D printed for evaluation. The printer's 3-color palette offered realistic bone and dental colorations. Preparations for direct and indirect restorations were performed on the master models. These prepared models were scanned to yield new images. These images were digitally "subtracted" from the original unprepared master models resulting in images of the indirect crown and inlay restorations. STL files of these images were milled from shaded acrylic resin pucks using a 5-axis milling unit (imes-icore 350i). Metallic silver, gold, and pink paints and markers added metallic and gutta percha effects. The restorations were luted with epoxy adhesive. Seven identical mock mass disaster kits, each with 23 jaw fragments and representing nine mock human casualties, were produced. A total of 161 models with 266 indirect restorations and 469 direct restorations were ultimately produced. The kits were distributed to bases in the United States and Europe and have been used successfully for mass casualty exercise training of dental officer staff and residents. This process could be replicated for federal, state, and local forensic dental teams to provide needed training on mass disasters.

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3D Technology; Mass Disaster; Forensic Odontology

H22 Odontometrics and Artificial Intelligence: A Comparative Study for Best Prediction of Facial Geometry in Medicolegal Cases

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Learning Overview: After attending this presentation, attendees will be able to identify and recognize the best-suited method of forensic facial approximation/prediction from morphometric dental data. The attendees will understand the application and importance of dental measurements as an indispensable tool for giving an identifiable face to charred, decomposed, commingled, skeletonized, and fragmented bodies.

Impact Statement: This presentation will impact the medicolegal community by providing highly significant multiple regression equations and artificial intelligence/machine learning-generated models for the prediction of facial geometry from various dental measurements that are essential for the identification of deceased in forensic and mass disaster cases. Moreover, this presentation will also explain that out of these two models, which methodology is best for the prediction of facial geometry from dental measurements.

Dental remains are an available type of evidence in cases of human remains and disfigured dead bodies resulting from mass fatality incidents (i.e., disasters, mass murders, fire, explosion, airplane crashes, train accidents, road accidents, etc.) and in single-person crimes (i.e., decomposed and unidentified dead bodies). The identification from teeth in terms of morphological and morphometric features has been explored by many researchers in the past.[1,2] The prediction of facial recognition from various teeth measurements is the latest approach to forensic identification of the deceased. For this purpose, various statistical tools and artificial intelligence software were utilized.[3] There is a lack of literature regarding a recognition of the most appropriate dental measurements for facial prediction and, hence, yield prediction models with high error rates. The present study evaluates specific dental measurements and will then propose statistically significant regression models and machine learning-generated models for facial prediction. Moreover, the study evaluates the comparative assessment of these two models.

The present study comprises 200 participants (93 males and 114 females) from the Haryana state of northern India. Ethical approval for the study has been obtained from the Panjab University Institutional Ethical Committee, Chandigarh, India (see approval no: PUIEC 230602-I-114 dated: 09/06/2023). The study includes obtaining the dental cast and the anthropometric facial measurements from the participants after obtaining informed consent. A sliding caliper was used to make six measurements that were recorded from the dental cast. Correlation and multiple regression analysis were evaluated in the IBM® SPSS statistics version 20. Further regression models were predicted for each measurement. Machine learning-based linear regression models were generated using Python® version 3.11.4. The Mean Absolute Error (MSE) was calculated for each model to evaluate the accuracy and reliability of the models.

Results depict that the regression models generated by the IBM® SPSS statistics version 20 from various teeth measurements showed less MSE as compared to the machine learning-generated models. The statistically predicted linear regression models for facial height, minimum frontal breadth, upper facial height, lower facial height, facial width, outer canthal distance, nasal width, and mouth width show the MSE between the range of $\pm$ -8.937 to $\pm$0.0066 (95% confidence level), whereas the machine learning-generated models show the MSE between the range of $\pm$0.25 to $\pm$0.65 (95% confidence level), which is far higher than the statistically predicted models. However, for the prediction of inner canthal distance, machine learning showed a lower MSE ($\pm$0.25) as compared to the $\pm$1.79 MSE obtained by statistical prediction. The present study concludes that the odontometrics/statistically generated models are better than the machine learning-generated models.

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Forensic Odontology; Artificial Intelligence; Personal Identification

H23   Advancing Forensic Odontology Standards: Insights and Updates

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Learning Overview: After attending this presentation, attendees will have gained comprehensive insights into the current state of discipline-specific standards within the realm of forensic odontology.

Impact Statement: This presentation will impact the forensic science community by fostering greater awareness of ongoing standards development initiatives within forensic odontology. Additionally, attendees will gain valuable knowledge regarding the training, tools, and resources available to support effective implementation, compliance monitoring, and enhanced overall understanding of developments occurring in standards development and forensic odontology.

The Organization of Scientific Area Committees (OSAC) for Forensic Science has a clear and important mission: to enhance the nation's use of forensic science by promoting and adopting discipline-specific standards. OSAC achieves this by creating standards that are then refined and published by Standards Developing Organizations (SDOs). Additionally, OSAC is responsible for reviewing and recognizing standards to ensure their quality. These standards are compiled in the OSAC Registry, serving as a centralized repository for high-quality, consensus-based, and technically sound standards.

The focus of this presentation is on significant updates and developments in the field of Forensic Odontology standards. Firstly, there are updates related to the Forensic Odontology Subcommittee Scope modifications. These changes aimed to improve the scope of the forensic odontology subcommittee in light of recent developments in the field.

Next, the presentation will cover ongoing projects by the OSAC Forensic Odontology Subcommittee. These projects include proposing a standard for the Photographic Documentation of Suspected Patterns of Dental Origin. The goal is to provide best practice recommendations for photographically documenting patterns by trained forensic specialists, allowing for future characterization and analysis. Another ongoing project is the proposed standard for Education and Training in Forensic Odontology, which outlines the minimum educational, training, and competency requirements for practicing in this field. This aims to ensure that professionals in forensic odontology have the necessary education to perform their roles effectively. Additionally, the OSAC Forensic Odontology Subcommittee is working on a proposed standard for Forensic Documentation for the Reporting by Oral Health Care Professionals of Suspected Human Abuse. This standard aims to establish guidelines for oral health care professionals when reporting cases of suspected cases, helping improve the documentation and accuracy of such reports.

The presentation also addresses updates to several existing standards. These include updates to American National Standards Institute/ Americans with Disabilities Act (ANSI/ADA) 1058-2010D Forensic Dental Data Set to incorporate International Organization for Standardization (ISO) CD 20888 Dentistry—Terminology for forensic orodental data, updates to ANSI/ADA 1088-2020 2010D Dentistry—Human Identification by Comparative Dental Analysis, and updates to ADA/ISO Specification No. 3950—Designation System for Teeth and Areas of the Oral Cavity. These updates further refine and enhance the existing standards in the field.

Finally, the presentation highlights international developments related to Forensic Odontology by ISO/TC 106 Dentistry and ISO/TC 272 Forensic Sciences. These developments include ISO/TC 106 ISO 5365 Dentistry—Designation system for tooth developmental stages, ISO/TC 106 ISO 10394 Dentistry—Designation system for supernumerary teeth, ISO/TC 106 ISO 24395 Dentistry—Classification of tooth restorations preparation, and ISO/TC 106 ISO NP 21611 Dentistry—Vocabulary for Source Conclusion for Human Identification by Dental Evidence. Additionally, ISO/TC 272 Forensic Sciences focuses on documentation, analysis, interpretation, and reporting of forensic data, with relevance to Forensic Odontology.

By engaging with this presentation, attendees will gain valuable insights into the latest updates and developments in forensic odontology standards. They will be better equipped to contribute to the advancement of these standards, ultimately raising the level of excellence within the discipline of forensic odontology.

Standards; Education; Forensic Science

H24   The Evolution of the American Board of Forensic Odontology Standards and Guidelines for Evaluating Bitemarks

Richard H. Fixott*, Redmond, OR

Learning Overview: After attending this presentation, attendees will understand what the current American Board of Forensic Odontology (ABFO) Standards and Guidelines for Evaluation Bitemarks are and how they impact casework and will also be aware of the number and types of case work in patterned injury investigation currently being conducted by Diplomates of the ABFO.

Impact Statement: This presentation will impact the forensic science community by adding clarity to current concerns regarding the recognition and investigation of human bite injuries.

Concerns over casework involving possible human bite injuries has been expressed by many for some time and even more so since the recent publication of the National Institute of Science and Technology. The concerns, especially regarding linkage of a specific dentition to an injury, are founded in exonerations of cases where bitemark evidence was presented. The overwhelming majority of the cases were litigated prior to 2000. The initial standards and guidelines were first published in the *ABFO Diplomates Reference Manual* (*DRM*) in the late 1970s-early 1980s.[1] The substance of those guidelines can currently be found in the archived *ABFO Forensic Odontology Workbook* published in 1980. The opinions noted in the Standards and Guidelines are biter, probable biter, not excluded as biter, excluded as biter, and non-conclusive. Assignment of a dentition to an injury with reasonable scientific or dental certainty was permitted.

In 2005, the guidelines were updated in response to increasing concern by the ABFO over DNA-based exonerations of cases where linkage of a dentition to an injury was stated as "reasonable dental certainty." Specifically: "Terms assuring unconditional identification of a perpetrator, or without doubt, are not sanctioned as a final conclusion." A stronger conclusion may be made in cases with a "closed" population. In 2013, terms relating to diagnosis of an injury as a bite were added: "human bitemark, suggestive, not a bitemark." From 2013-18, several algorithms were added and evolved. All basically limited further investigation of an injury to those injuries diagnosed as "human bitemark." In 2015, the linkage opinions were reduced to: "can't exclude, inconclusive, exclude." Another restriction was no longer should the term "biter" be used when considering a closed population. Most recently, the ABFO has included a guideline to obtain a second opinion on patterned injury casework.

A survey of ABFO Diplomates was conducted to gauge casework, performed since 2013, related to suspected human bite injuries. The data will be reviewed as part of this presentation.

The evolution of the ABFO Standards and Guidelines for Evaluating Bitemarks shows the Board's response to the grave concerns resulting from casework opinions proffered prior to 2005. No longer is linkage of a specific dentition to an injury allowed and as such is considered a Standard violation. Investigation of a suspected bite injury, under the current Standards and Guidelines, is of considerable value in excluding a suspect and may be of value in determining a possible suspect.

Reference:

[1.] Standards and Guidelines for Evaluating Bitemarks. *American Board of Forensic Odontology Diplomates Reference Manual* (February 2021). Section IV, Standards and Guidelines, pages 3-32 (www.abfo.org).

Standards; Bitemarks; Forensic Odontology

H25   Bitemarks: Can We Agree?

James F. Goodrich*, Cambridge, Waikato, New Zealand; Mark Eilers, Ochoco Dental, Prineville, OR

Learning Overview: After attending this presentation, attendees interested in observer agreement will have acquired up-to-date information as to whether or not expert bitemark practitioners are capable of general agreement when asked if a patterned injury suspected to be a bitemark should be taken forward for analysis and potential comparison.

Impact Statement: There has been considerable debate about the notion of expert consensus that effectively underpins the validity of the science. Previous attempts to test consensus have caused controversy, and this presentation will impact the forensic science community by hopefully answering some of the outstanding questions in this space.

Test subjects were paired with another expert and presented with a series of images of wounds on human skin. The sole question that they were asked about each image was: "Would you work this case up for potential comparison?" The understanding was that the image presented to them was the only evidence that was available for each case. The images represent a range of case evidence presented to odontologists in recent years. For the purposes of the study, "experts" were defined as diplomates of the American Board of Forensic Odontology.

This study attempts to provide a real-world setting for testing consensus among experts, whereby they are presented with an image of an injury on human skin, and they need to determine if they are confident to take that image to a potential comparison stage. The ability of experts to agree on this aspect of bitemark work is a fundamental aspect of bitemark work as a discipline. If there is no apparent agreement between expert pairs, then the validity of bitemark comparative analysis is questionable.

This study has been designed to remove as many of the confounding factors associated with testing expert consensus in this field as possible. The use of paired experts rather than individuals emulates "best practice" guidelines seen internationally. The single question asked also requires the test subjects to agree with respect to image quality, appropriateness of photographic scales, evidential value of the patterned injury, and of course if it satisfies whatever criteria that they use to be interpreted as a human bitemark. If the pair cannot agree, then the opinion devolves to a negative answer to the test question; this is also a real-world expectation.

This presentation will explain the results of the paired-expert study and provide insight as to whether or not expert consensus is prevalent, or indeed possible.

Forensic Odontology; Bitemarks; Consensus

H26   Insights From Wrongful Convictions Related to Bitemark Analysis

John Morgan*, Coptech Systems, Raleigh, NC

Learning Overview: After attending this presentation, attendees will be informed about the experience of wrongful convictions related to bitemark comparison, including the impact of testimony standards and the fundamental discriminatory power of bitemarks on human skin. The presenter has completed and published in the *Journal of Forensic Sciences* comprehensive research on wrongful convictions related to forensic evidence.[1,2] This session will provide new research and details on the specific issues in bitemark analysis, including the impact of changes in American Board of Forensic Odontology (ABFO) standards and the limits of ABFO governance processes. Attendees will learn the full breadth of issues encountered in wrongful convictions related to bitemarks using case studies and root cause analysis. The information will include discussion of scientific research and legal admissibility issues as they relate to lessons from wrongful convictions.

Impact Statement: This presentation will impact the forensic science community by providing unique information about bitemark analysis in light of the experience of wrongful convictions. Forensic science improvement should be closely tied to real-world experience and root cause analysis. This presentation provides a grounded, research-based approach to improvement that can be impactful in the bitemark discipline. It closely ties to the conference theme, Justice for All, which can be supported by the adoption of appropriate, evidence-based reforms that improve the reliability and effectiveness of bitemark analysis and its use by the criminal justice system.

A comprehensive analysis of wrongful convictions includes 28 cases in which bitemark analysis was used and 27 cases in which bitemark analysis was used in a false or misleading manner that contributed to the conviction. Although this incidence cannot be used to determine the overall error rate of bitemark analysis as a discipline, it can be used to elucidate system factors that may contribute to errors.

Of the 44 examinations that were analyzed in connection with the 28 cases, there were testimony errors in 32 instances as assessed with respect to the 2018 guidelines of the ABFO, which restricted the use of statements that imply inclusion of a bitemark within a population of sources. Most conforming testimony were exclusions. In most instances, the testimony did not conform to prior ABFO standards that were in place at the time of trial. For example, in 27 instances, examiners produced invalid individualization testimony, which was often presented with invalid statistical interpretations.

The experience of wrongful convictions demonstrates that the uncertainties in bitemark analysis may make it unsuitable for any type of inclusion or exclusion. Many observers have maintained that the weak scientific foundations of the discipline are unavoidable given the constraints on empirical research on bitemarks. That said, based on the descriptions by examiners, many presumed bitemarks included characteristics that were considered unusual and should have provided a basis for a reliable comparison, including missing and misaligned teeth. Also, even experienced and certified examiners—including leaders of the field—were associated with errors of classification or identification. Because wrongful convictions undermine public confidence more broadly, it is possible that continued use of bitemark analysis in alignment with the 2018 guidelines may undermine confidence in other aspects of odontology, including the valid use of presumed bitemarks to guide biological sampling.

Wrongful convictions associated with bitemark analysis also demonstrate the importance of governance mechanisms across forensic disciplines and jurisdictional contexts. Uncertified examiners produced many analyses that contributed to wrongful convictions. Certified examiners demonstrated variability in their conclusions, the basis for their conclusions, and reporting language. Like similar bodies in other disciplines, it is not clear if the ABFO has sufficient authority to prevent the use of invalid forensic science by law enforcement and the courts. The issue may be exacerbated within the field of bitemark analysis; in wrongful convictions, most examiners acted as part-time consultants and were not subject to oversight by an accredited forensic science organization. Proposed governance improvements will be discussed in the context of the history of wrongful convictions.

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Wrongful Conviction; Bitemarks; Reliability

H27   Expert Witness Testimony and the Standard of Care Involving Swallowed Dental Objects

Raymond G. Miller*, State University of New York at Buffalo School of Dental Medicine, Buffalo, NY

Learning Overview: After attending this presentation, attendees will understand the application of forensic odontology and standards of care to expert witness testimony. Specifically, this presentation involves incidents involving swallowed dental objects and the appropriate management of an occurrence.

Impact Statement: This presentation will impact the forensic science community by helping attendees better understand how forensic odontologists can serve as expert witnesses in casework specific to their fields of education and practice. Proper management of adverse circumstances with respect to swallowed dental objects and the defined application of standards of care will be discussed.

Forensic odontology deals with dental evidence that is evaluated and analyzed, when the need arises, to be examined at the request of the criminal justice system in both criminal and civil cases. Forensic odontologists, through experience and training, are capable and qualified to systematically and thoroughly evaluate and interpret both medical and dental records. Forensic odontologist, or forensic dentists, are familiar with courtroom testimony as well as standards of care that qualify them as expert witnesses in numerous clinical situations involving dental treatment and patient care. Historically, forensic odontologists are involved with victim identification by dental analysis, bitemark analysis, and dental age estimation. Another area where forensic odontologists have also contributed in an important role is through providing expert testimony when dental malpractice is alleged or a standard of care has been violated.

Standard of care refers to the degree of attentiveness, caution, and prudence that a reasonable person in the same circumstances would exercise. Failure to meet the standard is negligence, and the person who fails to meet the standard is liable for any damages caused by such negligence. The standard is not subject to a precise definition and is judged on a case-by-case basis. Courts have frequently upheld that the standard of care is what a minimally competent physician in the same field would do in the same situation, with the same resources. It is understood by the courts that bad outcomes are to be expected, and all entities cannot be expected to be diagnosed. However, reasonable actions and responses to mitigate harm must be incorporated into any decision making when a patient is at risk.

With respect to swallowed dental objects or devices, parameters have been established for a practitioner to make educated, prudent, and, if necessary, emergent decisions for appropriate management. A key primary determinant is the location of the object. Aspiration versus swallowing may not always be evident, and a radiograph protocol must be established. Another determinate that will dictate further potential treatments or recovery attempts is based on qualities of the object or device in question. These qualities are usually defined by size, shape, and sharpness. Although these descriptors are clear, there will be occasions when their application may not be specific to every individual or circumstance. The decision, however, by the practitioner on management may have significant consequences. With respect to swallowed objects, some large items may pass through the digestive track with no consequence, and small objects may cause serious life-threatening complications. This is also true of sharp objects. Having a management plan, with clear and accurate documentation concerning the object, patient response and condition, and, most importantly, the decision process to medically intervene or monitor, based on sound logic and science, is critical.

This presentation will present various cases involving various devices and objects as well as outcomes. Also, one case will be discussed where this presenter testified for the plaintiff. The goal will be to educate practitioners on standards and protocols and assist their determination regarding management. I will also encourage my forensic dental colleagues to incorporate this aspect of forensic litigation into their current practice. Besides clinical management of the situation, a forensic odontologist may be called upon to review records, determine if appropriate actions were implemented, or if negligence had occurred. This testimony may be for the defense or prosecution, but must be based on the facts, current understanding of standards of care, and the risk or benefits of action or inaction.

Odontology; Standards of Care; Swallowed

H28   Justice for Mariyah: Bitemark Analysis and the Power of Exclusion

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Learning Overview: After attending this presentation, attendees will understand how bitemark evidence can be properly incorporated into a criminal investigation. This will be a review of the standards set forth by the American Board of Forensic Odontology (ABFO) as it relates to this case presentation. Attendees will also learn how digital dentistry has also aided in bitemark evaluation using intraoral 3D scanners.

Impact Statement: With the on-going controversies regarding bitemark analysis, it is important to understand that bitemark evidence can be of value for criminal investigations. While the forensic community at large can agree that bitemark evidence should not be used to directly convict anyone of a crime, the debate exists whether it should be used at all. This presentation will impact the forensic science community by hopefully showing the community how excluding individuals from a pool of potential suspects can still be a crucial part of the investigative process.

In December of 2021, an infant girl was brought into Child Protective Services after sustaining numerous injuries at the hands of her teenage father. She had suffered a skull fracture, rib fractures, a femur fracture, and her body had bruises and several areas of patterned injuries on her arms, legs, stomach, and back. The father readily admitted to harming the child as he was playing video games and became frustrated, but denied inflicting any injuries on the child that would leave what appeared to be bitemarks.

After determining that the patterned injuries on the infant's body were made from the human dentition, each pattern was evaluated for its class and individual characteristics to determine which marks could be used, if any, for the examination. Several marks were of little evidentiary value, but a few of the marks did contain high value and thereby were able to be evaluated further for comparison.

The infant was only 8 weeks old at the time of the injury and had only been in the presence of her mother, father, and babysitter. All three individuals complied, and impressions and 3D intraoral scans were made by a blind third party, and models were subsequently printed using a 3D printer. The dentitions were then compared with the patterned injury using Adobe® Photoshop® and thus the exclusionary process was able to be conducted. Because this was a closed population of potential suspects, the lead detectives were able to utilize this information to continue their investigation with the case. As it became apparent, there were two individuals ultimately charged with the abuse of the child and subsequently were sentenced to time in prison. Had it not been for the bitemark evidence, only the father would have been charged as there was no other evidence supporting another individual involved with the abuse.

The ABFO guidelines for bitemark pattern analysis should continue to be followed because it is readily apparent in this case study that bitemark evidence has a place in forensic science.[1] When used properly, it can be valuable as an ethical approach to assisting with criminal investigations.

Reference:

1.   *ABFO Guidelines for Bitemark Evidence.* http://abfo.org/wp-content/uploads/2012/08/ABFO-Standards-Guidelines-for-Evaluating-Bitemarks-Feb-2018.pdf.

Bitemarks; Child Abuse; Conviction

H29 The Good, Bad, and Ugly of Bitemarks

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Learning Overview: After attending this presentation, attendees will have learned about bitemark evidence and the context surrounding its contentious nature in the legal system. This presentation will present information on both sides of the argument—the pros and cons of bitemarks—and allow attendees to make their own judgements about its value. Attendees will get a look into current expert opinion on bitemarks and empirical research results that contribute to its validation or invalidation as an evidence form.

Impact Statement: This presentation will impact the forensic science community by providing a comprehensive summary of the most updated literature around bitemark evidence. Bitemarks are an extremely controversial topic, and there is lack of consensus across the country whether they should continue to be used as evidence, if their role should be altered or diminished, or be retired altogether. The goal of this increased scrutiny of bitemarks is to better inform attendees on the topic and potentially help reach a consensus on their role in the legal system, with the ultimate goal of fair justice being served and victims getting the closure they deserve.

Introduction: Bitemarks are injuries most commonly associated with violent crimes like homicide, rape, and child abuse. They often serve as evidence in criminal cases and have been used to include and exclude suspects in said crimes. However, certain parts of the world are considering phasing out bitemarks as evidence toward suspect inclusion and argue for the cessation of its use. These criticisms are based on the examiner's work on past false convictions and lack of empirical evidence to substantiate its validity.

Objective: To analyze current literature on bitemark evidence and present pros and cons of its use in the legal system.

Methods: Searches were conducted on PubMed® and Google® Scholar databases and limited to full-text, free-access articles published since 2013 (within the past 10 years). Only the first 50 search results on Google® Scholar were screened to limit results and maintain search relevancy. Search keywords included "bitemark," "bitemark examination," "forensic," and "saliva."

Results: This study provides a comprehensive summary of current literature on bitemark evidence and contextualizes both sides of the contentious argument surrounding its use. Proponents of bitemarks support the idea of their uniqueness to the individual and the surrounding context it can provide as to when and how the injury was inflicted. Critics advocate for its abolishment in the legal system due to lack of reliability and specificity of skin as a substrate, lack of individuality across different people, and lack of expert consensus. A more reliable application for bitemarks through DNA analysis of saliva recovered from the mark appears to be most favorable.

Conclusions: Prevalent empirical evidence of the unreliability of bitemarks combined with lack of evidence in support of its validity suggests the need for a major reevaluation of its use in the legal system. Whether its role is limited or removed entirely, bitemark evidence should be interpreted with caution to prevent wrongful conviction and ensure justice will be served.

Bitemarks; Validation; Scientific Evidence

H30   Dental Age Assessment Through a Learning Machine Approach

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Learning Overview: After attending this presentation, attendees will be cognizant of the application of deep learning methods of machine learning, namely Convolutional Neural Network (CNN), in the process of dental age assessment. Attendees will also learn how often the chronological age is correctly classified for several age thresholds, namely 14, 17, 18, and 21 years of age.

Impact Statement: This presentation will impact the forensic science community as dental age assessment using artificial intelligence methodologies can increase the reliability of age assessment. Hence, the impact of this work covers the importance of accuracy of dental age assessment and the efficiency and applicability of machine learning procedures for all the methods included in this study. Employment of this advanced technology in forensic odontology enhances the precision and accuracy of dental age assessment, at the same time providing forensic practitioners with more reliable tools for decision-making. In conclusion, this study brings new opportunities and possibilities for integrating machine learning techniques in forensic odontology, expanding its application and potential in dental and forensic sciences Using machine learning will help forensic practitioners to save time and increase accuracy.

Background: Dental age assessment using CNNs remains unexplored and immature.[1-4] This pilot study aims to investigate the possibility of using artificial intelligence-based methods in age assessment in the Portuguese population.

Objective: The main objective is to establish the most suitable anatomical tooth stage to perform dental age assessment through several scoring systems and age estimation methods. To achieve this objective, deep learning techniques are used, namely CNN methodologies for age estimation. Once implemented, the accuracy and precision of the traditional methods can be compared with those obtained by the deep learning method.

Materials and Methods: We collected 1,250 Orthopantomographs (OPGs) from a Portuguese population (625 females and 625 males) aged between 10 to 24 years from the database of Lisbon North University Hospital Center, using a procedure approved by the Ethics Committee.

The following scoring systems were applied on the third molar: Demirjian (1973); Moorrees, Fanning, and Hunt (1963); Gleiser and Hunt (1955). Applied age estimation methods were: Haaviko (1970), Liversidge (2008), Kullman (1992), Lee et al. (2009), Caldas (2011), Mincer (1993), and Köhler (1994).

The OPGs analysis was allocated to two observers. After performing intra- and inter reliability validation, traditional and machine learning methods were applied to the data for age assessment. Hence, data were randomly divided into 80 per cent for training and the remaining 20 per cent for testing. After the learning procedure using the training data (model estimation), the classification reliability assessment was performed on the test data.

Results and Conclusion: The task of clustering and classification of OPGs and classification of radiographs using CNNs will enable the researchers to discover patterns on collected data images. These patterns will be useful not only for enriching classification and prediction models, but also for designing explainable models. If the application of CNNs is successful at classifying radiographs, a specific architecture, data processing, and hyperparameter values will be derived with another larger OPG sample. This model will be integrated into a system to be developed and made available.

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Dental Age Assessment; Convolutional Neural Network; Legal Age Thresholds

H31   The Age Assessment Procedure and Results of the Age Assessment: Where Is the Dental Age Assessment?

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Learning Overview: After attending this presentation, attendees will have a better understanding of the age assessment process and a number of challenges faced during the procedure, such as the limitations of the methods in use concerning to the non-accurate and non-precise results.

Impact Statement: This presentation will impact the forensic science community by providing a guidance on the consideration of the best interests of the child when assessing the need for age assessment and also when planning and performing an age assessment, using what is currently called a holistic and multidisciplinary approach, with particular attention to the need for accurate results.

Currently, the high increase of foreigners who travel, whether legally or illegally, to European countries increases the need for confirmation of the identity and age of these individuals. This presents a growing need for more reliable methods of control of who enters any country to promote safety.[1] With this in mind, age assessment plays a crucial role for living individuals in various scenarios such as criminal responsibility determination, adoption processes, birth certificate unavailability, sexual abuse/human trafficking, pornography, and murder. Age is an essential element of a child's identity.[2] The correct identification of an individual as a child or as an adult is crucial to ensure that children's rights are protected and guaranteed as well as to prevent adults from being placed among children in order to take advantage of additional rights or safeguards (such as access to education, appointment of a guardian/ representative) that are not afforded to them. The best interest of the child must be primarily considered in all actions concerning children. They are, therefore, to be applicable from the moment that it is considered that the applicant may be below 18 years of age, throughout the assessment of the age, if such assessment is necessary, and until conclusive results indicate that the applicant is an adult.[3]

A holistic and multidisciplinary approach combines or involves several academic disciplines or professional specializations in an approach to a topic or problem.[1] According to the European Asylum Office, a multidisciplinary approach for the purpose of age assessment would imply the exploration of different aspects or factors such as physical, psychological, developmental, environmental, and cultural ones.[4] What are the accurate medical methods? Can the dental age assessment as a medical method be applied without other medical methods? Is the dental age assessment an accurate method? Is the p-value of the research involving dental age assessment reliable? What is the effect size? Is the effect size important for dental age assessment?

The extraction of maximum useful information from statistical research data on dental age assessment helps the forensic expert to interpret the results of age estimation. Effect size estimates describe the observed effect and approaches to the practical relevance of the study: presenting the results of age estimation to the court. The main goal, in terms of the statistical significance test, is the emphasis of the power of the tests and the reduction of random errors of a mere sample variation. Generally, the larger the size, the larger the effect and impact caused by the dental age assessment methodology in age estimation. Effect size is a measure involving the concept of forensic significance, while the p-value involves that of statistical significance. Even though there are several methods for calculating the effect size, its major objectives for age estimation by dental approach are to validate the statistical significance test, and to allow for a comparison of results from different studies with each other. It is important to combine effect size and p-value to enhance interpretation and prevent misinterpretation of data and promote forensic decisions based on evidence-based studies for dental age assessment.

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Age Assessment; Dental Age Assessment; Age Assessment Procedure

H32 The Performance of Different Dental Age Estimation Methods on Saudi Children

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Learning Overview: The goal of this presentation is to describe the accuracy of dental age estimation using six different methods, namely: the Demirjian's method; the Moorrees, Fanning, and Hunt (MFH) method; the Chaillet et al. method; the Nolla's method; the Gleiser and Hunt method; and the Nicodemo et al. method.

Impact Statement: This presentation will impact the forensic science community by a first-of-its-kind study comparing six dental age estimation methods on a single population.

Dental age is of particular interest to many scientific and clinical fields of applications, including orthodontists and pediatric dentistry, in choosing a timing and treatment plan, forensic dentistry, and pediatric endocrinology studies. Dental age assessment is more reliable and genetically controlled compared to age assessment using skeletal indicators.

Aim: To evaluate and compare the performance of six dental age estimation methods (Moorrees, Fanning, and Hunt; Demirjian; Gleiser and Hunt; Nolla; Chaillet et al.; and Nicodemo et al.) on a sample of Saudi children.

Method: This cross-sectional study was based on the evaluation of the sample of 400 archived digital panoramic radiographs of healthy Saudi children (200 boys and 200 girls) between the ages of six to 15.99 years. Panoramic radiographs acquired in 2018–2021 were obtained from the Information Technology (IT) department of the Dental Hospital clinics at King Saud University, Riyadh, Saudi Arabia. Dental Age (DA) was evaluated using six dental age assessment methods (Moorrees, Fanning, and Hunt; Demirjian; Gleiser and Hunt; Nolla; Chaillet et al.; and Nicodemo et al.) on the developing permanent dentition in both jaws of the left side. The accuracy of each method was assessed in relation to Chronological Age (CA), and a comparison between these methods was made.

Results: For all tested methods, statistically significant differences were found between CA and Dental Age (DA) ($p < 0.001$). The mean difference between DA and CA was: -2.19 years for the Chaillet et al. method; 0.15 years for the Demirjian method; -1.01 years for the Moorrees, Fanning and Hunt method; -1.72 years for Nicodemo et al. method; -1.29 years for Nolla method; and -1.00 years for Gleiser and Hunt method.

Conclusion: Among the tested methods, the accuracy in the Saudi subjects was highest for Demirjian's method, followed by the MFH method. The Nicodemo et al. and Chaillet et al. methods were the least accurate.

Accuracy; Age Estimation; Forensic Odontology

H33 **A Comparison of Third Molar Development Between Hispanic Populations in Two Different Geographical Locations in the United States**

Jayakumar Jayaraman*, Virginia Commonwealth University, Richmond, VA

Learning Overview: After attending this presentation, attendees will understand the variations observed in the development of third molars between the same ethnic population living in different locations in Texas and California in the United States. This will be presented in the form of a research study that compares the average age at assessment of crown formation stages of the left side maxillary and mandibular third molars.

Impact Statement: This presentation will impact the forensic community by demonstrating variations observed in the development of third molars between females and males in the Hispanic population across two different states within the United States. This presentation also emphasizes the importance of using population- and sex-specific dental reference standards for reliable age estimation.

Background: Age estimation is an integral practice in forensic science, mainly employed to age children and adolescents who do not possess authentic birth documents. This is also used following suspicions about falsification of age for those who seek asylum or are involved in criminal offenses. Variations in dental development across different ethnic populations have already been established and it has been emphasized to use population-specific standards during the age estimation process. Several studies have demonstrated the importance of ethnicity on third molar development, and subsequently developed reference standards specific to a population.[1,2]. Most recently, population-specific dental reference standards have been reported for Hispanic children in Texas.[3,4] Although variations in third molar development have been well established in different ethnicities, including Hispanics, the influence of geographical location within the same ethnic groups has not been determined.

Aim: To evaluate the development of third molars in Hispanic children and adolescents in two geographical locations in the United States.

Methods: In total, 1,200 dental panoramic radiographs belonging to healthy children of Hispanic ethnicity were obtained from the University of Texas Health, San Antonio, TX, and the University of California, Los Angeles, CA, respectively. The sample comprised an equal number of males and females in each age group across 7.00 to 13.99 years. Two trained and calibrated examiners scored all left side third molars in the maxillary and mandibular arches based on Demirjian's classification system (A to D). The mean Age at Assessment (AaA) for each stage of development along with the number, and standard deviation were calculated. Statistical significance was set at $p<0.05$ and independent sample *t*-test was used to compare the AaA for individual stages of development for each third molar for crown formation stages and separately for females and males.

Results: No difference was observed in the development of left maxillary and mandibular third molars between the population ($p>0.05$). Among Hispanic children in California and Texas, the closest match in the average AaA was observed in the left maxillary third molar in males and the corresponding AaA for Stage A was 9.72 years vs. 9.73 years, Stage B was 10.17 years vs. 10.55 years, Stage C was 11.48 years vs. 11.43 years, and Stage D was 13.20 years vs. 13.30 years ($p>0.05$). Similarly, the difference in the average AaA was not statistically significant in the other maxillary and mandibular molars and between the sexes ($p>0.05$).

Conclusion: Similarity in third molar development was observed between Hispanic children in Texas and California. Further research is required to assess the development pattern of root formation stages of third molars and across other teeth between these populations.

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Age Assessment; Dental Identification; Hispanic

H35   A 3D CBCT Method for Adult Age Estimation Based on Pulp Chamber Narrowing of Central Incisors

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Learning Overview: After attending this presentation, attendees will have a better understanding of the adult age assessment method based on the geometric approximation of dental volumes and its reliability in different age cohorts and adult populations.

Impact Statement: This presentation will impact the forensic science community by providing a reliable age assessment method for those cohorts of adult age for which other methods usually fail or are not applicable.

The deposition of secondary dentin throughout the life of an adult individual causes a progressive narrowing of the volume of the pulp. Previous studies demonstrated a significant correlation between secondary dentin deposition (pulp narrowing) and adult age; Pinchi et al. proposed a new, simple, and non-invasive method based on a geometric approximation of tooth volumes using Cone Beam Computed Tomography (CBCT) with good accuracy, especially for cohorts of age 30-59.[1,2]

Since recent literature highlights the need to validate experimental methods for adult age assessment and to produce reliable population-specific results for forensic purposes, this study aims to improve the accuracy of the Pinchi et al. method applied to a larger sample.[3] The secondary scope of this study is to analyze the influence of inter-operator variability, radiography technique, and other confounding factors on age estimation results.

A total of 500 CBCTs from healthy subjects aged from 18 to 90 years were collected and only the upper left central incisor was chosen to evaluate pulp/tooth volume, the most reliable tooth according to previous review.[4] Only sound teeth were included in the study. Two experienced forensic odontologists performed blinded measurements of dental volumes: the root, the pulp, and the crown were assimilated to elliptical-based solid cones, and the volume was calculated by taking nine measurements (three linear and six areas) on Digital Imaging and Communications in Medicine (DICOM) images using RadiAnt™ software. The ratio between the Pulp volume and the Hard tissues volume (PHr) was assumed as the significant variable for age estimation in order to reduce errors according to the following formula: $PHr=V_{pulp}/V_{ht}[V_{ht}=V_{tot}-V_{pulp}]$ (V_{pulp} is the volume of the pulp, V_{ht} is the volume of dental hard tissues, and V_{tot} the total volume of the tooth).

The analysis of the outliers was applied to identify the presence of any anomalous values/families of values. Four different methods (one-class SVM with non-linear kernel (RBF) - covariance estimator - local outlier factor - isolation forest) were used to verify the non-randomness of the results, imposing that the data identified as outliers were 5% of the total. Hierarchical cluster analysis techniques combined with the WARD algorithm for graph construction were then applied to verify the presence of any hidden clusters within the sample.

Preliminary results show high variability, especially in 30–60 year samples, but interesting different linear dynamics of the relationship between tooth volume and age cohorts (<30 years, between 30–60, and >60 years). These results are consistent with the biological trend of dental "aging" for the first two age cohorts (almost absent before 30–35 years, then significant in the 40-50-60 years) but extremely promising in the third age cohort (>60–65 years) in which the other age assessment methods become less reliable because of the plateau in which the aging processes slow down.

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Adults Age Estimation; Pulp Narrowing; CBCT

H36   A Dental Age Comparison of North Indian Children and Adolescents

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Learning Overview: This presentation will discuss and highlight the variations in the error rate in the estimated dental age of children and adolescents belonging to a North Indian population. This study indicates that there may be a need to formulate different methods or scores for children or adolescents for estimating age and establish the responsible factors as well as their effect on variations.

Impact Statement: Currently, the dental age for non-adults is primarily estimated using morphological methods based on calcification and the eruption pattern of teeth. However, the analysis conducted for children and adolescents separately showed clear differences in their error range. This presentation will impact the forensic science community through results that will focus on the need to identify the cause of these variations in these two non-adult age groups for formulation of more precise methods in future.

Age estimation is one of the crucial components of biological profiling and key requirement of some forensic investigations. Dental age assessments form an important class of estimation methods. Inherent properties of the teeth such as a storehouse of biological information, no-remodeling, and higher resistance to external influences make them an ideal element for forensic investigations.[1] Radiographical analysis of teeth is one such non-invasive and non-destructive technique that can be applied to the living as well as deceased individuals. In addition to identification of the dead, age is also estimated for living individuals in cases like child-pornography, frauds to avail age-related legal relaxations or benefits, etc. Estimating age in subadults is an easier task since the development stages follow a more-or-less definite pattern, unlike adults, where teeth undergo degenerative changes at a much slower rate than the formation stage and wide variations are depicted due to environmental, nutritional, and genetic factors.[2] Hence, dental age assessments are considered to be less precise in adults. Despite this, variations in the accuracy of the dental age assessments have been reported in subadults. These variations, often stated to be due to population factors, have not been much analyzed for children and adolescents, separately.

The present study is based on the evaluation and comparison of dental age variations, if any, between children and adolescents of a North Indian population. Digital panoramic radiographs of 166 participants, 80 and 88 of the children (aged 3–9 years) and adolescents (10–16 years), respectively, were collected from an oral health center. Following the Indian Council of Medical Research (ICMR) guidelines, these radiographs were collected with the written informed consent as well as assent form from the participants and their guardians. Ethical clearance from the Institutional Ethics Committee of the Government Medical College and Hospital (GMCH), Chandigarh, was duly taken. Collected images were analyzed using ImageJ® software. Dental age was estimated using Nolla's, Demirjian's and Willem's method, and variation in the accuracy rate for each dental age method was observed in both age groups. Mean Absolute Error (MAE) was less than one year in children but more than one year in adolescents for estimated dental ages using all three methods. A significant difference was found between the errors presented by the three estimated dental ages in children and adolescents. For all methods, more than 95% of the dental ages in children showed error up to ±2 years, whereas it was slightly more than 75% in cases of adolescents. Willem's method was found to be better suited for both children and adolescents. However, irrespective of the method used, variations were observed in the accuracy of the estimated ages in children and adolescents. The reason for decreased accuracy in adolescents is unclear and the role of puberty hormones as one of the factors is a plausible theory and needs to be ascertained through extensive research in other populations as well.[3]

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Age Estimation; Forensic Odontology; Adolescent

H37 Dental Assessment of Unaccompanied Refugees: A Mediterranean Perspective

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Learning Overview: The goal of this presentation is to compare the various methodological European age evaluation approaches to find the major adherence to both scientific and ethical issues.

Impact Statement: This presentation will impact the forensic science community by offering a reflection on a global phenomenon that could be uniformly regulated and a clear view on how different legal frameworks affect forensic analyses.

In recent years, migration has increasingly affected the Mediterranean, and the European Union (EU) has dealt with increasing numbers of unaccompanied refugees, mainly leaving from countries experiencing dictatorship, war, and abuses of human rights.[1] For 2023 alone, 109,688 arrivals have been registered.

The arrival of unaccompanied people is bound to become increasingly important because of climate change and conflict. Although it is a phenomenon of global concern, specific reception rules vary among countries.[3] Since minors are eligible for protection under the United Nations' Convention on the Rights of the Child, age assessment is widely used in Europe, and forensic medicine has gone through a rising demand to assess the age of adolescents and young adults.[4] Nevertheless, the assessment procedure among the various European countries is not uniform. This also reflects the absence of a protocol that is universally accepted by the scientific world. EU Member States use a combination of techniques to determine the age of a minor and to certify minor status, including interviews and documentation, physical examinations, sexual maturity assessment, psychological, sociological assessment, and radiological tests (carpal, dental, or collar bone X-rays).[5] There are many ethical repercussions to the methodology of age ascertainment, and safeguarding patient rights focuses importantly on informed consent.

The use of radiological examinations only for forensic and not clinical purposes leads to doubts as to whether to inform the person in case of a pathological finding. In addition, irradiating minors without parental or patient consent/assent violates the rights of the parents/patients to make an informed decision about their care.[6] Finally, utilizing X-rays raises the issue of risk for women who are or may be pregnant.

Starting from an Italian case history, the various ascertainment methodologies adopted in other European countries will be reviewed, with the aim of highlighting the pros and cons of the various evaluative approaches until arriving at a summary proposal that takes into account aspects of scientific validity and ethical relevance.

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Refugees; Age Assessment; Dental Identification

H38   The Application of ABFO Standards and Guidelines in Diagnosing Human Bite Injuries

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WITHDRAWN

H39   When the Biter Leaves a Tooth Behind

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Learning Overview: This presentation will discuss the collaboration between odontology, radiology, and forensic pathology along with the use of digital radiology and computerized tomography to locate and identify foreign objects during postmortem examinations. After attending this presentation, attendees will better understand the importance of the use of this technology and its value during forensic pathology investigations.

Impact Statement: This presentation will impact the forensic science community by displaying the importance of collaborations between forensic disciplines to aid in forensic pathology investigations.

Forensic pathology is the medical specialty where physicians examine the deceased to determine cause and manner of death. This is primarily conducted through the autopsy. Radiology can significantly enhance and complement the autopsy and odontology examinations through identifying and locating retained foreign objects that may have not otherwise been visualized during the autopsy.

We report a case where a military service member was recovered off the coast a day after supposedly drowning.[1] Witnesses reported that the individual was swimming, went under the water, and never resurfaced. Upon recovery, the individual's body demonstrated traumatic injury that was believed to be from animal predation. This individual's wounds involved extensive loss of soft tissue over multiple parts of the body, including the upper and lower back, left buttock, left scrotum, and the left lower limb. During radiological imaging of the body, the following findings were noted: fluid in sinus cavities of the skull and a retained foreign object within one of the areas of soft tissue loss on the lower extremity. The retained foreign object had a density similar to that of bone, but no fractures of the leg were noted. The autopsy confirmed the extensive tissue loss and fluid-filled sinuses. Dissection of the soft tissue of the lower leg allowed for recovery of the retained foreign object. Upon further analysis and examination, the foreign object was confirmed to be a shark tooth. The identification and location of the retained foreign object and subsequent dissection with recovery allowed for a greater understanding of the events prior to death. The confirmation of the shark tooth prevented an inaccurate assumption of small animal predation. It is unknown if the initial witnessed event was related to an attack. Advisories were made regarding the local swimming conditions and the presence of sharks, potentially saving others from injury or death.

Radiology continues to be an invaluable complement to the autopsy. The two modalities of radiology and forensic pathology proved invaluable in providing closure to the victim's family during this unfortunate accident.

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Radiology; 3D Technology; Autopsy

I1   Fatal Pesticide Ingestion Involving Boric Acid: A Case Report and Review of the Literature

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Learning Overview: After attending this presentation, attendees will better understand the toxic effects of boron-containing compounds, including boric acid.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of recognizing the toxic nature of boron-containing compounds as well as the signs and symptoms related to acute toxicity, particularly as they impact the determination of cause and manner of death.

Boron is a non-metallic element that is thought to play a role in biologic functions such as bone formation, immunity, and neurologic function. Boron exists endogenously in the human body and its common form is the borate anion. Boric acid is a boron-containing compound that has been used in a wide variety of applications, including medications, household products, and pesticides. It is readily absorbed from the gastrointestinal tract, mucous membranes, and abraded skin. Boric acid toxicity is associated with adverse effects on the gastrointestinal, central nervous, cutaneous, and renal systems, including hypotension, metabolic acidosis, and seizures. High doses of boron can be fatal.

Here we report a case of a biologic male in the fourth decade of life who died following the ingestion of boron in the form of a boric acid-containing roach killer. The decedent was found in a shaded outdoor area, near a motor vehicle registered to his name. Initial investigation showed no evidence of trauma or acute drug intoxication. Autopsy examination showed no lethal trauma and no significant natural disease. Initial toxicologic analysis of peripheral blood revealed cannabinoids and a non-lethal level of diphenhydramine. Subsequent requested follow-up investigation revealed that the motor vehicle contained a bottle of roach killer with an active ingredient of boric acid. Directed testing of peripheral blood for boron revealed an elevated level (42mcg/mL; reference range 0.025 to 0.036mcg/mL). Cause of death was attributed to boron toxicity.

A literature search was performed on the PubMed® database, using combinations of search terms including boron, boric acid, toxicity, poison, ingestion, fatal, and death. A review of the results is discussed. In the available literature, 13 adult (18 years of age or older) cases of boron exposure resulting in death were identified. Six of the fatal cases reported blood levels of boron (atomic weight 10.81 g/mol) or boric acid (atomic weight 61.8 g/mol). The average boron concentration in fatal cases of exposure was 210mcg/mL (range 6.6mcg/mL to 619mcg/mL). This case highlights the importance of careful scene investigation. Failure of the forensic pathologist to recognize the toxic nature of boron-containing compounds may have significant implications for determining cause and manner of death.

Pesticide; Toxicity; Scene Investigation

I2   **A Study of Corpus Callosum Expression Trajectories of Human Oligodendrocyte Progenitor Cells (OPCs) in Traumatic Head Injury Cases for Estimation of Agonal Period**

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WITHDRAWN

I3 A Sudden Unexpected Death From a Rupture of the Pulmonary Artery in Patients With Systemic Autoimmune Diseases

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Learning Overview: After attending this presentation, attendees will be aware that although heart wall and aorta ruptures are more frequent, a pulmonary artery rupture can also lead to hemopericardium and consequently cardiac tamponade. This cause of death can be related to systemic autoimmune diseases, and they may result both in an increased strain of the pulmonary circulation and in the endothelial dysfunction, which are typically linked to vascular lesions.

Impact Statement: As few cases were accounted for in the literature, collecting a case history of similar deaths would be desirable and would allow the forensic science community to assess the actual impact of the pathologic causes contributing in pulmonary artery rupture resulting in hemopericardium and consequently cardiac tamponade.

Spontaneous (non-traumatic, non-infectious) rupture and/or dissection of the main pulmonary trunk or secondary pulmonary arteries is a very uncommon event, and it has rarely been reported to cause sudden death.[1] This condition is more common in females, with a male-to-female ratio of 1:1.2. Generally, there is a bimodal incidence with peaks in the third and sixth decades.[2,3]

Rupture of the pulmonary artery usually represents an unusual complication of pulmonary hypertension that may be associated with heart and chronic obstructive pulmonary diseases.[4] It is manifested as sudden death or cardiogenic shock, and it is usually diagnosed postmortem.[5]

We report the case of a 71-year-old-woman with a relevant medical history: lupus, hypertension, moderate aortic, mitralic and tricuspid insufficiency, rheumatoid arthritis, dermatomyositis, thyroid disease, celiac disease, and connective tissue disease. She was found dead at home, so an autopsy was performed 48 hours postmortem to ascertain the cause of death. The gross external examination was unremarkable. About 375cc of partially coagulated blood and a clot of 20g were extracted from the pericardial sac (hemopericardium). The heart weight was 350g. The right coronary artery was patented with hemorrhagic infarction of the neighboring tissues up to the region relating to the pulmonary artery. The descending branch of the left coronary artery showed a concentric plaque, which occluded the vessel for approximately 30-40%. The proximal segment of the main pulmonary trunk appeared hemorrhagic with a full-thickness tear of the vessel wall (about 0.4cm, above the level of the valve cusps); furthermore, a second full-thickness punctiform tear was detectable next to the first one. Despite these lesions, the pulmonary artery didn't show any aneurism or false lumen; on the contrary, the entire aortic tract was dissected, but undamaged.

Systemic autoimmune diseases could be considered a contributing factor to the rupture of the pulmonary artery; they may increase the fragility of the vascular system by their negative effect on collagen formation and connective tissue strength, therefore making vessel rupture more likely.[6,7] This pathogenetic mechanism also led to aortic and mitralic valves insufficiency, resulting in unperforming left heart chambers, which caused right chambers overload.[8] A plausible theory of pulmonary artery rupture can be related to both the increased strain of the pulmonary circulation and the endothelial dysfunction linked to systemic autoimmune diseases, as confirmed by the histopathological evaluation we made to corroborate our hypothesis. As few cases were accounted for the Literature, collecting a case history of similar deaths would be desirable and would allow the scientific community to assess the actual impact of the pathologic causes contributing in pulmonary artery rupture resulting into hemopericardium and consequently cardiac tamponade.

In conclusion, the fact that the diagnosis is often made during the autopsy highlights the fundamental role of the forensic pathologist in the definition and pathological characterization of this disease.

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Sudden Death; Pulmonary Artery Rupture; Autoimmune Diseases

I4 An 11-Year Review of Fall Deaths Due to Head Trauma Reported in Shelby County, TN

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Learning Overview: This presentation will utilize forensic death investigative information and decedent medical records to determine the demographics of those most commonly affected by fall deaths due to head trauma. This information can then be of utility in public health initiatives that educate senior adults and their families about preventative measures that can be taken to promote fall safety and awareness. This presentation will also bring to light the importance of pharmacologic and non-pharmacologic management of conditions that affect the cardiovascular system in an effort to aid in fall death prevention.

Impact Statement: This presentation will impact the forensic science community by shedding light on fatal falls with resultant head trauma and intracranial hemorrhage in the elderly by taking a closer look at significant risk factors and suggesting preventative measures that can be taken to prevent fall deaths.

Falls were the number one cause of visits to United States emergency departments due to unintentional injuries from 2017–2018 and were responsible for almost 41,000 deaths in people aged 45 and older in the United States in 2020.[1,2] Overall medical spending for fatal falls amounted to $754 million in 2015.[3] While ending fall fatalities in the elderly population would be a lofty goal, using available decedent information such as demographics and medical records to determine risk factors associated with these fall fatalities can prove to be beneficial. Among fatal falls, head injury is the most common cause of death, with the second being complications of hip fracture.[4] This project attempts to identify risk factors from demographics to medical history that place an individual at risk for intracranial hemorrhage when falling and striking their head.

In this study, 342 fall death cases, the majority of which were same level falls, with evidence of head trauma and intracranial hemorrhage were reviewed. Of these, 322 (94%) were found to have subdural hematomas, 16 (5%) had subarachnoid hemorrhage, 3 (1%) had intracerebral hemorrhage not further specified, and 1 case (< 1%) had extra-axial hemorrhage not further specified. It was found that falls resulting in head trauma/intracranial hemorrhage and ultimately death were most commonly reported among White males, accounting for 130 (38%) of the cases reviewed. The majority of the falls, 243 (71%), occurred in the decedent's own residence, while 63 (18%) occurred in a medical facility such as an inpatient hospital, emergency department, assisted living facility, or doctor's office. The remainder of the falls, 33 cases (10%), occurred in another location (i.e., work, a family member's residence), and 3 (1%) were unable to be determined. A documented history of hypertension was determined to be the most common risk factor, reported in 255 (75%) of cases reviewed. In addition, prescription of an antiplatelet or anticoagulant regimen was found among 191 (56%) cases, ranking as the second most-common risk factor. Ninety-eight (29%) of the cases reviewed also had a documented history of falls prior to the fatal (most recent) fall. These may or may not have resulted in prior head trauma.

Falls pose an enormous risk to the elderly population not only due to their physical and emotional repercussions, but also due to their vast economic burden. By examining decedent medical records for significant risk factors, forensic pathologists can gain a greater understanding of what the significant risk factors are for suffering head trauma and intracranial hemorrhage due to falling. Going forward, the information gleaned can be used as grounds for educational initiatives by highlighting the importance of fall education, especially in individuals with hypertension and/or those prescribed an anticoagulation/antiplatelet regimen. Providing access to mobility devices and implementing safety modifications to the residence may also help mitigate the risk of fall deaths.

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Head Trauma; Risk Assessment; Vascular

I5   Cause and Manner of Death in New Mexico's Unhoused Population, 2014–2018

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Learning Overview: This presentation will use cause and manner of death data from the New Mexico Office of the Medical Investigator as well as the New Mexico Department of Health to investigate the ways by which unhoused people die in New Mexico and to compare some of the more common causes/manners of death to the general New Mexico population. After attending this presentation, attendees will be able to explain the most common causes of death among the unhoused population and will have an understanding of how the vulnerable status of the unhoused population puts them at higher risk for accidental overdoses, environmental exposure, motor vehicle collisions, suicides, and homicides as compared to the general population. Attendees will also learn about difficulties that medical examiners offices face in determining housing status postmortem.

Impact Statement: As the homelessness crisis in New Mexico continues to grow, a more detailed understanding of why this subgroup dies at higher rates than the general population may guide public health initiatives in addressing health disparities among this vulnerable group. This presentation will impact the forensic science community by providing a framework for understanding how rates of certain causes and manners of death differ between the housed and unhoused population and will emphasize the importance of determining housing status postmortem.

People Experiencing Homelessness (PEH) have a higher mortality rate than the general population, and public health research focuses on explanations for this disparity.[1-4] This population experiences an increased prevalence of chronic medical conditions, psychiatric conditions, and substance use.[5-9] Due in part to high levels of substance use and decreased access to health care, PEH have an increased risk for certain infections such as HIV, Hepatitis C, and other infections associated with impaired immunity.[10] Consistent inability to access shelter puts PEH at risk for exposure to extremes of temperature and leaves this population vulnerable to experiencing violence, including sexual and physical assault.[11-14] While any of these risk factors can contribute, it has also been shown that homelessness itself is an independent risk factor for mortality.[15] Multiple, international studies have examined trends in cause of death among PEH, citing heart disease, cancer, and psychoactive substance use as common causes.[16-19]

To investigate mortality trends of PEH in New Mexico, cases in which the decedent was suspected or confirmed to be unhoused in the database of the New Mexico Office of the Medical Investigator (OMI) during the years 2014–2018 were identified and reviewed. Cause and manner of death for the general New Mexico population from the same time period was obtained from reports generated by the New Mexico Department of Health (DOH) and compared to the population of interest using two-tailed chi-squared analysis with a significance level of 0.05 or less.

A total of 367 cases were identified during the years of 2014–2018. This is likely an undercount as determining a decedent's housing status can be challenging, especially in cases where decedents are unidentified and/or decomposed. In the study population, 79.8% of decedents were male and 20.2% were female, with the average age of the group being 49.84 years; 75.5% of decedents were White, 19.8% Native American, 2.7% Black, and 2.0% race unknown; 24.4% of decedents were Hispanic. The most common manner of death was unintentional injury (accidents accounted for 44% of deaths), which included overdoses (25.61% of total deaths) and motor vehicle collisions (6.52%); 40% of deaths were deemed to be natural in manner, with major cardiovascular disease and chronic liver disease/cirrhosis comprising the majority of natural deaths.

The unhoused population was more likely to die from accidental overdoses (25.61% versus 2.65%, p<0.0001). Additionally, this population was more likely to die via suicide (6.52% versus 2.66%, p<0.0001) and was more likely to be a victim of homicide (4.89% versus 0.94%, p<0.0001). This population was also more likely to die via a motor vehicle collision (6.52% versus 2.09%, p<0.0001) or via environmental exposure (5.71% versus 0.67%, p<0.0001). The population was less likely than the general population to die from certain natural diseases such as cardiovascular disease, diabetes mellitus, chronic obstructive pulmonary disease, and carcinoma.

The unhoused population in New Mexico is at increased risk compared to the general population from multiple causes/manners of death, highlighting their vulnerable status in society. Continuing to identify these vulnerabilities may prove useful in policy development to address homelessness on a state, as well as national, level. A significant limitation to this research is determining housing status postmortem, with multiple factors playing a role in this difficult classification.[21] Refining the means by which medical examiners offices determine housing status will allow for a better understanding of this population.

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Cause of Death; Homelessness; Population

I6 Under Pandemic Pressure: A Four-Year Review of Suicidal Deaths in Shelby County, Tennessee, With Emphasis on the Effect of COVID-19

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Learning Overview: By examining the impact of the COVID-19 pandemic on suicide rates in Shelby County, TN, attendees will gain insights into the complex relationship between crises and mental health outcomes. Attendees will develop an understanding of the potential influence of social isolation, fear, and financial instability on suicide rates during a pandemic.

Impact Statement: By analyzing suicide cases during the COVID-19 pandemic and investigating the influence of various risk factors such as depression, domestic disputes, and COVID-related factors, this research provides insights into the complex dynamics surrounding suicide. This presentation will impact the forensic science community by highlighting the need for ongoing mental health support for individuals and encourages the forensic community to incorporate a holistic perspective when assessing suicidal death.

Suicide is a complex and pressing public health concern that continues to pose significant challenges in the United States, affecting families across various socioeconomic backgrounds, and cultures. The COVID-19 pandemic introduced many disruptions to society, including having an impact on homicide and overdose rates, widespread social isolation, economic unrest, and psychological distress.[1] The West Tennessee Regional Forensic Center is located in Memphis, TN, the largest city in Shelby County. Tennessee's suicide rate at the height of the pandemic was 17.5 per 100,000 population, which was 25% higher than the national rate.[2] Preceding the COVID-19 pandemic, Shelby County suicide deaths increased annually between the years of 2013-2018.[2-3] The objective of this retrospective study is to investigate the impact of the COVID-19 pandemic on suicidal deaths within Shelby County, TN.

Data was obtained from the Death Investigation and Decedent Information (DIDI) database by reviewing all deaths where the manner was classified as suicide reported between March 8, 2018, and March 9, 2022. The circumstances of each death were extensively reviewed to include death investigator notes, autopsy reports, police reports, and photographs in order to evaluate possible risk factors, associations, or inciting events. The dataset comprised a total of 488 cases where the manner of death was classified as suicide, with 81% (396 cases) of cases meeting the study criteria.

Suicidal deaths in Shelby County during the COVID-19 pandemic decreased 9.4% between the pre-pandemic and the COVID-19 pandemic periods. Cause of death reported between the pre/post-pandemic time frames demonstrated that self-inflicted gunshot wounds remain the primary method of suicide within Shelby County. A review of the dataset showed depression (24%) and domestic issues (19%) were reported precluding suicidal deaths. COVID-19 as a potential association was identified in 13% of cases (31 cases), and 35% of these cases (11 cases) were directly associated with COVID-19 in which the decedent was infected with COVID-19, exposed to COVID-19, or related to the reported fear of COVID-19. Notably, unemployment and financial instability were identified as prevalent factors in deaths associated with COVID-19.

Contrary to initial expectations, our findings did not reveal a significant increase in suicidal deaths during the COVID-19 pandemic when compared to the pre-pandemic period. A subset of deaths was associated with COVID-19, whether directly or indirectly, which demonstrates the impact the pandemic had on suicidal deaths. Determination of a preceding event or association with suicidal deaths is subjective and the authors acknowledge this fact, which is certainly a limitation to the study. A further limitation is the subset of cases with no clear direct or indirect preceding event or circumstances. We aim to further examine the dataset to determine any association with COVID-19 variant-related spikes.

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Suicide; COVID-19; Mental Health

I7   A Case of Death Resulting From an Unrecognized Wilms Tumor in a 4-Year-Old Child

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Learning Overview: This presentation presents a case of alleged diagnostic delay of a Wilms tumor that led to the death of a 4½-year-old child. The goal of this presentation is to draw attention to the importance of a comprehensive autopsy examination, coupled with a careful analysis of medical documentation, in determining the existence and different potential degrees of professional responsibility in cases of death.

Impact Statement: This presentation will impact the forensic science community by highlighting the entirety of the forensic pathologist's duties in analyzing a death likely related to professional responsibility. It serves as an example of how a careful analysis of medical documentation, in conjunction with autopsy and other postmortem examinations, is crucial in defining potential profiles of professional liability.

Wilms tumor, or nephroblastoma, is the most common primary tumor in childhood, accounting for over 90% of primary renal tumors in patients under the age of 20 and 5% of all childhood tumors.[1] Histology is a crucial factor in the prognostic definition of this pathology. In fact, 5-10% of nephroblastomas exhibit anaplastic histology, characterized by the presence of atypical and polypoid mitotic figures, enlarged nuclear size, and hyperchromasia. Tumors with anaplastic histology, also known as unfavorable, have significantly lower survival rates compared to non-anaplastic forms.[2,3]

In this study, the authors present a case of the death of a 4½-year -old child affected by stage IV Wilms tumor where postmortem medicolegal analysis played a crucial role in recognizing a potential diagnostic delay of the neoplasm.

According to the mother, the child had been experiencing fever, loss of appetite, and abdominal pain for about eight months until the situation worsened with the presentation of a painful, tense, and swollen abdomen. Due to this condition, the patient was sent to the emergency room. An abdominal ultrasound was performed, revealing a mass measuring approximately 100mm in diameter in the right hypochondrium, infiltrating the liver and compressing the right kidney. Consequently, the patient was immediately admitted to the hospital. During the hospitalization, a biopsy of the abdominal mass was performed, and several cycles of chemotherapy were administered. However, surgical intervention was not possible due to the extent of the neoplastic disease. The biopsy revealed the following immunophenotypic profile: Beta-catenin +, EMA +, Vimentin +, WT1 +, Inhibin +, Wilms Tumor +, leading to a diagnosis of poorly differentiated nephroblastoma.

The child passed away after just over a month of hospitalization due to the development of a systemic septic condition on top of the pre-existing terminal state. The autopsy revealed a voluminous neoplastic mass with reduced consistency, lacunar appearance, and necrotic-hemorrhagic characteristics, protruding into the upper right renal pole and invading the right lobe of the liver, causing complete disruption of the parenchyma of the affected organs. Metastases were also observed on the diaphragm, serosal surfaces of the stomach and intestines, as well as in the lungs. Postmortem anatomopathological examinations, although challenging to interpret due to advanced postmortem changes and previous cycles of chemotherapy, confirmed the diagnosis of nephroblastoma. The cause of death was attributed to acute multi-organ failure in a patient with poorly differentiated and metastatic Wilms tumor (stage IV) with terminal septic shock.

In this case, all postmortem examinations conducted, including a careful analysis of the medical documentation, allowed for differentiation of the levels of professional responsibility among the physicians who treated the child in relation to a presumed diagnostic delay of the neoplastic disease that led to the child's death.

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Wilms Tumor; Diagnostic Delay; Professional Responsibility

I8 Considerations of Decedent General Health Parameters During Determination of Cause of Death in Persons Abusing Substances

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Learning Overview: After attending this presentation, attendees will have learned more about the necessary role of autopsy in drug-related death investigation. This study examined the incidence of severe underlying disease and the demographics of those who died drug-related deaths. We offer that natural disease should be considered in drug deaths and that a complete postmortem examination is essential.

Impact Statement: This presentation will impact the forensic science community by highlighting the necessity of autopsy in drug-related death investigation in order to account for contributing and confounding factors.

In 2019, the Centers for Disease Control and Prevention (CDC) provided modern guidance to medicolegal death professionals about investigating suspected drug-related deaths. Some of that guidance was explicit. However, the interpretation of toxicologic findings was largely mandated to the professional opinion of the certifier.[1] Despite the authority offered by this guidance, medicolegal professionals know that there is considerable uncertainty in the interpretation of the blood concentrations and presumed effects of mixed substances detected at autopsy.[2] In the context of that uncertainty, other possible causes of and contributors to death need to be considered. Such consideration may not be possible through limited examination (toxicology only).[3]

The goal of this study was to investigate how often persons who died while abusing drugs also had other significant medical disease of the severity to have been an alternate explanation for sudden unexpected death. From a series of 1,162 complete autopsies, we considered 462 cases in which acute drug toxicity was a significant component in the cause of death. Not considered were deaths by suicide or homicide or in which traumatic injuries were also significant contributors to death. The cases were evaluated for known history and/or gross and microscopic indicators of significant pre-existing cardiac disease and/or significant acute pneumonia. Of the 462 cases, 164 had significant heart disease, 31 had acute pneumonia, and 13 had both; 45% of the decedents had significant natural disease sufficient to have caused death. The average age of the decedents in this group was 41 years. The average age of decedents without significant disease was 37 years, and the average age of decedents with significant disease was 46 years. The means were significantly different (p<0.001), but the age ranges of the groups overlapped. The large majority of persons in this study died after the abuse of opioids and other mixed substances. We did not find significant differences between average estimated morphine equivalents in the two groups.

Based on the results of this study, it seems clear that natural disease must also be considered in the interpretation of drug-related deaths. The alternative would be to arbitrarily exclude information that may differentiate persons who die accidentally during drug abuse from chronic abusers who die in the context of overwhelming natural disease. Investigation of all drug-related deaths should include complete postmortem examination.

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Autopsy; Drug Abuse; Cardiac Death

I9   Fat Embolism Syndrome in a Patient With Osteogenesis Imperfecta: A Textbook Case

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Learning Overview: The goal of this presentation is to report the case of a 33-year-old man with osteogenesis imperfecta who died a few days after hospital discharge. The present study will describe the full forensic assessment of Fat Embolism Syndrome (FES). This presentation shows the clinical and postmortem diagnostic workups of fat embolism syndrome and points out the hypothetical link between osteogenesis imperfecta and FES.

Impact Statement: FES diagnosis requires a complex workup and can be challenging for pathologists. This presentation will impact the forensic science community by presenting a brief review of FES diagnostic criteria showing the postmortem diagnostic workup and discussing the hypothetical link between osteogenesis imperfecta and FES.

This presentation will report the case of a 33-year-old man with osteogenesis imperfecta who died a few days after hospital discharge. The present study will describe the full forensic assessment of fat embolism syndrome. The presentation shows the clinical and postmortem diagnostic workups of fat embolism syndrome. The authors also pointed out the hypothetical link between osteogenesis imperfecta and fat embolism syndrome.

Since it was first described by Zenker in 1863, fat embolism syndrome (FES) has posed a serious challenge because of the wide range of possible clinical presentations.[1] FES comprises respiratory, neurological, cutaneous, and hematologic manifestations related to intravascular embolization of fat arising from within the fractured bone marrow space. Symptoms usually appear several days after a major trauma involving the pelvis or lower extremities and may rapidly lead to death. Many authors have proposed different diagnostic criteria for FES, owing to its unpredictable clinical presentation.[2] Many cases of FES are misdiagnosed, and postmortem examination should provide sufficient means for appropriate diagnosis.[3]

This is the case of a 33-year-old man who died of FES one week after a car accident. He suffered multiple fractures but was hemodynamically stable and showed no neurological changes. The patient died a few days after hospital discharge. Additionally, he had Osteogenesis Imperfecta type III, a genetic disorder associated with bone fragility. To the best of our knowledge, no direct association was observed between osteogenesis imperfecta and FES. However, due to the frequency of fractures suffered by osteogenesis imperfecta patients, the possible association with FES should deserve further evaluation. FES diagnosis requires a complex workup and can be challenging for pathologists. Due to its heterogeneous manifestations, there are many different clinical and histological diagnostic criteria of FES; each one includes a deep case history assessment and specific histological examinations. Hence, the clinical suspect should drive the postmortem assessment.

We present a brief review of FES diagnostic criteria, show the postmortem diagnostic workup, and discuss the hypothetical link between osteogenesis imperfecta and FES.

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Forensic Science; Medical Liability; Cause of Death

I10   Ligature Strangulation Utilizing Cable Ties: An Unusual Means of Suicide in Italy

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Learning Overview: The goal of this presentation is to present an unusual case of a rare type of suicide: ligature self-strangulation through the use of cable ties. This presentation is intended to draw the attendees' attention to examine the possible dynamics leading to the event according to a hypothesis of homicide or suicide.

Impact Statement: By drawing attention to a particularly infrequent topic, this presentation will impact the forensic science community as it is a cue to start collaborations between various forensic pathology centers, also at international level, in order to find codified scientific methods to rule out homicide.

The data given by Italy's National Institute of Statistics (ISTAT) for the period 2015–2020 suggests that the number of suicides among people ages of 35–64 is completed by hanging and other forms of asphyxia is 6301.[1] Strangulation is a type of mechanical asphyxia produced by constriction of the neck using ligature material, without suspending the body.[2-6] Cases of suicidal self-strangulation are uncommon; there are currently no cases reported in Italian scientific literature that occurred through the use of cable ties.

We describe a case of a 61-year-old man who was found lying in a supine position on the floor of his electrician shed. The decedent had two rows of white plastic lock-cable ties, four per row, completely encircling his neck. During the autopsy, removal of the ligatures revealed two horizontal 0.5cm-wide furrows completely surrounding the neck. There were signs of asphyxia, such as facial congestion, subconjunctival hemorrhage, and bilateral subpleural petechiae. There was hemorrhagic infiltration of the soft tissues of the neck without any injuries of the hyoid bone.

In the left parieto-temporal region was a depressed 6x1cm-wide excoriation and in the right fronto-parietal area was observed a 2.5cm-wide laceration with clear margins. Below, there was a superficial hemorrhagic infiltration, even though the cranial theca was intact and the brain had no injuries. Toxicological screening tests revealed no drugs assumption. The cause of death was certified as violent mechanical asphyxia due to strangulation. Given the circumstantial evidence, the deceased allegedly manifested suicidal ideation, which is a symptom of depression, even though he hadn't received a diagnosis. The investigation of the scene showed no signs of forced entry, nor upon autopsy, any signs of a struggle. The presence of a double row of cable ties also pointed toward a self-inflicted death.

However, signs of vitality of the wound on the head suggested examining the possible dynamics leading to the event. In the hypothesis of homicide, first the victim could have been hit in the head, then strangled. Considering the suicide hypothesis, the wound could be consequential to a fall on an angled surface that occurred shortly before death, even as a result of an unconservative act. Therefore, it was necessary to reanalyze the scene to confirm the compatibility of the vital wound with the presence of sharp profiles in the electrician shed. Searching for DNA samples on cable ties could be misleading, since these are items continuously manipulated, so foreign DNA must always be expected. Searching for fingerprints on the cable ties could not have been successful either, because of the surface of the cable ties themselves.

In conclusion, in cases of strangulation using uncommon means, it is advisable to supplement the circumstantial evidence with data obtained from on-site investigation, autopsy, and toxicological examinations to rule out the hypothesis of homicide, as was possible in this case.

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Self-Strangulation; Cable Ties; Uncommon Suicide Method

I11   Chasing Euphoria: When Butane Inhalation Leads to Death

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Learning Overview: Volatile substance abuse is widespread among adolescents due to its easy availability and methods of consumption. Inhalant abuse represents a cause of significant morbidity and mortality due to direct toxicity on several target organs and gas displacement, resulting in a lack of oxygen. As a result of its relatively unknown use, butane is not often considered when a toxicological analysis is performed. Therefore, cases of acute butane intoxication are probably underestimated because it is not part of the routine toxicological screening. The goal of this presentation is to depict a case of butane abuse in a convict in an Italian prison in which toxicological investigations proved to be essential to determine the cause of death.

Impact Statement: When death by butane inhalation is hypothesized, autopsy and histological findings may be non-specific. Therefore, toxicological investigations assume a crucial role in solving the case. This presentation will impact the forensic science community by describing the importance of collaboration among different forensic departments and, even more, the importance of analytical and precise methods to collect biological samples to reduce significant post-mortal alterations.

This presentation shows the case of a 41-year-old man who was found unresponsive on the floor of a bathroom cell of an Italian prison near a gas camping stove. Prompt resuscitation techniques were performed by medical personnel of the penitentiary while emergency services were called to the scene. Despite their efforts, the man was declared dead after an episode of ventricular fibrillation. It was later found that he had a history of substance abuse, for which he was treated with methadone, and previous hospitalizations due to an overdose of benzodiazepines and inhalants.

The external examination of the corpse revealed a minimal lesion consisting of minor ecchymotic bruising and a superficial laceration located on the left eyebrow. The autopsy only revealed imponent polivisceral congestion and increased blood thinness. Given the circumstantial data and history of substance abuse, upon internal examination, a sample of bronchi air was collected using a gastight vial. Furthermore, several different matrix samples, comprehensive of blood, vitreous humor, urine, fat tissue, lung, brain, kidney, and liver, were collected and stored at a temperature between -20°C and -30°C. Histopathologic analyses of the cardiac tissue revealed the presence of interstitial and perivascular fibrosis, necrosis, and congestion. Moreover, the hepatic and lung tissues showed significant congestion.

Toxicological analyses of the samples collected during the autopsy and analyzed with Gas Chromatography/Mass Spectrometry (GC-/MS) showed butane levels compatible with acute butane intoxication. Consequently, the cause of death was determined to be acute butane intoxication, which led to a dual mechanism: on the one hand, anoxia due to the depletion of oxygen; on the other hand, the cardiac arrhythmia as a result of massive catecholamine release and sensibilization.

Butane is an odorless, colorless gas commonly used as a solvent and domestic fuel. In fact, it is used as liquefied gas in cigarette lighters, camping stoves, gasoline, and fuel gas.[1] Butane abuse is becoming more and more common, especially among adolescents, due to its wide availability and its low cost.[2] Nonetheless, butane intoxication is often underestimated and undetected on routine toxicological screenings.

This work demonstrates that when butane intoxication is suspected, in order to reach an accurate cause of death, the investigation must include a thorough crime scene analysis, autopsy, and specific and rigorous toxicological analysis. This ever-evolving phenomenon gives rise to the need for stricter regulation of the sale of this substance because the use of butane as a recreational substance of abuse is becoming widespread, especially among the young.

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Butane; Inhalant Abuse; Toxicological Analyses

I12   Acute or Chronic Myocarditis? Or Is It Budd-Chiari Syndrome? A Peculiar Case of Multi-Organ Failure in a Subject With SARS-CoV2 Infection

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Learning Overview: Since the year 2020, COVID-19 has claimed millions of lives all over the world. Even though the virus is a well-known cause of acute myocarditis, the development of acute myocarditis on a previous layer of chronic myocarditis has been described in only a few cases. The goal of this presentation is to present a case of death caused by a rapid multiorgan failure in a COVID-positive patient affected by acute myocarditis arising from a previously unidentified condition of chronic myocardial inflammation and Budd-Chiari Syndrome.

Impact Statement: This presentation will impact the forensic science community by showing how the systemic inflammatory response to Sars-Cov-2 can overwhelm already-damaged organs while exacerbating previously undiagnosed clinical conditions, thus leading to a fatal and rapid multiorgan failure. Moreover, it is essential to emphasize the importance of histological examinations in subjects who died from COVID-19, with the objective of describing all the complications of this disease and trying to prevent them.

Sars-Cov-2 has been responsible for the deaths of approximately seven million people worldwide since 2020.[1] From the first phase of the pandemic, it was widely known the role the virus had in stimulating autoimmunity processes and inflammation in the respiratory and cardiovascular systems. Cardiomyopathy is a disease concurrently developed during COVID-19, though pathogenesis and the cause-effect relationship between viral infection and myocarditis are challenging to demonstrate.[2] On the other hand, Budd-Chiari Syndrome is a relatively rare disorder (1/100000 born alive) characterized by the thrombotic or non-thrombotic obstruction of hepatic venous outflow, which can be diagnosed after massive ascitic episodes, fulminant hepatitis, and unexplained chronic liver disease.[3,4] Some authors have also reported the possibility of diagnosing Budd-Chiari Syndrome during a COVID-19 infection and describe the development of the subsequent hepatic symptomatology as a negative predictive factor for the outcome of the primary viral disease [5].

This work presents the case of a 58-year-old man, positive for Sars-Cov-2 infection over the last two days, was admitted to the emergency department for asthenia and thoracic pain described as "stingy." His pathological remote anamnesis was negative in all its aspects, while the physical examination showed no signs of cardiac and pulmonary sufferance; the thoracic radiography was negative for acute pulmonary or pleuritic inflammatory processes. The following cardiologic and echocardiographic evaluation highlighted a smaller-than-average left ventricular chamber, with an ejection fraction <40% and an increased width of the ventricular wall. Laboratory exams reported acute renal and hepatic insufficiency. The following morning, the patient developed a sudden decrease in blood pressure, whose lowest detected value was 65/45mmHg; after two cycles of cardio-pulmonary resuscitation maneuvers that lasted more than 50 minutes, the doctors declared the man's death.

The autopsy didn't show relevant macroscopic alterations of the heart, liver, and kidneys; furthermore, no signs of large vessel thrombosis were detected. On the contrary, histopathologic analysis of the cardiac tissue revealed the presence of diffuse myocytolysis, intracytoplasmic lipofuscin deposits, and lymphocyte infiltration. Furthermore, some areas revealed the presence of fibroblasts, recent and old adipose tissue deposition, and sporadic lymphocytes. The hepatic tissue showed significant sinusoidal congestion, cellular necrosis, and diffuse and almost complete thrombosis of small hepatic veins. Finally, toxicological analyzes excluded a state of acute intoxication from substances of abuse. The cause of death was determined to be a multi-organ failure in a patient suffering from acute myocarditis on a substrate of previously undetected chronic myocarditis and Budd-Chiari Syndrome.

This work shows how in the case of SARS-CoV-2 infection and multiorgan failure, pathologies with a rare incidence such as Budd Chiari Syndrome or conditions with hyperacute development such as myocarditis should not be forgotten nor overlooked.

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Cardiac Death; COVID-19; Sudden Death

I13 Anosmia and COVID-19: The Search for the Virus in the Brain Through Immunohistochemical Investigations With the Anti-Sars-Cov-2 Antibody

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Learning Overview: The goal of this presentation is to support the search for the cause of anosmia in patients affected by the Sars-Cov-2 virus. Through immunohistochemical investigations, we have highlighted the presence of the virus in postmortem brain samples from areas involved in the olfactory system.

Impact Statement: This presentation will impact the forensic scientific community by showing the presence of the Sars-Cov-2 virus in the meninges and around the nerve cells of the olfactory system. Immunohistochemistry plays a central role in studying the mechanism of infection, now and in the future.

The Sars-Cov-2 pandemic has upset everyone's lives. Each person had to change their lifestyle habits to avoid viral infection. In these two years, thanks to the rapid progress of research, scientists have understood the viral pathogenetic mechanisms and have developed new vaccines. Sars-Cov-2 presents numerous symptoms, often completely non-specific, such as cough, fever, and dyspnea. Anosmia and ageusia are peculiar symptoms of this infection. Patients suffering from COVID-19 suddenly lose the sense of smell in 38.2% of cases and of taste in 36.6.[1] These senses are recovered spontaneously after two or three weeks, in some cases after several months. The pathogenetic mechanism underlying the loss of smell is not known. The virus could attack the nerve cells of the nervous and olfactory system, as happens in other organs through the ACE2-receptor. The olfactory dysfunctions induced by the coronavirus can be a direct effect of the virus on the function of the supporting cells, or it can cause metabolic dysfunctions of the olfactory system receptors, as the supporting cells have trophic functions.[1]

In an experiment with hamsters, the experimenters observed desquamation of the olfactory epithelium, infection of supporting cells (preferred to neurons), and intense immune response.[2]. Indeed, olfactory epithelium damage can be aggravated by an inflammatory response, leading to cell death. Loss of smell may be due to olfactory bulb inflammation triggered by virus infection. SARS-CoV-2 can infect the central nervous system through the synapses, using the olfactory nerve afferents to reach the olfactory bulb, raising the possibility of SARS-CoV-2 utilizing this infection path as well.[3]

We have selected 14 cases of deceased infected by the Sars-Cov-2 virus from the autopsy case histories of the Institute of Forensic Medicine of University of Foggia. The causes of death were not necessarily infectious. In each autopsy, the whole brain was taken and fixed in formalin; samples of each organ were also taken. Once fixed in formalin, the olfactory striae, the amygdala, the olfactory trine, and the thalamus have been identified and sampled in the brain. These areas are a part of the olfactory system: from the olfactory bulb originate the striae, which form the olfactory trine. The striae carry the olfactory nerve fibers toward the primary olfactory cortex and the amygdala. The amygdala and the thalamus are part of the limbic system; these areas play an important role in evoking memories and emotions related to smells. All samples were stained with H&E and anti-COVID19 antibodies and examined with an optical microscope. We found widespread positivity of anti-COVID-19 in brain samples, with perineuronal accumulations of the autoantibody. In the collection of olfactory striae, positivity is present at the level of the meninx.

These findings could support the hypothesis that the virus infects the olfactory nerve cells by entering through the olfactory nerve. These first encouraging results may guide research by evaluating whether the virus that has reached the brain induces an inflammatory response only hypothesized so far. The scientific community should investigate the presence of inflammation through further immunohistochemical investigations.

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COVID-19; Microscopy; Histological

I14 **Oxidative Stress Markers in the Human Brain and Placenta May Reveal the Timing of Hypoxic-Ischemic Injury: Evidence From an Immunohistochemical Study**

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Learning Overview: The goal of this presentation, from the point of view of forensic pathology, is to provide scientific evidence in cases of medical liability in the obstetric field to support the clinical data of the timing of Hypoxic Ischemic Encephalopathy (HIE).

Impact Statement: This presentation will impact the forensic science community by providing valuable insights into the role of Reactive Oxygen Species (ROS) as crucial signaling molecules during pregnancy, particularly concerning fetoplacental circulatory physiology. Moreover, this presentation highlights the association between oxidative stress and the pathogenesis and progression of HIE.

ROS are physiologically produced from cell metabolisms and play a dual role as both beneficial and toxic compounds. At physiological concentrations, ROS plays a role in defense against pathogens, cell signaling, and mitogenic responses. At higher concentrations (e.g., in case of either overload of ROS or depletion of natural antioxidants), ROS cause Oxidative Stress (OS), potentially leading to impaired cell function and decreased cell viability due to damage to proteins, DNA, and cellular lipids. During pregnancy, ROS serves as crucial signaling molecules for fetoplacental circulatory physiology. Oxidative stress was supposed to sustain the pathogenesis and progression of HIE.

A retrospective study was performed on the brains and placentas of fetuses and newborns, between 36–42 weeks of gestation (Group 1: Fetal intrauterine deaths; Group 2: Intrapartum deaths; Group 3: Post-partum deaths; Control group sudden neonatal death, all groups were further divided into two subgroups (Subgroup_B [brain] and Subgroup_P [placenta]) and it was conducted, through the immunohistochemical investigations of markers of oxidative stress (NOX2, 8-OHdG, NT, iNOS), IL-6, and only on brain samples, AQP4.

The results for the brain samples suggest that NOX2, 8OHdG, NT, iNOS, and IL-6 were statistically significantly expressed in cases compared with Controls. iNOS is more expressed in Fetal intrauterine death (Group 1) and a little less expressed in post-partum death (Group 3), while in Intra-partum death (Group 2) the immunoreactivity was very low. IL-6 showed in Fetal intrauterine death (Group 1) the highest expression in the brain cortex, while Intra-partum death (Group 2) and Post-partum death (Group 3) showed weak immunoreactivity. Post-partum death placentas (Group 3) showed the highest immunoreactivity to NOX2, which was almost double compared to Fetal intrauterine death (Group 1) and Intra-partum death (Group 2) placentas. Placental tissues of Fetal intrauterine death (Group 1) and Intra-partum death (Group 2) showed higher expression of iNOS than Post-partum death (Group 3), while the IL-6 expression was higher in Fetal intrauterine death (Group 1) than Post-partum death (Group 3). The AQP4 was discarded as a possible marker because the immunohistochemical reaction in the three Groups of cases and the Control Group was negative.

Forensic Science; Histological; Pregnancy

I15 Starvation-Induced Hepatocyte Changes: Light and Electron Microscopy Findings in Two Pediatric Homicide Cases

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Learning Overview: After attending this presentation, attendees will understand that starvation induces changes in hepatocytes that can be seen at the light and electron microscopic levels and that these changes are critical in the body's response to starvation.

Impact Statement: This presentation will impact the forensic science community by providing pathophysiological evidence for starvation-induced changes that can serve in the investigation of fatal pediatric starvation cases

Fatal starvation is a rare but important cause of death in cases of child abuse and neglect. A thorough scene investigation, autopsy, toxicology, metabolic screen, radiographic and microbiologic studies, vitreous chemistry, and light microscopic examination are essential in establishing the cause and manner of death. The microscopic findings in cases of pediatric starvation are sparse and non-specific, and they include hepatic microvesicular steatosis, brown fat transformation, thymic involution, thymic calcification of Hassall's corpuscles, and thymic fibrofatty replacement.[1,2] The pathophysiology of starvation is complex and has been proposed to involve autophagy, which is the process of degradation of cellular components through a regulated lysosome dependent mechanism.[3]

This presentation describes two cases of homicidal pediatric death caused by starvation utilizing electron microscopy to further characterize the light microscopic findings of the liver. The first case involved an 8-year-old male who had reportedly been feeling sick the prior two days. The autopsy revealed generalized muscle and adipose tissue atrophy, bilateral temporal muscle wasting, and a marked decrease in body weight (<3rd percentile for age and gender). On the internal exam, there was atrophy of the liver, kidneys, and spleen, and dilation of the heart. Light microscopic examination of the liver revealed round eosinophilic material in the cytoplasm of hepatocytes and in the extracellular space. Special stains, including PAS, Masson's trichrome, and reticulin, showed depletion of hepatocyte glycogen but no other abnormal findings. Electron microscopic examination of the liver showed the cytoplasmic vacuoles containing amorphous material and occasional fragments of degenerated membranes, very prominent lipofuscin inclusions, and abundant mitochondria that showed various degree of swelling and loss of mitochondrial cristae. Few profiles of rough endoplasmic reticulum cisterna showed various degrees of swelling. There were numerous small vesicles with components of smooth endoplasmic reticulum or Golgi apparatus and occasional peroxisomes. The ancillary tests, including metabolic screening, were non-contributary.

The second case involved a 2-year-old male who was found unresponsive at home in a crib. The autopsy revealed severe generalized muscle and adipose tissue atrophy, bilateral temporal muscle wasting, marked decrease in body weight (<3rd percentile for age and gender), gross evidence of dehydration, and multiple inflicted blunt force injuries in various stages of healing. On the internal exam, there was severe atrophy of all organs. There was also an old non-space occupying subdural hemorrhage and chronic subdural hygroma due to brain atrophy. Light microscopic examination of the lungs revealed pneumonia. The liver showed small cytoplasmic vacuoles with non-homogenous contents. Special stains with ubiquitin and PAS showed glycogen depletion in the hepatocytes. Electron microscopic examination of the liver showed hepatocytes with swollen giant mitochondria. There were a few stacks of rough endoplasmic reticulum and various vesicles representing the smooth endoplasmic reticulum. Approximately 10% of hepatocytes contained one or two cytoplasmic inclusions that were roughly round and appeared to be membranes bound. These vesicles contained amorphous/granular electron lucent material and rare dense partially crystallized precipitates.

The two cases reported here showed ultrastructural evidence of autophagy occurring in the liver in the setting of pediatric fatal starvation. This is the first report of swollen and giant mitochondria and electron microscopic findings consistent with autophagy in human cases of homicidal starvation. The ultrastructural findings in these two cases add to the current literature surrounding starvation and the mechanisms of cellular injury in response to starvation.

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Pediatric; Homicide; Microscopy

I16 Macrophage Activation Syndrome Following Administration of the ChAdOx1 nCov-19 Vaccine: A Case Study

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Learning Overview: The goal of this presentation is to highlight a potential correlation between the administration of the ChAdOx1 nCov-19 vaccine and an intense inflammatory response with Macrophage Activation Syndrome (MAS).

Impact Statement: This presentation will impact the forensic community by showing the findings of a COVID-19 vaccine-related death to increase the awareness of such correlation and the clinical management of such conditions.

COVID-19 and anti-SARS-CoV-2 vaccines are associated with intense activation of the inflammatory system, often referred to as the "cytokine storm."[1,2] Among the adverse reactions reported, there have been cases resembling MAS in severe COVID-19 cases and following vaccination.[1-4] This report presents a case of MAS found during a judicial autopsy of a 63-year-old subject with a history of arterial hypertension, who experienced symptoms after receiving the ChAdOx1 nCov-19 Vaccine (first dose). In this presentation, we present a case study involving a 63-year-old subject with a positive history of arterial hypertension, who was seemingly in good health. Shortly after receiving the ChAdOx1 nCov-19 vaccine (first dose), the patient developed symptoms, including fever, asthenia, and an episode of fainting. He sought medical attention at the emergency department due to diffuse abdominal pain and presented with elevated levels of inflammatory markers (D-Dimer, troponin, CRP), intense monocytosis, erythrocytosis, and thrombocytopenia. Despite undergoing multiple clinical tests, the exact cause of the symptoms remained uncertain.

Approximately 15 hours after admission, the patient experienced distributive shock, necessitating maximal support therapy. Despite efforts, the patient's condition deteriorated, leading to a transfer to the intensive care unit, where he passed away during transport. An autopsy performed seven days after death revealed a picture of polyvisceral stasis, massive hemorrhagic pulmonary edema, and extensive sequestration of fluids in the third space. Histological and immunohistochemical examination of the pulmonary tissues and spleen (CD 68) demonstrated numerous blood clots in the pulmonary vessels rich in monocytes, along with the presence of monoblasts and macrophagic desquamative alveolitis. Additionally, monocyte/macrophage inflammatory foci were observed in the brain, heart, kidney, and lymph nodes, indicating systemic involvement with hypoxic/ischemic damage. Laboratory findings on blood samples showed a remarkable increase of inflammatory markers.

The clinical, laboratory, and histopathological findings showed significant similarities with cases previously reported in the literature, linking deaths to the administration of the COVID-19 vaccine.[2-4] The cause of death in this case was attributed to macrophage activation syndrome, accompanied by acute hemorrhagic pulmonary edema, multi-organ hypoxic-ischemic injury, and diffuse myocardial reperfusion necrosis. The literature and World Health Organization (WHO) causality assessment methods were applied and a correlation between vaccine administration and death has been assessed[5].

This case study highlights a potential correlation between the administration of the ChAdOx1 nCov-19 vaccine and an intense inflammatory response with MAS. As researchers and the scientific community, it is imperative to recognize and consider such adverse events in order to develop necessary preventive and therapeutic measures. Standardized diagnostic algorithms should be employed to accurately assess causal relationships between vaccinations and adverse outcomes, ultimately ensuring the safety and efficacy of vaccination programs.

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COVID-19; Vaccine; Autopsy

I17   **A Delayed Traumatic Intracranial Hemorrhage With Antithrombotic Medication: A Report of an Autopsy Case and Verification in an Animal Model**

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Learning Overview: Traumatic brain injury is exacerbated in patients using antithrombotic medications, including anticoagulants and antiplatelet agents. After attending this presentation, attendees will have learned about the mechanisms of delayed deterioration of traumatic brain injury in patients using antithrombotic medications who have sustained head trauma.

Impact Statement: The mechanism of delayed deterioration from traumatic brain injury is crucial to understanding the causal relationship between head injury and death from brain injury. This presentation will impact the forensic science community by discussing how this study is useful for forensic autopsies to diagnose the causal relationship between head trauma and delayed deterioration of brain injury.

Introduction: Intracranial hemorrhage after a head injury may be exacerbated by anticoagulant medications. We present an autopsy case of delayed traumatic intracranial hemorrhage in a patient using the anticoagulant warfarin. We also report an animal model of traumatic brain injury treated with warfarin to investigate the mechanism of exacerbation of traumatic intracranial hemorrhage during anticoagulant medication.

Autopsy case: An elderly man was involved in a bicycle-car collision, leading to a fall. He was found unconscious at home the following day and died at the transport hospital. During the autopsy, a linear fracture of the occipital skull, subdural hematoma, cerebral contusion in the left and right anterior temporal lobes of the cerebrum, brain herniation, and secondary bleeding in the brainstem were observed. Additionally, warfarin was detected in the blood. It was noted that the cerebral contusion was aggravated half a day to one day after the injury, resulting in a subdural hematoma.

Animal model: Mice (C57BL/6J, 8–10wk old), fed with a vitamin K-deficient diet, were orally administered warfarin (low dose 0.35mg/kg/24h, high dose 0.70mg/kg/24h), after which focal brain damage was created in the cerebral cortex using a brain contusion creation device. The brain contusion device (Impact One, Leica®) was used to create a focal brain injury in the cerebral cortex. The results were compared to mice that received no warfarin or only a scalp incision. Blood prothrombin time, cerebral hemorrhage volume, and blood levels of warfarin and 7-hydroxylated warfarin were measured 2h, 1 day, and 3 days after the injury. A trend toward prolonged blood prothrombin time, increased cerebral hemorrhage volume, and higher blood warfarin and 7-hydroxylated warfarin concentrations was observed one day after the injury in the high-dose warfarin group.

Discussion: The results indicate that when warfarin was administered, cerebral hemorrhage associated with cerebral contusion increased on the first day after injury. Post-injury-impaired blood coagulation and elevated warfarin levels in the bloodstream may be involved in the mechanism of exacerbation of traumatic brain injury.

Brain; Injury; Neuropathology

I18   Delayed Traumatic Rupture of the Spleen in a Patient with Mantle Cell Non-Hodgkin Lymphoma: Histopathological and Immunohistochemistry Aspects

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Learning Overview: This presentation aims to explore the histopathological and immunohistochemical aspects involved in diagnosing traumatic splenic rupture in a patient with mantle cell Non-Hodgkin Lymphoma (NHL) who died ten days after a fall in the hospital.

Impact Statement: This presentation will impact the forensic community by highlighting the importance of considering underlying hematological malignancies when investigating delayed traumatic splenic rupture. Moreover, histopathological and immunohistochemistry analyses play a crucial role in establishing accurate diagnoses and time estimation of splenic hematoma, leading to a better understanding of potential complications, guiding appropriate patient management, and evaluating health care workers' liability.

Splenic rupture and hematoma are significant complications that can arise in patients with NHL.[1] NHL is a type of hematological malignancy characterized by abnormal lymphoid cell proliferation. In some cases, NHL can cause splenomegaly, an enlargement of the spleen, making it more susceptible to rupture due to trauma or even spontaneously.[2] The rupture may lead to intraperitoneal bleeding, forming a hematoma. Histopathological and immunohistochemistry analyses play a crucial role in diagnosing NHL and assessing the extent of spleen involvement. Identifying such complications is essential for timely intervention and optimal patient management.[3] A thorough understanding of these associations can aid hematological researchers in enhancing patient care and outcomes.

A judicial autopsy was requested by the prosecutor's office for a malpractice claim due to a fall in the hospital and a lack of surveillance. A 72-year-old male was admitted to the hospital with a history of asthenia and hypotension for several days. While hospitalized, he suffered a lacerated contusion wound to the frontal region of the head after falling off the gurney. However, no other symptoms were reported, and a Computed Tomography (CT) scan of the face and brain showed no abnormalities. Nine days after discharge, the patient presented with abdominal pain, and he was taken to the emergency room. Abdominal CT revealed splenomegaly, splenic rupture, and hemoperitoneum. An emergent open splenectomy was performed, but the patient showed signs of shock and hemodynamic instability and, subsequently, the death occurred.

The autopsy revealed an increased volume of paratracheal and mesenteric lymph nodes and blood coagulation in the splenic lodge. Macroscopic examination of the spleen showed splenomegaly with capsular laceration and multiple areas of subcapsular hemorrhagic infarction.

Histopathological examination of the spleen revealed an abundance of abnormal tumor cells. Based on immunohistochemical analyses, the patient was diagnosed with mantle cell NHL, as evidenced by the presence of CD20, CD5, Bcl-2, and Ki-67 (>80%) positive cells. Staining with hematoxylin-eosin revealed a monomorphic lymphoid proliferation of small- to medium-sized cells with irregular nuclear contours. A histopathological time estimation of the subcapsular hematoma was performed. The van Gieson and CD 68 staining was particularly useful in this case, highlighting the timing of subcapsular hematoma and necrotic infarction of splenic parenchyma. Spontaneous rupture has been excluded. The findings strongly suggest that the splenic rupture experienced after trauma may have been associated with the patient's pre-existing hematological malignancy of the spleen, namely mantle cell NHL.

This case highlights the importance of considering underlying hematological malignancies when investigating delayed traumatic splenic rupture. Histopathological and immunohistochemistry analyses play a crucial role in establishing accurate diagnoses, leading to a better understanding of potential complications and guiding appropriate patient management.

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Spleen Rupture; Lymphoma; Autopsy

I19 Combined Mechanical Asphyxia by Strangulation and Immobilization of the Chest in a Case of Matricide

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Learning Overview: After attending this presentation, attendees will understand the importance of possible mechanisms of airway obstruction in cases of asphyxia and violent deaths.

Impact Statement: This presentation will impact the forensic science community by demonstrating the importance of forensic and autopsy investigation in the study of mechanical asphyxia by combined strangulation and immobilization of the chest.

The case presented concerns the autopsy investigation of an asphyxia-related death from combined mechanism of airway obstruction. An elderly woman was found dead in the bedroom of the home she shared with her son, and a small, coiled cord was found on the dresser in the same room. The victim presented at the neck the lesion of an atypical strangulation, with a transversal cutaneous compressive ligature mark at the neck, and, at the thoracic region, an introflexion of the rib cage with multiple rib fractures, bilateral, and mostly on the right, with signs of vital reactions. The neck injury was limited to the posterior and left lateral cervical region, resulting in incomplete constriction of the upper airways located in the neck. Thoracic injury was indicative of compression asphyxia/immobilization of the chest, determining the impossibility of performing respiratory acts.

These two types of injuries to the neck and to the chest allowed a reconstruction of the aggression dynamic in which the victim was supine on the ground and her aggressor bent over her, likely kneeling on her chest in an attempt to immobilize the victim with his own weight, while performing the strangulation maneuver. The force exerted by leaning on her knee resulted in a limitation of lung expansion through compression of the chest and at the same time allowed for an increase in the pulling force of the ligature placed around her neck, thus determining a combined asphyxia mechanism.

The forensic investigations were performed and identified the cause of death as a mechanical asphyxia by occlusion of the upper airways through the mechanism of strangulation, namely, compression of the neck by a ligature, and immobilization of the chest, namely, impediment of respiratory acts by compression of the chest.

The asphyxiation had non-rapid times in relation to the incomplete airway obstruction from atypical strangulation, which also gave a reason for the multiple injuries found on the victim's corpse in addition to the ligature mark caused by the aggressor with the intent to hasten the death by strangulation and the facial injuries (scratches) found on the body of the aggressor that represented an extreme defense of the victim before the loss of consciousness. Indeed, genetic investigations allowed the existence of traces of the aggressor's DNA under the victim's fingernails. As frequently occurs, matricide was perpetrated by a subject with psychiatric disorders, with poor adherence to appropriate pharmacological treatment and without valid clinical intervention and psychological support.

In conclusion, the strangulation mechanism, which can be defined as atypical based on the characteristics of the ligature mark, may have insufficient and ineffective lethal action due to incomplete compression and airway obstruction at the neck level. The autopsy investigations identified the genesis of the mechanical asphyxia and the cause of death in a dual mechanism, combined strangulation and immobilization of the chest.

Asphyxiation; Forensic Investigation; Strangulation

I20   Unknown Heart Disease and Sudden Cardiac Death: The Role of Postmortem Investigations

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Learning Overview: After attending this presentation, attendees will understand the importance of defined protocols in postmortem forensic investigations for the diagnosis and correct analysis of cases of Sudden Cardiac Death (SCD).

Impact Statement: This presentation will impact the forensic science community by demonstrating the need to use autopsy data for the creation of special National Registers aimed at epidemiological control and prevention of SCDs.

Introduction: This study was carried out on 15 cases with the aim of evaluating how the heart appears from a macroscopic point of view in SCD, identifying investigation protocols to be carried out postmortem in order to create National Registries with free access to local health structures.

Materials and Methods: All cases under examination were subjected to autopsy. The gross heart examination was performed with macroscopic visualization of the coronaries, cardiac chambers, and microscopic evaluation after formaldehyde fixation. Histological, microbiological, toxicological, biochemical, and genetic investigations were integrated.

Results and Discussion: The series includes subjects between 2179 years old. Among the causes of death, myocardial ischemia predominates (53%). From the macroscopic analysis of the heart: increase in size in 66% of cases, hypertrophy of the left ventricle in 60%, hypertrophy of the septum in 53.3%, increase in weight in 73.3%. An inflammatory infiltrate was found in 5 out of 15 cases, two attributable to myocarditis, one due to vasculitis, and one case with infiltrate in the intermyofibrillar connective tissue and the other in the pericardial adipose tissue.

In three cases, the diagnosis of ischemia was supported by biochemical investigations since the histological examination did not reveal necrosis or hypoxic/ischemic suffering. The histopathological examination cannot confirm an evaluable microscopic finding on the myocardium in the first hours of the onset of a myocardial infarction, given the times necessary to visualize the coagulative necrosis with hypereosinophilia; therefore, in these cases, the diagnosis must be based on the autopsy data integrated with the clinical-instrumental data. CK-MB, Myoglobin (Myo), Troponin I (TnI) expressed in ng/ml were evaluated in cases 8, 13, and 14 whose values were found to be altered to support predominantly myocardial damage.Results were: Case 8: CK-MB 2,6; Myo 221↑; TnI 646↑; Case 13: CK-MB 45↑; Myo 132↑; TnI >30↑; and Case 14: CK-MB >80↑; Myo < 5; TnI >30↑. The coronaries were free in five cases (33.33%). Hypostases on the head and neck were identified in 40% of cases, while thrombotic and/or embolic phenomena were identified in 33.3%. In Case 6, a mutation was identified in the fibrillin 1 gene, responsible for S. Marfan, a genetic pathology that correlates with the risk of developing aneurysms that can undergo potentially fatal rupture. The diagnosis in this case is fundamental; therefore, it is necessary to communicate the results of the autopsy examination and direct the relatives of the victim to the screening programs.

The aim is to propose a study protocol that should be applied in all those cases of SCD that allows the cause of death to be traced and screening investigations to be carried out in the closest family members: when a sudden death is ascertained, the doctors request for the execution of an autopsy/diagnostic check by filling in a form that includes the patient's details, circumstances, and manner of death. The body is received by the Unit of Forensic Medicine, which will carry out the autopsy alongside additional investigations. We want to confirm the fundamental role of the autopsy in cases of SCD in order to identify a clear cause of death through a postmortem diagnosis process and the importance of integrating the autopsy data with histological, laboratory, and genetic data. The latter is crucial to recognize a hereditary disease as the cause of death, which implies the inclusion of relatives of the victim in screening programs in order to prevent a further fatal outcome from occurring.

The use of the data obtained for the creation of National Registers could give insight into the significant impact these deaths have in today's society and enhance the importance of cardiological prevention.

Sudden Death; Autopsy; Forensic Science

I21 Sudden Cardiac Death in a Man With a Functioning Implantable Cardioverter-Defibrillator

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Learning Overview: The goal of this presentation is to highlight the role of a multidisciplinary approach in the Sudden Cardiac Death (SCD) in patients with a functioning Implantable Cardioverter-Defibrillator (ICD).

Impact Statement: This presentation will impact the forensic science community by helping attendees understand that in cases of sudden death in patients with an implanted defibrillator, a close cooperation between forensic pathologist, cardiologist electrophysiologist, and biomedical engineering company of the ICD, is essential in order to evaluate the correct functioning of the device.

SCD is defined as an unexpected natural death from a cardiac cause that takes place during the first hour after onset of symptoms in apparently healthy or in chronically ill individuals. The ICD is the most effective method we have to prevent death from sSCD.

Case Report: In April 2021, a 51-year-old man suffering from post-ischemic dilated cardiomyopathy, permanent atrial fibrillation, arterial hypertension, and a wearer of an ICD alerted the emergency services for the defibrillator leaking from the skin pocket. The patient did not have cardiological examinations for years due to the COVID pandemic. Subsequently, the emergency workers placed several bandages in the chest wall and carried the patient out by using the stairs as the building was not equipped with a lift. During the transport, the patient underwent cardio-circulatory arrest and died despite Cardiopulmonary Resuscitation (CPR).

A complete forensic investigation was performed. The external examination confirmed the presence in the left side of the thorax of a cutaneous lesion from which the electro-catheter of ICD came out. A postmortem Computed Tomography (CT) scan was performed and showed the terminal portion of the electro-catheter in the context of the left pectoralis major muscle. It was also confirmed during the autopsy examination. Moreover, the macroscopic examination of the heart showed a dilation of the left ventricular chamber. Histological examinations showed areas of fibrotic replacement in samples from the left ventricular wall of the heart. Blood stasis and grade I colliquative myocytolysis were also found. The histological study of the coronaries showed the presence of atheromatous plaques of the anterior interventricular artery lumen reaching 75%. No significative alteration of other organs was found. The Boston Scientific sent a letter to share the analysis of ICD functionally of the days preceding the death. In addition, a cardiologist did an evaluation of the ICD by connecting it to a programmer. Both studies confirmed the correct functioning of the device, but, due to the malposition of the lead, the defibrillator did not play its therapeutic role.

The autopsy findings and histopathological studies were fundamental for the root cause of death. In addition, a multidisciplinary approach with a cardiologist made it possible to determine if the ICD was active or not. The postmortem examination of the ICD is mandatory in order to assess the proper functioning of the device and perhaps communicate any bias to the companies related.

Sudden Death; ICD; Cardiology

I22   Unraveling Unusual Mediastinal Anatomy Alterations Through Postmortem CT and Multiparametric 3-D Reconstructions: A Case Series of Major Trauma

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Learning Overview: The aim of this presentation is to evaluate postmortem Computed Tomography (CT) with a 3D multiparametric reconstruction unusual findings scan with alteration of mediastinic anatomy and then compare with those of traditional autopsy in a case series of major trauma.

Impact Statement: This presentation will impact the forensic community by providing valuable insights into the subversion of mediastinal and thorax anatomy and reinforce the significance of employing a multidimensional approach in forensic investigations of major trauma cases by performing, in selected cases, a postmortem CT and multiparametric 3D reconstructions.

Major trauma resulting from high-intensity actions, such as traffic accidents and crush injuries, often leads to fatal injuries with complex and diverse mechanisms affecting various body regions. In such cases, postmortem CT, also known as virtopsy, complements traditional autopsies, providing valuable insights into subtle signs that may be challenging to detect using conventional methods.[1,2] This presentation explores a case series of major trauma involving three unique incidents of destruction to physiological mediastinal anatomy.

In the first case, a 29-year-old man suffered a fatal injury when an iron bar pierced his chest during a vehicular collision with iron fences. Postmortem CT revealed extensive cutaneous and subcutaneous tissue damage, detachment of subscapular fascial planes, and modest dorsal dislocation of the scapular body, along with fractures of the posterior arch of the VIII and IX left ribs. The iron bar had passed through the mediastinum, causing significant disruption to large mediastinal vessels and total alteration of cardiac anatomy. The complementary use of postmortem CT and traditional autopsy confirmed chest injuries and emphasized the path of the iron bar, while traditional autopsy focused on vital organ and large vessel injuries.

In the second case, a 48-year-old man sustained fatal injuries from an industrial machine crush during work. External examination did not reveal significant injuries, but postmortem CT unveiled a complete destruction of physiological mediastinal anatomy, with the heart dislocated into the left pleural cavity and detachment of large cardiac vessels. Postmortem CT also detected rib fractures, vertebra burst fractures, and pneumothorax. Traditional autopsy further confirmed heart valvular wall injury, pericardial injury, lung injuries, rib fractures, and vertebra fractures.

The third case involves a 60-year-old man who suffered of a car crash. The postmortem CT was performed after the cadaver exhumation, four months after death. Hemorrhage of the thorax with the involvement of the perisuclavian area was observed as a consequence of multiple rib fractures. The CT findings were crucial to help the autopsy procedure and assess the cause and manner of death in a cadaver under advanced decomposition.

The synergistic use of virtual and traditional autopsies, and 3D multiparametric reconstructions proves to be essential in forensic science. While traditional autopsies excel at revealing vital organ thoracic injuries, they have limitations in studying injuries to the splanchnocranium, vertebral column, and pelvis. The contemporary utilization of both techniques, particularly in cases of significant trauma, promises to enhance the forensic approach to judicial autopsies.

This presentation highlights the uniqueness of major trauma cases with unusual mediastinal anatomy findings, underscoring the importance of combining traditional and virtual autopsy methods to accurately assess injuries and determine the mechanisms of death.

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3D Technology; Postmortem CT; Major Trauma

I23   **The Role of Optical Coherence Tomography in Sudden Cardiac Death: Insights and Future Perspectives in Forensic Sciences Through a Case Series of Spontaneous Coronary Artery Dissection**

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Learning Overview: This presentation will describe the role of Optical Coherence Tomography (OCT) in forensic investigations as a complementary postmortem imaging technique in cases of sudden cardiac death by being able to better display coronary vessel walls.

Impact Statement: This presentation will highlight the usefulness of an OCT-based approach in evaluating coronary arteries. We will describe a case series of spontaneous coronary artery dissection in which OCT helped to find the cause of death.

Sudden Cardiac Death (SCD) is frequently observed in forensic autopsies; therefore, it is one of the most-studied topics in forensic pathology. The uncertainty in the diagnosis of SCD-related causes of death has led over the years to apply histological, histochemical, and imaging techniques. However, ordinary methods used in forensic investigations for SCD often do not lead to certain results due to the presence of many non-specific findings. This uncertainty in defining the exact causes of death has pushed research to test new methodologies in order to reach a more accurate diagnosis. Among these, OCT has recently gained relevance.

The working principle of OCT is based on the measurement of the optical dispersion of a low-coherence light beam, carried by optical fibers, passing through biological tissues. This method generates real-time high-resolution images (1–15μm), thus resulting in a kind of "optical biopsy" of tissues. Starting with ophthalmology applications in the mid 1990s, it has been successfully implemented into coronary imaging.

Since OCT tomography is a non-destructive examination, it preserves tissue integrity before classic transverse dissection. On the other hand, OCT-obtained images are much more detailed in comparison with other imaging methods currently used in the forensic routine (Postmortem Computed Tomography [PMCT], Postmortem Computed Tomography Angiography [PMCTA], Postmortem Magnetic Resonance [PMMR], Postmortem Magnetic Resonance Angiography [PMMRA]). Moreover, OCT imaging can be performed on fresh specimens besides formalin-fixed ones, which could prevent the formation of formalin-related artifacts. A minimally invasive approach to postmortem examination by using OCT imaging has been described in the literature too. However, despite this and its large-scale use in intravascular and endoscopic imaging from cardiologists, there is currently little literature in the forensic field.

This presentation will describe a case series in which OCT imaging revealed the presence of a spontaneous coronary artery dissection. Subsequently, we have compared the OCT findings with the classic histological examinations to highlight the reliability of the method and its usefulness in forensic diagnoses.

Postmortem Magnetic Resonance Angiography

I24 The Role of Radiological Investigations and the Postmortem Laboratory in an Enigmatic Suicide Case: Between Doubts and Certainties

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Learning Overview: After attending this presentation, attendees will understand the role of a multidisciplinary forensic approach in reconstructing the dynamics of a traumatic case of suicide.

Impact Statement: This presentation will impact the forensic science community by highlighting how a multidisciplinary forensic approach and the use of postmortem laboratory investigations can help to discriminate suicides from homicides or accidental deaths.

To date, all over the world, suicide prevention is not considered enough; this is because suicide is not perceived as a public health issue. In some societies, it is still a taboo and is difficult to talk about it. Today, suicide is one of the top three causes of death among subjects aged 15–44 years old. From a forensic point of view, it is often difficult to reconstruct the dynamics of a suicide, especially when there are circumstantial, doubtful data that make the job hard even for an expert pathologist.

We report the case of a fall from a great height that occurred from the fire escape stairs of a southern Italian hospital. The victim's body was lying on the ground in a puddle with an important polytrauma; injuries were compatible with a fall. We proceeded with the analysis of the potential point of fall, a railing on the ninth floor of the hospital, accessible from the fire escapes. An accidental fall was also hypothesized due to the low height of the safety railing. This hypothesis was advanced due to conflicting testimonies regarding the hypothesis of an illness that could have preceded and caused the fall. An investigation of previous pathologies was carried out through the analysis of the health databases of the hospital in which the subject had been hospitalized.

A multislice 3D Computed Tomography (CT) scan was performed before the autopsy with evidence of a mobile phone in the jacket's victim. The discovery of the phone eliminated the hypothesis of a possible theft ending in a fatal dispute. The autopsy revealed a chest trauma and a head trauma with laceration of internal organs. Skull fractures with cerebral hemorrhage were detected. The victim had multiple rib fractures, aortic dissection, and vertebral burst fracture with myelic involvement. The abdominal organs were translocated to the thorax due to traumatic rupture of the diaphragm. In the abdomen, there were multiple hepatic lacerations, an intestinal hemorrhagic infiltration with involvement of the celiac trunk. The left renal capsule was hemorrhagic. Toxicological investigations were negative for drugs in biological samples. In order to investigate any cardiac illness such as causing loss of consciousness with a subsequent accidental fall, the dosage of cardiac biomarkers was carried out. This investigation showed that the victim was alive at the moment of impact with the ground. The trauma and the multiple chest injuries determined the increase of the cardiac damage biomarkers (CKMB, MYO, TNI). A psychological autopsy was carried out through an interview with the victim's family members; from this it emerged that the subject presented a depressed humor due to financial problems. For this reason, the victim was short-tempered and grumpy.

Suicides still remain a great forensic enigma due to the difficult reconstruction of the dynamics of the event. Therefore, it is crucial in suicide cases to carry out radiological and laboratory investigations; these insights provide useful information that allows the forensic pathologist to eliminate doubts.

Suicide; Forensic Analysis; Forensic Psychiatry

I25 A Case of Dismemberment in Italy: A Multidisciplinary Approach to Put the Pieces Together

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Learning Overview: After attending this presentation, attendees will understand how the work of a multidisciplinary team is critical to the approach and resolution of complicated cases involving the discovery of dismembered bodies.

Impact Statement: This presentation will impact the forensic science community by showing how multidisciplinary work has been effective in solving the tangled case of a dismembered corpse.

The purpose of this presentation is to show the importance of a multidisciplinary approach in studying dismembered corpses. These findings usually present interpretative difficulties for identification, diagnosis of death, and determination of wound vitality due to the body's condition, the scarcity of useful thanatological parameters, and the absence of uniform investigative protocols.

Corpse dismemberment is an uncommon event, usually performed after homicides in order to make the body easily transportable and/or conceal it. The head and the limbs are often detached from the torso, and the pieces are taken to different locations to make them more complicated to find. After the discovery of the pieces, the first need is to figure out whether they all belong to the same corpse or to multiple individuals. Second, the identification of the individual(s) is critical, and no less important is to then understand what happened before the dismemberment.

In July of 2022, several plastic bags were found in various stretches of a river in northern Italy. The bags contained body parts in advanced putrefaction: head, four limbs, two hemithoraxes, a left hemiabdomen as well as a bag with organs and viscera. Preliminary radiological investigations (X-rays and Computed Tomography [CT] scans) were carried out to look for useful elements for recognition, identify fractures, and try to match the various parts of the skeleton.

The autopsy was performed over several days and allowed accurate analysis of the pieces' margins to conclude that the various parts belonged to the same individual: a Caucasian male about 65–80 years old. Bone margins were carefully exposed, and lesion areas were dissected to look for macroscopically evident hemorrhagic infiltration and to study any bone discontinuity. No biological fluids were present. Multiple skin, bone, and visceral portions were collected for histological investigations. Various bone segments were taken for additional radiological investigations by micro-CT. Fingerprint study, biological sampling (teeth, hair, nails), and collection of dipteran larvae were also carried out.

Histological preparations, stained with hematoxylin-eosin, identified microscopic blood hemorrhagic infiltration, a sign of a vital reaction, in head lesions and in the margins of the neck dismemberment areas. The immunohistochemical study with antibodies anti-glycophorin A was positive in skin samples taken in the posterior-cervical, left lateral-cervical, and sub-occipital regions, in the left mandibular region, in the left ear, in the C2 area, and in the left thoracic region. Micro-CT studies two sections of the cervical and dorsal spine, identifying three types of lesions (deepening incisions, false starts, complete bone sections), attributable to two types of bladed weapons.

The autoptic examination determined that the body pieces were in the water three to ten days before discovery, but the postmortem interval could not be established due to the unreliability of thanotochronological parameters and microfauna colonization. However, it was possible to attribute the cause of death to acute hemorrhagic-hypovolemic shock and traumatic spinal cord shock from penetrating-lacerative trauma of the left lateral-cervical and submandibular region caused by the action of an axe. Two types of bladed weapons (axe and knife/cleaver) were used for the dismemberment, which occurred after death.

After the police investigation and the identification of the body, the prosecutor arrested the victim's wife, a psychiatric patient, as the alleged perpetrator of the murder. She claimed that she killed her husband because he beat her and wanted to cheat on her with a woman who spoke to him through a microchip in his ear.

Dismemberment; Histological; Computed Tomography

I26   A Rare Case of Homicide by Air Rifle

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Learning Overview: The goal of this presentation is to highlight not only the potential dangers of air-powered guns, but also to show the potential damage to the human body in a case of homicide.

Impact Statement: This presentation will impact the forensic science community by making attendees aware that although the wounds from air-powered guns are less dramatic than from firearms, recognition of their lethal potential is an important part of death investigation, medicolegal autopsy, and proper assignment of manner and cause of death.

Background: Air rifles and guns (air weapons) are a class of weapons that propel a projectile with compressed gas. This may be through spring or piston compression or compressed gas cylinders.[1,2] Like firearms, air weapons may be smooth bored or rifled and fire a variety of projectiles in multiple calibers, including 0.22, 0.177, and 0.20.[1,3] Projectile types include spherical BBs, hour-glass-shaped Diabolo pellets, and pointed conical Sheridan pellets.[1] Hollow, flat, and rounded point varieties as well as forms with an attached point to increase penetrating power also exist.[4] Velocity depends on the projectile, manner of discharge, and the weapon quality.[1,5,6] Velocities of up to 940ft/s, 1,000ft/s, and 1,200ft/s have been published.[1,5,7] Depending on the source cited, the velocity to penetrate skin ranges from 125 to 331ft/s, and for bone 350ft/s.[5,7-9] The point of impact obviously factors into whether penetrating trauma occurs and the extent of internal injury sustained. Entrance wounds from these weapons look like those from firearms, although stippling, soot deposition, and radiating lacerations do not occur. An exit wound would be an exceptional occurrence. In addition to thoracic trauma, often to the heart or great vessels, cases of air weapon fatal head injuries do occur in both the pediatric population and skeletally mature adults.[3] Accidental death and suicide by air weapon is uncommon and homicide is very rare.[1,3] Understanding the potential lethality of air weapons and recognizing the projectiles and injury characteristics is important in differentiation from firearm injury.

Case: A 57-year-old female was found deceased in her significant other's home under suspicious circumstances. The subject was found wrapped in a blanket with trash bags over her body. Blood spatter was noted on the floor, ceiling, and walls near the body. An air rifle was in the residence. The rifle had dried blood and hair on it.

Radiographs demonstrated multiple radiopaque objects within the cranium and soft tissues of the scalp. External examination of the body showed abrasions and contusions on the torso and upper and lower extremities. The head and neck were remarkable for multiple entrance gunshot wounds. No exit wounds were present. Defects measured 3/16 x 3/16 up to 1/2 x 1/4 inch. Abrasion of the wound margins measured up to 1/8 inch. Stippling and soot were not present. Numerous flattened metallic projectiles and orange tips were recovered from the soft tissues of the scalp and outer table of the calvarium with underling skull fractures and cerebral contusion. The temporal and frontal skull had penetrating trauma with internal beveling. Additional projectiles were recovered from the cranial cavity and superficial cerebral cortex. The cause of death was multiple gunshot wounds. The manner of death was homicide.

Conclusion: Air weapon injuries are more common in children than adults and mostly involve young boys.[2,3,6-9] Fatalities are uncommon. Fatalities tend to occur with injury to the thorax or neck, commonly when projectiles travel through the intercostal space and injure the heart or great vessels.[2,3,5,10] Intracranial injury usually occurs with penetration of the orbit or temporal bone as other areas of the skull usually resist penetrating injury by these weapons. Suicidal temporal injury has been reported.[2,3] In the forensic setting, it is important to recognize that the injury pattern and projectiles may be from an air weapon, as this may be relevant to the investigation. Projectiles from air weapons should be treated like those from firearms and collected carefully as potential ballistic evidence.

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Homicide; Accident; Weapon Analysis

I27   The Cerulean Man: A Case of Argyria

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Learning Overview: After attending this presentation, attendees will be aware of the history, physiopathology, and differential diagnosis for a rare condition called argyria.

Impact Statement: The presentation will impact the forensic science community by helping attendees recognize this rare condition.

A 69-year-old White male was discovered unresponsive on a street in Pittsburgh by a passerby. He was pronounced by a responding emergency medical team who observed a distinctive blue-gray discoloration of the skin, mucosal surfaces, and conjunctiva. The case was referred to the Allegheny County Office of the Medical Examiner (ACOME). The decedent had a past medical history of argyria or argyriosis, cardiac disease, intestinal issues, and atrial fibrillation. The autopsy confirmed an intense blue mucocutaneous discoloration and moderate to severe cardiovascular pathology consistent with past medical history. Histologic sections (H&E) showed brown-black pigment granules embedded in the dermis and internal organs. The cause of death was ruled as hypertensive and atherosclerotic cardiovascular disease and the manner of death was natural.

The uncommon condition known as argyria is acquired and is brought on by exposure to or ingestion of silver-containing products. The word argyria is derived from the Greek word for silver, argyros. Even when the physical look does not improve after the exposure is removed, the issue is not life-threatening. Research is not definitive, but the literature suggests that people with argyria can develop decreased kidney function or significant impairment in night vision due to damaged rods in the eyes. Silver poisoning can happen and even be fatal if significant amounts of silver-containing products are acutely ingested. The differential diagnoses for blue skin discoloration are multiple, including central hypoxemia with cyanosis, methylene blue poisoning, hemochromatosis, methemoglobinemia due to poisoning with sodium nitrate, and others.

Silver is used in a variety of medical devices due to its antimicrobial characteristics caused by the oligodynamic effect. Colloidal silver, a liquid suspension of minute silver particles, has traditionally been used internally to cure a variety of diseases. In the 1940s, they were superseded by the introduction of prescription antibiotics such as penicillin. Colloidal silver is now being illegally promoted as a medicine; however, it is ineffective and may interfere with the operation of some pharmaceuticals, such as antibiotics and thyroxine.

The authors invite the readers to review with us an actual case of argyria, interesting cases throughout history, the physiopathology behind the blue discoloration, and the differential diagnoses in the practice of forensic pathology.

Diagnosis; Forensic Analysis; Skin

I28   Analysis of a Case of Neonatal Precipitation: Feticide or Infanticide?

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Learning Overview: After attending this presentation, attendees will have significant insights into the forensic investigation of feticide and infanticide cases, a crime whose frequency has been steadily decreasing due to radical cultural and legislative changes.

Impact Statement: This presentation will impact the forensic science community by explaining the usefulness of the crime scene investigation and through an account of the peculiarities of feticide and infanticide.

Here we present an unusual case of an infanticide accomplished by defenestration, throwing a newborn girl out of a window (from an height of about ten meters). Death was due to the mechanism of precipitation, and the cause of death was traumatic brain injury with burst fractures of the skull cap and multiple cerebral hemorrhagic foci.

The corpse of the newborn girl was found on the ground lying among the broken branches of a perimetrical hedge of pittosporum about 230cm high, in a fenced condominium area. The perimetrical hedge showed bushy outcrops and broken branches with blood traces describing the passage of the little body during the fall. The infant corpse showed a series of lacerated-contused wounds, excoriated contusions, and ecchymosis, almost parallel to each other and concentrated in the areas of impact with the elements of the bush. The place where the corpse was found was about five meters from the building from which she was thrown.

These circumstances made it possible to reconstruct the dynamics of the event as a homicidal precipitation. The injuries found at the autopsy unequivocally confirmed a traumatic event. In fact, the injury to the skull, the cause of death, was indicative of a fall on the vertex, with impact of the head against a hedge spike, resulting in the cutaneous laceration and contusion of the left fronto-temporo-parietal region, fractures of the underlying bone and cerebral injuries. Other injuries were found on the infant's little body, all of which were referable to its passage through the hedge and caused by the impact and/or crawling of its body surface against the roughness of the branches.

Establishing the cause of death and reconstructing the dynamic of the event, the forensic medical investigation found that the fetus had been born alive and vital, with a level of maturity compatible with a full-term pregnancy, and that death had occurred in the hours immediately after birth. This ruled out the possibility that homicidal violence was carried out on a fetus during childbirth, before the extra-uterine life, a circumstance that would have ruled in the crime of feticide.

The puerpera had an I° grade vagino-perineal injury, with a dilated uterine cervix and the presence of a small periurethral laceration. The birth had been carried out at home, unassisted, and the placenta was hidden.

In conclusion, in the hypothesis of infanticide, it is a complete forensic medical investigation, with analysis of both the infant and the mother, and of the places. The first in order to verify, in addition to the time, the cause and means of death, the vitality by docimasic study, the maturity of the infant; the second in order to verify the signs of a recent birth, temporally compatible with the extra-uterine life span of the infant (forensic gynecology) and the conditions of moral and material abandonment of the woman (forensic psychopathology); and the third in order to verify the dynamics of the event.

Forensic Investigation; Fall From Height; Infant Death

I29   Death Due to Aggression by Maremman Dogs: The Role of Forensic Sciences in the Reconstruction of the Case

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Learning Overview: After attending this presentation, attendees will better understand the role of a multidisciplinary forensic approach in reconstructing the dynamics of a forensic dog assault case.

Impact Statement: This presentation will impact the forensic science community by highlighting how a multidisciplinary forensic approach can discriminate between intentional rather than accidental deaths.

Introduction: To date, the victims of aggression by the common domestic dog are numerous. The attacks most frequently affect the population of the elderly and children.

Case Report: We report the case of a young girl attacked by a pack of dogs during a walk in a mountain area. During the crime scene inspection, 18 Maremma dogs were caught.

Materials and Methods: The autopsy was performed, and all the wounds present on the body were analyzed. The injuries were photographed and the distances between the bite lesions were measured. The hairs found on the victim's hands were cataloged. The autopsy results were evaluated by a forensic anthropologist and a forensic zoologist. Some videos of the moments preceding the attack were analyzed.

Results: On external examination of the cadaver, the body presented numerous lesions on the head, trunk, and limbs. On the head, there was a large lacerated and bruised wound with loss of the scalp and exposure of the cranial vault. On examination of the body, 44 injuries of a mixed type were found. The lesions were represented by particular punctiform holes in the shape of a "V" and skin lesions with irregular edges. Skin abrasions and superficial linear abrasions, consistent with nail marks, were also present on the back. The pelvis and groin region were deeply lacerated. Death occurred from hemorrhagic shock due to internal and external injuries consistent with a dog pack aggression. Evaluation of the bite marks revealed the presence of at least three dogs. Dog bite marks have a typical "hole and tear" appearance and are produced in two phases: the hole reproduces the penetration of the teeth into the skin, followed by the tearing of the tissues by the shaking of the head. The study of the crime scene, the analysis of the body, and the evaluation of the lesions (location, number, and type) can allow clarification of the dynamics of the event and the number of animals. In the case under examination, the multidisciplinary approach made it possible to reconstruct the dynamics of the event by dividing it into different phases. In the first phase, the victim tried to escape, but she was caught and pushed to the ground. Then the victim lay on the ground, in a prone position, and was bitten at the level of the lower limbs by the dogs that tried to turn her body upside down (as evidenced by the excoriations on the back). Once in the supine position, the victim was bitten in the groin region and lower limbs, with the femoral artery severed and massive hemorrhage. Finally, the dogs fed on the limbs of the victim by dragging the body with a partial removal of the scalp. The analysis of the videos showed that before the attack, some dogs of the pack had only approached and only when a larger dog arrived from the woods was the attack activated against the victim.

Discussion: From this analysis, it emerged that the pack of dogs attacked the girl for reasons of dominance of the territory and for hunger. The evaluation of the bite marks (wounds left by the imprint of the canines) made it possible to distinguish at least one large dog, one medium dog, and one of a small size. We therefore highlight the importance of the role of a multidisciplinary forensic approach in fatal attacks by packs of dogs, which can allow not only the identification of the responsible animals and therefore of the owners responsible for the omitted custody, but also the intentional or accidental nature of the event.

Autopsy; Dog Bites; Forensic Analysis

I30   A Case of Fatal Hemorrhage: Natural or Violent Death? A Deceptive Crime Scene

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Learning Overview: After attending this presentation, attendees will understand that an accurate crime scene investigation is essential, especially when the scene appears to be indicative of a violent crime.

Impact Statement: This presentation will impact the forensic science community by showing the importance of an accurate inspection of the crime scene and a complete approach on the cadaver. This case raised issues concerning the homicidal, accidental, or suicidal nature of the act. The aspects considered in the management of the case are discussed, along with a compared approach to the data provided by literature.

Surveys at the site of recovery represent the first approach to the discovery of a corpse, and since they represent an unrepeatable investigation, the association of a forensic pathologist to the law enforcement officers is of utmost importance in order to correctly collect elements/samples/information useful for the reconstruction of the case.[1]

An 80-year-old woman who lived alone was found dead in her home. Her body was slumped in the chair with a huge bloodstain on the floor. The crime scene was characterized by small and large bloodstains on the floor of many rooms, corridors, bathroom, with some shoe prints. There were medications in the living room, including blood-thinning pills. A judicial autopsy was performed to establish the cause of death and rule out the possibility of a crime.

On external examination, there were no traumatic injuries; only on the lateral malleolus of the left foot was a regular, rounded skin laceration, with a diameter of 0.5cm, penetrating for a few mm, involving the skin and subcutaneous tissue. A plane-by-plane (single) anatomical dissection was performed. The dissection of the dorsum of the foot and malleolar regions showed the dorsal metatarsal veins, dorsal cutaneous venous arch, lateral marginal vein, minor saphenous vein, and dorsal cutaneous nerve of the lateral vein with evidence of partial laceration of a lateral branch of the small saphenous vein. The skin lesion of the left leg was also sampled. Internal examination revealed pale organs. The complete autopsy procedure and the histological and toxicological examination of the organ tissues ruled out any alternative causes of death, so the death was ultimately attributed to hemorrhagic shock (death from a common pathological cause, triggered by an accidental traumatic event).

Conclusion. The analysis of the reviewed articles confirms not only the importance of the presence of a forensic examiner at the site of recovery but, in selected cases, also the need of a complete cadaver approach to shed light on the dynamics of the events.

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Fatal Hemorrhage; Crime Scene Investigation; Forensic Pathology

I31   Transorbital Orbitocranial Penetrating Injuries (TOPI): A Report of Two Cases

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Learning Overview: This presentation examines two cases of TOPI and performs a review of the literature. This presentation will highlight a very unusual type of injury and stress the importance of autopsy in the accurate identification of damaged endocranial structures.

Impact Statement: This presentation will impact the forensic science community and pathologists in particular by bringing attention to injuries caused by penetrating, low-velocity objects. In these cases, compared with a minimal external injury of orbit, for the contiguity between the anatomical structures of the cranial cavities, the injury can cause relevant intracranial damages and the death. The study describes two unusual homicide case perpetrated by TOPI.

TOPIs are uncommon. The prevalence of TOPI is 24% of penetrating injuries in adults and 45% in children, occurring more often in boys than girls. A younger age is correlated with a lower mortality rate.[1] Accidents, suicide attempts, or assaults can cause TOPI.[2] The severity of trauma depends on the velocity and trajectory. The penetrating object in these cases is usually longer than 5cm because the whole depth of the adult orbital cavity (4 to 4.5cm) must be transversed before penetrating the cranium. Furthermore, due to the thin bony walls of the orbit, it is simple penetration in the cranial cavity. The penetration of the object can result in severe damage to the eye, optic nerves, brain, and cerebral vasculature.

This study describes the autopsy findings of two homicide cases by TOPI cases observed at the Departments of Forensic Sciences of the University of Foggia and Rome.

The first case is about a 22-year-old female who was pierced in the left eye by the tip of an umbrella wielded by a young woman. At the external examination, the left orbital region showed a sharp cut and a small abrasion on the lower edge placed inside of a red-bluish ecchymotic context. The left was undamaged but with a conjunctival hemorrhage in the upper quadrant. The autopsy revealed fractures of the medial wall of the left orbit, of the smaller wing of the left sphenoid, of the medial wall of the ethmoid, and of the back of the sella turcica. It traced a rectilinear path, approximately 7.0cm long, from front to back. The brain had a subarachnoid hemorrhage, especially spread at the base and cerebellum. There was a full-thickness subtotal laceration of the basilar artery.

The second case concerns a 37-year-old man hit with a long, sharp object in the brow region during a robbery in his cafe. He went to the hospital and died almost one month later. The external examination showed a sutured cut in the left brow region, and the left orbital region was extensively ecchymotic. The autopsy revealed a small incision of the upper margin of the left orbital arch. The brain showed a subdural hematoma, subarachnoid hemorrhage, and intraparenchymal hemorrhages. There was a bone fracture of the left sphenoid wing.

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Autopsy; Forensic Science; Injury

I32 Complex Suicide by Gunshot and Hanging

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Learning Overview: After attending this presentation, attendees will be aware that a complex suicide may be mislabeled as a homicide, so an accurate examination of the corpse, together with the inspection of the death scene, are essential to ascertain the most accurate cause and manner of death.

Impact Statement: This presentation will impact the forensic science community by sharing a case of complex suicide through self-inflicted gunshot and hanging in order to provide a better understanding of this unusual circumstance.

Complex suicide is defined as the use of more than one suicide method to induce death. This procedure can be "planned" if previously conceived by the victim, or "unplanned" when the first attempt fails or proves to be too painful.[1] It has been estimated that complex suicides account for 1.5–5.6% of all suicides worldwide.[2,3]

In the presented case, the victim was a 68-year-old man found on the ground floor of his household, located in a small town in northern Italy. The inspection of the scene revealed the corpse with a gunshot wound at the head, hanged with a noose made of steel cables coated in rubber, used as bicycle locks, tied to the balustrade of the building's staircase. A drum gun and a box of ammo, belonging to the man, were found on a step of the staircase next to the corpse.

The external examination of the body highlighted the presence of a wound in the right fronto-temporal region with an excoriated and ecchymotic border, consistent with a firearm entry wound. At dissection, the bullet went through the pericranial soft tissue and stopped in correspondence of the ipsilateral temporal bone. At the brain examination, a small area of subarachnoid hemorrhage in the right fronto-temporal region was found. Signs of asphyxia were also found at autopsy. Data collected from the scene and autopsy suggest that the man put the noose around his neck before shooting himself. The gunshot wound was not suitable to cause death by itself, which was due to acute mechanical asphyxia from hanging.

In complex suicides, every clue should be analyzed critically to clarify the dynamics of events and if there is any chance of homicide. A careful inspection of the body's place of discovery is essential to ascertain the circumstances of death, as well as an accurate examination of the corpse at the autopsy.

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Suicide; Hanging; Head Trauma

I33 Black Esophagus and Diabetic Ketoacidosis: A Report of an Autopsy Case

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Learning Overview: The goal of this presentation is to inform attendees about Acute Esophageal Necrosis (AEN), also known as "black esophagus," a rare and still poorly understood condition. It is often found during autopsy, and it is related to various pathologies, of which diabetes is the most common.

Impact Statement: The role of the forensic pathologist in the definition, and pathological characterization of this disease is fundamental. This presentation will impact the forensic science community by confirming the close correlation between diabetic ketoacidosis and AEN and how further analyses of autopsy cases of "black esophagus" are needed to elucidate the pathogenesis and pathophysiology of this condition.

Black esophagus, also known as AEN, is a rare condition, and its pathogenesis is not completely known. AEN is macroscopically characterized by a black discoloration of the esophageal mucosa that sharply stops at the gastro-esophageal junction. In endoscopic studies, its prevalence appears to be between 0.1 to 0.3% but it could be underestimated.[1] The patient usually presents multiple comorbidities, the most common being diabetes mellitus (38%), hypertension (37%), alcohol abuse (25%), chronic kidney disease (16%), and chronic liver disease (15%).[2] Diagnosis is based on acute hematemesis or melena with abdominal pain, black discoloration of the esophageal mucosa, and exclusion of other causes of esophageal injuries, for example caustic ingestion. The pathogenesis of esophagitis isn't totally clear; it probably involves a combination of multiple mechanisms, including the esophageal ischemia usually seen in hemodynamic compromise and low-flow states, backflow injury from gastric acid, and impaired mucosal reparative mechanisms present in debilitated physical states. AEN has a poor prognostic factor: the mortality rate in the most recent literature review stands around 32%.[3]

We report the case of a 40-year-old man with a relevant clinical history, including intellectual disability, chronic inflammatory demyelinating polyneuropathy, and type II diabetes who was admitted into the emergency department for vomiting, hematemesis, and deterioration of his conditions. At the hospital, he underwent a digestive endoscopy that reported massive necrosis of the esophageal mucosa with suspected caustic ingestion in a dehydrated subject with diabetic ketoacidosis (blood glucose over 370mg/dl), while a Computed Tomography (CT) scan of the chest revealed an ongoing COVID-19 (confirmed by molecular analysis) -related bronchopneumonia process. The day after hospital access, the patient went into a coma and died 11 days after admission with no clinical improvement.

Based on a suspected caustic ingestion with criminal intent and to ascertain the cause of death, a forensic autopsy was performed two days after death. External examination didn't show significant lesions other than those compatible with hospitalization, as well as the exploration of the oral cavity. The lungs revealed massive edema and a semi-solid yellowish purulent material come out of all lung lobes. The pharynx showed nothing significant, and the esophageal mucosa showed a blackish discoloration that ended sharply at the gastro-esophageal junction; the stomach mucosa was normal. Histologic examinations confirmed the presence of bilateral bronchopneumonia associated with interstitial pneumonia, as well as an important chronic full-thickness ulcerative esophagitis in various stages in absence of exogenous material. The cause of death was attributed to a multi-organ failure secondary to septic shock and bilateral bronchopneumonia in a patient with diabetic ketoacidosis.

The diagnosis is often made during the autopsy: the role of the forensic pathologist in the definition and pathological characterization of this disease is fundamental, even in patients with severe clinical conditions, as the patient involved in our study.

In conclusion, the findings in our case suggest that AEN is a condition with onset shortly before death in combination with other pathologies. Our report specifically confirms the close correlation between diabetic ketoacidosis and AEN and how further analyses of autopsy cases of black esophagus are needed to elucidate the pathogenesis.

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Black Esophagus; Diabetic Ketoacidosis; Autopsy

I34　A Blunt Force Injury to the Chest Caused by Firework Misuse: A Case Report

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Learning Overview: The goal of this presentation is to describe autopsy findings in a death due to fireworks misuse and share some statistics from the 2022 Consumer Product Safety Commission (CPSC) annual report.

Impact Statement: This presentation will impact the forensic science community by bringing awareness regarding misuse of fireworks and highlighting some autopsy findings from a case report.

Approximately 73% of fireworks injuries in the United States occurred in the four weeks surrounding the Fourth of July holiday in 2022. There were more than 10,000 fireworks-related injuries that were treated in emergency rooms in the United States last year.[1]

We report a case of a blunt force trauma to the chest in an otherwise healthy 20-year-old male due to a misuse of fireworks. Per police investigation, the decedent had gathered behind a residence with a group of friends to set off fireworks. The witness statements describe that the decedent had ignited a mortar-type firework, and after lighting it, he picked it up and placed it in close proximity to his chest. While holding the mortar tube, it unexpectedly exploded, knocking the decedent to the ground onto his back. He was unable to be resuscitated despite resuscitative efforts and was pronounced deceased at a local hospital. Autopsy examination showed an ovoid defect of the mid chest with surrounding abrasion and contusion, and scattered abrasions of the chest, anterior neck, and face. Additionally, the underlying sternum was fractured, and the pericardial sac was notably distended and contained approximately 400ml frank blood corresponding to a 2" transmural laceration of the anterior right ventricle of the heart. Bilateral pulmonary contusions were also noted. The right hand showed ragged avulsed laceration, apparent thermal injury of skeletal muscle tissues, and bony fractures.

Firework fatalities are uncommon in the United States. According to the United States Consumer Product Safety Commission 2022 Fireworks Annual Report, 11 non-occupational related fatalities were reported in 2022, mostly associated with mortar-style devices, with non-fatal injuries being significantly more common.[1,2] Five of the deaths were associated with firework misuse.[1] This case report highlights the autopsy findings in a death due to fireworks misuse.

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Blunt Force; Fireworks; Mortar

I35   Frail Elder Abuse in Long-Term Care Facilities: A Suspected Case of Atypical Asphyxia

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Learning Overview: The goal of this presentation is to describe an unusual case of atypical asphyxia due to three combined factors. The case concerns an elderly psychiatric patient who was found dead in a nursing home as a result of the restraining measures on suspicion of neglect constituting maltreatment. This presentation is intended to draw the attendees' attention to the importance of a shrewd forensic medical approach in suspected elder abuse, especially when the critical issues arise from a lack of appropriate actions.

Impact Statement: By drawing attention to a particular seldom-discussed topic, this presentation will impact the forensic science community by providing a cue to both create a network of protection for the elderly involving medical and judicial specialists and to train health care professionals to better recognize and report cases of maltreatment.

The increasing number of frail elderly individuals in long-term care facilities, coupled with the issue of a reduced health care workforce in Italy, has a marked impact on their management. To preserve their mental and physical integrity, it is often necessary to employ restraint measures; however, if not properly applied, they can themselves become a risk factor for patient safety.

In this study, we describe the case of an 89-year-old woman, living in a nursing home, suffering from sarcopenia, vascular dementia, and previous psychotic decompensation. She was found dead near the edge of the sanitary bed, kneeling on the floor, with her chest and head resting lying between bed bars. A restraining belt was wrapped around her chest, exerting traction on it and, minimally, on her neck.

Analysis of video surveillance images and clinical documentation raised suspicions of negligent behavior, protracted over time, by the staff of the nursing home. As a result, an autopsy was then performed, revealing figured ecchymoses in the left supraclavicular region, on the chest, and in the submammary region bilaterally. These injuries were attributable to the restraint belt and compression against the side bed bars, without signs of asphyxia. The autopsy also showed numerous rib fractures on the left hemithorax, associated with pulmonary emphysema and congestion, especially at the apices, in the absence of petechiae.

These findings led to the declaration that the cause of death was "violent mechanical asphyxia derived from three combined factors: positional factor, chest compression, and neck traction by the restraining belt, in a sarcopenic elderly." The above result suggests that compressive (thoracic and cervical) and positional factors, associated with neurogenic and baroceptive stimulation, were simultaneously involved in the development of acute respiratory failure, which occurred while the woman was attempting to remove the restraints. Death was ascertained at 2:30 a.m., approximately 7 hours after the last access by health care personnel to the patient's room. The time of death was estimated between 4.5 and 2.5 hours before the corpse was found (between 10:00 p.m. and 12:00 a.m. of the previous day).

Properly selecting restraint devices tailored to each patient and increasing the frequency of checking their correct positioning, coupled with pharmacological restraint in the context of timely monitoring of the psychic condition, could have prevented the patient's death. Such neglect could amount to elder abuse, defined by the World Health Organization as "lack of appropriate action, occurring within any relationship where there is an expectation of trust which causes harm or distress to an older person."

This study highlights the importance of a shrewd forensic medical approach in suspected elder abuse, especially when the critical issues arise from a lack of appropriate actions. In conclusion, attention is drawn to the necessity to create a network of medical and judicial specialists in order to protect the frail elderly with psychiatric problems within long-term care services; moreover, we point out the importance of training health care professionals to better recognize and report cases of maltreatment.[1-4]

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Elder Abuse; Atypical Asphyxia; Nursing Homes

I36 Sudden Death Secondary to Colloid Cyst of the Third Ventricle: A Case Review

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Learning Overview: After attending this presentation, attendees will be knowledgeable about the general characteristics and the presentation of colloid cysts, sudden death associated with colloid cysts, and current research involving the risk factors associated with sudden death secondary to colloid cysts.

Impact Statement: This fascinating case review presentation can significantly impact the forensic science community by raising awareness, improving detection and diagnosis, and guiding future investigations in cases of sudden and unexplained deaths. Furthermore, this presentation will elucidate the risks associated with sudden deaths secondary to colloid cysts and illuminate potential areas of future research.

Sudden and unexpected deaths pose significant challenges to forensic pathologists in accurately determining the cause and manner of death. We present a case review of a 26-year-old male who died suddenly and unexpectedly. The decedent's medical and social histories were carefully analyzed to ascertain potential contributing factors to his sudden death. Past medical history was notable only for migraine headaches. The decedent had been evaluated by neurology several years prior to death, but no head imaging had been performed. Family members were unaware of any changes in migraine symptoms or any suicidal ideations, and they only noted that he had a viral gastrointestinal illness the week prior. The father noted the decedent to be a social drinker, and he was unaware of any illicit drug or tobacco use. Death investigators responding to the scene noted an abundant amount of empty alcohol containers throughout the decedent's living quarters.

The postmortem Computed Tomography (CT) revealed the presence of ventriculomegaly and a hyperdense mass lesion within the third ventricle, prompting further investigation. A comprehensive autopsy was performed, and the brain was prepared for neuropathology examination. Gross examination of the brain revealed a 1.5x 1.4x 1.3cm mass within the third ventricle to be the cause of the hydrocephalus. On microscopic examination, the mass was determined to be a colloid cyst. The cyst's location and size raised suspicions of its involvement in the sudden death.

Colloid cysts within the third ventricle are a rare but potentially life-threatening benign congenital lesion and are not considered a true neoplasm.[1] Diagnosing colloid cysts is challenging given the wide variability of presentation observed among patients. Symptoms may be non-specific, vary with rate of hydrocephalus development, present simply as worsening headaches, or not even be discovered until autopsy examination after a sudden death.[2] This case underscores the importance of thorough postmortem imaging, especially in sudden death cases where external examination alone may not yield conclusive results. This case also demonstrates the need for further research involving the risk factors associated with sudden death due to colloid cysts.

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Brain; Sudden Death; Computed Tomography

I37 Marjolin's Ulcers in the Forensic Setting: When Cancer Becomes a Homicide

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Learning Overview: After attending this presentation, attendees will have learned to include Marjolin's ulcer to their list of differentials in cases where the decedent has remote injuries.

Impact Statement: This presentation will impact the forensic science community by bringing awareness to the association between Marjolin's ulcers and remote injuries in a forensic setting.

Background: A Marjolin's ulcer is a rare aggressive cutaneous malignancy that develops from an area of skin with a pre-existing scar or chronic injury. The leading causes are scars associated with burns and fistulae associated with osteomyelitis, but it can also arise in the setting of pressure ulcers. Squamous cell carcinoma arises more than 90% of the time, but other cutaneous malignancies can also develop. It more commonly develops in men with a 36-year average reported latency period between the time of injury to the onset of malignant transformation.[1-3]

Case Descriptions: We report three cases of delayed homicides associated with Marjolin's ulcer development due to paraplegia secondary to gunshot wounds. The first decedent was a 47-year-old male who developed sacral decubitus ulcers associated with paraplegia after a gunshot wound of the torso injured his T10 vertebra and spinal cord. Twenty-six years after his initial injury, he was diagnosed with metastatic squamous cell carcinoma arising from an ischial pressure ulcer and died a year later. The second decedent was a 39-year-old male who developed extensive pressure ulcers associated with paraplegia due to a gunshot wound of the chest which injured his T10 vertebra and spinal cord. Twenty-two years after his initial injury, he died due to complications, including metastatic squamous cell carcinoma arising from a chronic wound in the groin area. The third decedent was a 46-year-old male who developed sacral decubitus ulcers associated with paraplegia due to a gunshot wound to the right shoulder that injured his spinal cord at the T8-T9 level. Eighteen years after the initial injury, he died of complications of paraplegia, including metastatic squamous cell carcinoma arising within a sacral decubitus ulcer.

Discussion and Conclusion: The average age of the decedents was 44 years. A history of chronic osteomyelitis at the site of the primary cancer was found in the past medical history of all three of the decedents. The average latency period from the time of decubitus ulcer formation and malignant transformation could not be ascertained with certainty, but the average time from injury resulting in paraplegia to death was 22 years. Squamous cell carcinoma is the most prevalent malignancy identified in Marjolin's ulcers and was confirmed in all cases in this study. In conclusion, Marjolin's ulcers should be in the differential when working cases of decedents with chronic wounds and paraplegia, including those associated with remote injuries.

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Cancer; Homicide; Cause of Death

I38 Oxidative Stress in Sepsis: A Focus on Cardiac Pathology

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Learning Overview: The goal of this presentation is to demonstrate the oxidative stress role in the etiopathogenesis and/or exacerbation of sepsis in order to prove the importance of the postmortem examination and histological analysis, not only for understanding the pathological mechanism but also the impact on human life, consequently supporting the rationale for including therapies targeting redox abnormalities in the management of septic patients.

Impact Statement: This presentation will impact the forensic science community by demonstrating a correlation between oxidative and nitrosative stress imbalance and the pathophysiology of cardiac dysfunction in sepsis. Therefore, subsequent studies will focus on the expression of oxidative stress markers in other organs and tissues as well as on the involvement of the intracellular pattern of apoptosis to better clarify the complex pathogenesis of multi-organ failure, leading to the consideration of the potential therapeutic effects of antioxidant agents in routine therapy in these patients.

Background: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response following an infection. Therefore, the widely accepted theory that reported sepsis as an uncontrolled inflammatory response to a pathogen that was rather a bystander than the real insult has been questioned. Indeed, the failure of numerous studies using anti-inflammatory agents has cast doubt on the hypothesis of hyperinflammation. A growing body of evidence suggests that the cellular inability to consume oxygen could play a crucial role in the pathogenesis of sepsis.[1-11] The present research project aims: (1) to evaluate the existence or extent of oxidative stress in subjects whose cause of death has been traced to sepsis; (2) to demonstrate its role in the etiopathogenesis and/or exacerbation of sepsis; and, consequently, (3) to support the rationale for including therapies targeting redox abnormalities in the management of septic patients.

Materials and Methods: The ten cases selected for the present study had died in hospitals with antemortem clinical-laboratory signs diagnosing septic shock. A Negative Control (NC) case dealing with a presumed healthy heart of a 22-year-old male who expired due to head trauma from a shotgun was also included. All the samples of tissue were fixed in formalin 10% for 48 hours, then processed and included in paraffin. For each sample, one section has been stained with Haematoxylin and Eosin (H&E) and the other sections were used for the immunohistochemical study (NOX2, NT, iNOS, 8OHdG).

Results and Discussion: The present study demonstrated that the selected markers showed a positive reaction in all the samples examined compared to the control case. In detail, the inducible NOS (iNOS/NOS2) is the enzyme responsible for the production of NO in sepsis in response to endotoxins, cytokines, and other mediators.[12,13] iNOS is, in fact, inactive under physiological conditions and is not constitutively expressed in cells. The increased NO production was correlated with arterial hypotension and increased cardiac work, favoring the onset of septic-induced cardiomyopathy. On the other hand, the production of $ONOO^-$ is a crucial pathophysiological event that occurs during sepsis since it represents a cytotoxic factor in tissue damage mediated by oxidative stress. $ONOO^-$ and its derivatives can enter the cell membrane and consequently oxidize multiple target molecules, either directly or through the generation of highly reactive radicals. $ONOO^-$ can lead to the production of nitrogen dioxide (NO_2), which can lead to redox reactions with the consequent formation of nitrate species, such as nitrotyrosine. Another relevant interaction of $ONOO^-$ occurs with nucleic acids, with the production of 8-hydroxydeoxyguanosine. On the other hand, some studies have shown that the gp91phox subunit of NADPH oxidase involved in the production of ROS would determine a critical role in myocardial depression induced by endotoxemia and contribute to the expression of TNF-α, which in turn exerts a negative inotropic effect.[14] A further elucidation deserves the study of the samples of subjects who died from Sars-Cov-2 infection that showed an intense positivity especially for the oxidative stress products (8-OHdG and NT), higher than the other preparations. This could suggest a greater involvement of oxidative stress in the pathogenesis of septic development.

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Histological; Disease; Heart

I39 Blunt Force Trauma and Aspiration of Gastric Contents: What Is the Cause of Death? An Unusual Case

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Learning Overview: After attending this presentation, attendees will understand that aspiration of gastric contents can indeed occur shortly after a craniocephalic trauma, resulting in a synergic effect of the two pathophysiological processes in the causation of death. When the results of the blunt trauma alone are not sufficiently lethal to explain the event, other findings need to be investigated by performing a postmortem Computed Tomography (CT) exam, a full autopsy, and a microscopic analysis with immunohistochemistry panels to confirm death by gastric contents aspiration.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of providing an effective protocol based on our experience: an accurate crime scene investigation, followed by gross examination of the affected systems and organs, together with histological and immunohistochemical analysis of the damaged tissues are mandatory.

Vomiting and aspiration of gastric contents into the airways and lungs are a well-known clinical-pathological entity, which includes airway obstruction, the development of chemical pneumonia, the onset of secondary infections in the airways and lung parenchyma, and in some cases, death.[1] In general, aspiration tends to occur in individuals with a decreased level of consciousness or in those with predisposing conditions.[2] Cases of sudden death due to massive aspiration of gastric contents have been described in certain individuals, such as pregnant women and individuals with significant alcohol intoxication (above 150mg/100ml), in the absence of other causes.[3,4]

In forensic pathology, the presence of gastric material in the respiratory tract is not necessarily significant, as it has been demonstrated that reflux of gastric material from the stomach into the airways can occur in the postmortem period.[4-7] Hence, in the absence of clear evidence, the diagnosis of death from aspiration of gastric contents can be quite challenging in the forensic practice, especially in complex cases, such as homicides, where the reconstruction of the physiopathology plays a crucial role.[4-8]

This study deals with the case of a 39-year-old man who died following a bare-handed assault that took place on the street. Emergency services were immediately contacted by bystanders, and therefore, the circumstantial data regarding the timeline of the incident appeared clear. During the crime scene investigation, vomit was found in the proximity of the dead body, and, upon closer inspection, the oral cavity was filled by fluid with gastric contents. The clothing was stained by a similar material as well. The external examination revealed diffuse contusion and abrasion injuries, mostly located at the level of the head and the face.

Prior to the autoptic examination, a CT scan was performed, which revealed mild subdural hemorrhage at the level of the temporal lobes, while the esophagus and the upper and lower airways were filled by an amorphous foamy material mixed with fluid. Furthermore, the lungs showed diffused parenchymal and subpleural opacities, mostly evident on the pulmonary apices and the bases. The autopsy showed a picture of mild subdural hemorrhage, with minimal foci of subarachnoid hemorrhage. The lungs showed diffuse petechial hemorrhages, clustered in the subpleural layers. Examination of the airways and lungs confirmed the CT findings, showing bronchial obstruction down to the smaller branches. Overdistension of the gastric wall was observed as well.

Microscopically, histological examination with hematoxylin-eosin highlighted diffuse macrophage infiltration in the alveolar spaces, which were filled with cellular debris and amorphous material, as in bronchoaspiration. Immunohistochemical staining with β-APP in brain tissue and HIF in lung samples was positive. Toxicological investigations showed ethanol concentrations slightly below 150mg/100ml. The cause of death was attributed to acute cardiorespiratory failure secondary to internal mechanical asphyxia from massive inhalation of gastric material (vomit) in a subject with cranioencephalic trauma.

This case demonstrates the necessity of using multiple investigations in controversial deaths, such as massive vomit inhalation, especially in a homicide case. The combination of several conditions, including mild cerebral hemorrhage, acute alcohol intoxication, and a full stomach, makes this diagnosis one of the most challenging to establish, requiring multiple objective pieces of evidence. This becomes even more problematic in cases of homicide where multiple questionable findings overlap (such as cerebral hemorrhage, alcohol intoxication, and gastric distension). Therefore, in the event of suspicion of massive inhalation of vomit, additional complementary investigations are necessary, starting from postmortem imaging up until immunohistochemistry, in order to provide an accurate reconstruction.

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Asphyxiation; Blunt Force; CT Scan

I40   Dealing With a Massive Number of Victims Due to an Accidental Fire

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Learning Overview: After attending this presentation, attendees will be familiar with the identification of victims of an accidental fire.

Impact Statement: This presentation will impact the forensic science community by describing victim identification in the case of an accidental fire in Baghdad, Iraq.

On Saturday, April 4, 2021, around 11:00 p.m., the Ibn Al-Khatib hospital in Baghdad, which is devoted to the treatment of COVID-19 cases, was set on fire due to an electrical fault. Many people were injured and some even died. The medicolegal directorate in Baghdad started to receive all of the dead cases and an effort was made to overcome this catastrophic disaster by grouping the received cases in the mortuary into known (recognizable)—having enough external criteria to be recognized—and unknown (unrecognizable)—completely charred group. The known group was even subdivided according to cause of death into: death due to burns, death due to asphyxia by Carbon Monoxide (CO). Cases that couldn't be recognized were also dealt with by taking charred blood mixed with distal water that was put on an FTA card and referred to the DNA section within the directorate to complete the work, taking blood samples from families who had missing relatives who were hospitalized in the Ibn Al-Khatib hospital at the time of the incident. The total number of received cases was 56; of these, 3 were known and 53 were unknown.

For unrecognizable cases the work was performed in two stages. Stage one compared charred blood samples from an FTA card of the deceased with a blood sample from families of missing relatives, allowing the identification of 42 of the unknown cases. Stage two involved matching charred blood samples of the deceased with each other as many relatives were hospitalized at the same hospital at the time of incident. This latter technique provided invaluable information and allowed us to identify 11 of the deceased cases.

The end result of this effective work was that the total number of received cases was 56; of these, 3 cases were immediately identified, 42 cases were identified by matching with relatives, and 11 cases were identified by matching with other deceased cases.

Accident; Burn; DNA

I41   Bee-Ware of MDPHP: A Case of Intoxication by Synthetic Cathinones Resulting in Hundreds of Bee Stings

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Learning Overview: After attending this presentation, attendees will be aware of a more uncommon way bee stings can cause death rather than anaphylactic shock—by mass envenomation provoking arrhythmic cardiac arrest in a subject who had taken drugs of abuse.

Impact Statement: This presentation will impact the forensic science community by reporting a case of abuse of a relatively unknown designer drug (MDPHP) resulting in an incident involving mass envenomation by bees.

In a small city in northern Italy, some bystanders saw a 53-year-old man running by for a distance of about 4 kilometers (2.5 miles); he had his pants lowered to the knees and his genitalia exposed. He then bumped into an apiary, whose honeybees immediately began to chase and sting him. After a few meters, he fell down, still surrounded by bees. The rescue team, alerted by the witnesses, was able to disperse the bees a few hours later and finally reached the man, who was declared dead.

At the scene inspection performed by the law enforcement, the body presented with hundreds of bee stings and a bleeding wound to the head; additionally, bloodstains were found along the path he covered. A forensic autopsy was requested and performed, including histological, toxicological, and genetic investigation.

External examination of the body confirmed the presence of a large number of bee stings all over the skin and even in the mouth, much of them with the stinger still in site; several wounds were also noted, in particular the large lacerated and contused one in the parietal region of the head with a length of 5.5cm circa. Autoptic examination most notably showed no skull fractures, widespread coronary stenosis, and edematous lungs with hemorrhagic areas; the larynx did not appear edematous. Histopathological examination was performed and didn't show any evidence of allergic reaction in the tongue nor in the larynx sample but confirmed the coronary sclerosis.

Genetic tests confirmed the blood collected on the street belonged to the man in question. A toxicological exam conducted on blood samples was positive for MDPHP at a concentration thought to be fatal (398ng/ml).[1] Moreover, benzoylecgonine (cocaine's metabolite) and paroxetine (regularly prescribed by his family doctor) were found in blood.

MDPHP is a designer drug of the cathinone class, thus having a strong stimulant action.[2] In literature, very few cases of fatal overdose by MDPHP are reported, and its effects are not yet fully known. Overall, synthetic cathinones are very powerful drugs whose stimulating effect are able to deeply affect the cardiovascular and nervous system. On the other hand, deaths caused by bee and other Hymenoptera stings occur more often because of allergic reactions to venom components in the face of a limited number of stings; however, deaths have also been reported to be caused by mass envenomation when hundreds of insects sting a subject in a limited span of time, injecting a series of toxic compounds whose effect is neglectable when only few stings occur.[3]

Overall, the absence of macro and microscopic signs of anaphylactic shock is suggestive of a much rarer way by which bee stings can cause death.[4] In our case, the massive dose of venom injected by hundreds of stings was considered to be enough to cause an arrhythmic cardiac arrest, particularly because the subject's heart function was already severely impaired by drugs of abuse.

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Cause of Death; Honeybees; Drug Abuse

I42   When a Nursing Home Turns Into a Killing Home—Forensic Aspects of Serial Murder in Health Care: A Case of a Serial Killer Nurse

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Learning Overview: Serial murders in health care facilities are rare events and their immorality imposes a firm condemnation on the ethical and professional code. The goal of this presentation is to show that the collaboration between forensic sciences (forensic pathology, forensic toxicology, and forensic pharmacology) and forensic investigations is crucial in solving such an intricate and complex case, where the deaths took place inside a facility that was considered, until proven otherwise, safe for patients.

Impact Statement: This presentation will impact the forensic science community be informing attendees that to solve a complicated case like the one presented, characterized by a series of homicidal events and an attempted homicide, plus the pharmacological and toxicological results, careful forensic evaluation is fundamental. Only in this way was it possible to compare the symptomatologic clinical data with the toxicological results (when available), finally noting an effective correlation between the administration of drugs and the deaths.

Serial murders committed by health care professionals in health facilities are a barely understood phenomenon that have come to light in recent years.[1-8] Among health care personnel, nurses are the most involved, primarily because of the easy access to drugs they have as their main role is the administration of therapies.[9] Furthermore, the nursing staff often assists highly vulnerable patients such as the elderly, terminal, and disabled patients who are often in critical condition and therefore objectively unable to defend themselves.

Drug injection is the major route used to perform murders, followed by air embolus. Injection is primarily used for drugs that are frequently already prescribed for that patient and whose effects are generally masked by the symptomatology of the treated disease. Moreover, the analysis of their postmortem concentration may be difficult to interpret as the differentiation from endogenic or therapeutic values (for example, insulin, potassium, and epinephrine) could be complicated.[10]

This presentation showcases a series of murders and attempted murders committed by a nurse in a health care residence in eastern Italy. All the victims were fragile patients: most were elderly and suffered from neurological and cardiac pathologies. Specifically, three patients died after repeated and inexplicably serious hypoglycemic events, unresponsive to glucose therapy. In these cases, the toxicological investigations conducted on ante- and postmortem blood samples and the pharmacological forensic analysis established that a long-acting insulin was used as the drug to perform the homicides. This hypothesis has become predominant because of the trends that the blood sugar curves of these patients had assumed in the days before the deaths. Only one patient survived the insulin administration, and this case constituted, among all presented, the case of attempted homicide. Four other patients died after episodes of obnubilation and coma that were preceded by some psychomotor agitation crisis. Even in these cases, thanks to forensic and toxicological investigations, it was established that the drug used was the promazine.

Thanks to the forensic investigation and on the basis of the toxicological and pharmacological results, the trial ended with the nurse being sentenced to life imprisonment for seven murders and one attempted murder, with the recognition of aggravating circumstances.

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Serial Killer; Nurse; Toxicology

I43　Respiratory Illness Surveillance in Suspected Drug-Related Deaths

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Learning Overview: After attending this presentation, attendees will be familiar with the risk of pulmonary infections in individuals with Substance Use Disorder (SUD) and the value of a full autopsy in cases of suspected drug-related deaths.

Impact Statement: This presentation will impact the forensic science community by illustrating the value of interpreting postmortem respiratory pathogen test results in the full context of the medicolegal death investigation.

Introduction: Individuals with SUD are at an increased risk for pulmonary infections for multiple reasons, including substance abuse-related cardio-pulmonary morbidities, compromised immunity, housing instability, and inadequate access to health care.[1] The authors sought to identify whether a correlation exists between respiratory illness and suspected drug-related deaths, as these findings may help to inform future public health response efforts.

Methods: Western Michigan University Homer Stryker M.D. School of Medicine (WMed) serves as the Medical Examiner's Office for 12 Michigan counties. Per performance standards set forth by the National Association of Medical Examiners for accredited offices, all suspected drug-related deaths are investigated, and these decedents are examined by a forensic pathologist. In February of 2021, postmortem laboratory testing for deaths occurring outside of a health care facility began as a part of a multi-site study funded by the Centers for Disease Control and Prevention and the Council of State and Territorial Epidemiologists. Laboratory testing was performed at the Michigan Department of Health and Human Services Bureau of Laboratories (BOL).

To better understand the potential correlation between respiratory illness(es) and suspected drug-related deaths, postmortem nasopharyngeal swabs were collected from these decedents throughout 2021, 2022, and 2023. Nasopharyngeal swabs were then tested for SARS-CoV-2, Influenza A, and Influenza B. A subset of these samples was also submitted for an expanded respiratory panel.

Results: During the project period, samples were collected at autopsy from 369 suspected drug-related deaths and were tested as previously described. Twenty-four (6.5%) decedents tested positive for SARS-CoV-2, while only one of these deaths indicated SARS-CoV-2 as contributory on the death certificate. All 24 decedents that tested positive for SARS-CoV-2 were confirmed to be drug-related deaths. Of the decedents with SARS-CoV-2 detected, nine (37.5%) experienced a least one symptom before death, four (16.7%) were asymptomatic, and eleven (45.8%) had either undocumented or unknown symptoms. Only three (12.5%) of these decedents had at least one vaccination against SARS-CoV-2.

Three additional individuals out of the 369 suspected drug-related deaths tested positive for respiratory pathogens other than SARS-CoV-2, including one decedent positive for Influenza B, one decedent positive for Rhinovirus/Enterovirus, and one decedent positive for both Coronavirus OC43 and Rhinovirus/Enterovirus. There were also two SARS-CoV-2 cases with multiple pathogens present, including Adenovirus and Rhinovirus/Enterovirus. None of the additional respiratory pathogens were deemed contributory to the death of any of these decedents.

Conclusion: People with SUD are at an increased risk for pulmonary infections. While several decedents that were suspected to have died due to drug use also tested positive for respiratory pathogens, the majority of these pathogens were deemed non-contributory to the death. Without a full autopsy, including gross and microscopic examination of the lungs and other organs, many of these deaths may have been attributed to a respiratory infection. This study highlights the need for a comprehensive postmortem examination of all suspected drug-related deaths so that the results of ancillary studies may be appropriately interpreted by the forensic pathologist.

Reference:

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COVID-19; Drug Abuse; Autopsy

I44   Comparing Manner of Death in Georgia Prison and Jail Populations

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Learning Overview: After attending this presentation, attendees will know about the Georgia prison and jail populations and how Manner of Death (MoD) differences between them have evolved.

Impact Statement: This presentation will impact the forensic science community by providing evidence of how correctional facility deaths occur in a disproportionate manner based on incarceration environment and by proposing future studies to address concerning trends.

Background: The increasing amount of deaths in state prisons are of growing concern. In 2005, the Bureau of Justice Statistics (BJS) published a comparison of homicide and suicide in United States state prisons and jails with evidence that suggested the rates of suicide were higher in jail than in prison and that rates of homicide were similar in both environments in 2002. Since then, numerous studies indicate worsening death rates in United States prisons, causing this office to assess MoD within the incarcerated population of Georgia.

Methods: In this retrospective study, we extracted MoD information from autopsy and investigative reports in all prison population deaths that were reported to the Georgia Bureau of Investigation (GBI) from November 2018 to December 2021. After assessing the difference in demographic variables (age, race, sex) between the prison death and jail death populations, the difference in MoD was assessed.

Results: A total of 300 decedents, aged 18–86 years (mean age of 48 in jail; 50 in prison) were identified. A total of 288 (96%) were male; 12 (4%) were female. A total of 2 (<1%) were Asian, 150 (50%) were Black, 7 (2.3% were Hispanic), 139 (46.3%) were White, and 2 (<1%) were unknown. In Georgia prisons, the breakdown of MoD is as follows: 142 natural, 14 accidental, 35 suicide, 37 homicide, and 4 undetermined. In Georgia jails, the breakdown of MoD is: 37 natural, 10 accidental, 20 suicide, 1 homicide, and 0 undetermined. We discovered that inmate deaths are significantly ($p=0.008$) more likely to occur by homicide in the Georgia prison population than in the jail population. Inmate deaths in jail are significantly ($p=0.02$) more likely to occur by suicide than deaths in prison. We also discovered a significant association ($p=0.007$) between race and death in prison vs. jail.

Conclusion: There is an association between the incarceration environment and MoD in Georgia. However, this data was collected during the COVID-19 pandemic and more studies are necessary to understand if there was a role in the COVID-19 pandemic in this observed association.

Reference:
1.   Mumola CJ. Suicide and Homicide in State Prisons and Local Jails. *Bureau of Justice Statistics*, Aug 2005, NCJ 210036.

Prison Death; Jail; Manner of Death

I45 The Effect of Preservation, Time, and Temperature on DNA Recovery of Blow Fly Specimens (Diptera: Calliphoridae)

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Learning Overview: After attending this presentation, attendees will discover the ways that preservation, time, and temperature can play a role in how DNA is recovered from insect evidence, in particular blow fly specimens.

Impact Statement: This presentation will impact the forensic science community by further strengthening the standard operating procedures in the forensic entomology community.

Correct blow fly (Diptera: Calliphoridae) species identification is a fundamental prerequisite of any minimum time of death investigation using insect evidence. To successfully identify preserved samples, Standard Operating Procedures (SOP) must be followed. If these SOPs are not followed, this can affect the integrity of the samples and, therefore, affect the success rate of species identification. In situations where specimens are stored incorrectly, or poorly preserved/damaged, genomic, and mitochondrial DNA is relied upon to amplify, sequence, and analyze a barcoding region for species identity.

While it is commonly agreed in the forensic entomology community that 70–80% ethanol after hot water killing of larval specimens should be used, the effect of temperature, time, and preservative has not been extensively examined in relation to the success rate of retrieving and sequencing DNA. This project aimed to examine the effect that different preservation treatments and time had on the success rate of recovering DNA for amplification and species identification.

The larvae of *Calliphora latifrons* Hough, 1899 (Diptera: Calliphoridae) were sampled and underwent a set number of treatments. Four preservatives (formalin, Carnoy's solution, 70% ethanol, and 100% ethanol), time in preservation (6 months, 3 months, 1 month, 1 week, and 1 day), and temperature (-20ºC, 4ºC, and 20ºC) were examined. Using a modified QIAGEN® DNeasy® method, DNA was extracted and quantified using Qubit® fluorimetry. An approximate 600-base pair region of the *cytochrome oxidase I* was amplified to determine successful amplification of the species barcoding region. A subset of successful amplicons was sequenced using Sanger sequencing at Arizona State University and inputted onto Blastn for species assignment validation. Statistical analysis was applied to DNA quantification data and found that preservatives had a significant effect on the recovery and success rate of amplification. Future work is expected to examine the effect of longer time intervals, more preservatives, and different temperatures.

Entomology; DNA; Insects

I46 **The Succession of Invertebrate Communities Involved in Swine Decomposition in Freshwater Environments in Italy**

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Learning Overview: After attending this presentation, attendees will be informed about general concepts pertaining to decomposition in water. Main thanatological characteristics of each stage will be discussed as well as the biotic and abiotic factors that play a role in the process of decay. Moreover, attendees will learn about arthropod colonization of corpses in water and how the communities of invertebrates that visit carcasses decomposing in freshwater are significantly different across the different stages. Differences in these communities will be accompanied by explanations pertaining to their trophic role and the thanatological phenomena recorded on the bodies.

Impact Statement: The forensic science communities, especially pathologists, death investigators, and forensic entomologists, are usually exposed to terrestrial decomposition and indeed the majority of the literature produced on these topics focuses on terrestrial scenarios. Decomposition studies in water environments are in fact quite complex and difficult to execute due to the challenges presented by water itself when it comes to recovering remains and evidence. This study will impact the forensic science community by contributing to knowledge about decomposition in water and will show how ecological succession, first noticed by Mégnin in the 1800s, can also apply to aquatic ecosystems; furthermore, by gaining knowledge of aquatic decompositions, this information will be helpful for investigators and the outcomes of their investigations, given that the decay is different from the terrestrial one.

Aquatic freshwater environments are a unique and diverse habitat that can influence the properties of major ecological processes such as decomposition. Carrion decomposition patterns in aqueous environments may be similar to what has been observed in terrestrial ecosystems, but there are some major differences that are fundamentally constrained to aquatic ecosystems. Understanding the processes driving freshwater ecosystems requires not only insight into the dynamics of the organisms under study, but also how those organisms interact with their abiotic environment. For these ecosystems, the hydrochemistry itself (e.g., relative pH, salinity, temperature, humidity, etc.) is the primary abiotic influence that drives the distribution and community composition of aquatic organisms that contribute to decomposition.

Similar to soil burials, aquatic decomposition is considerably slower than that on the soil surface, but the entomofauna is quite different; to better understand the relationship between the aquatic carrion decomposition and its necrobiome, it is important to know the stages of decay, which are different from terrestrial decomposition. Aquatic decay is divided into five stages: submerged fresh, early floating, early floating decay, advanced floating decay, and sunken remains. There does not appear to be a standardized carrion decomposer community from a taxonomic standpoint, and researchers have observed a range of aquatic insects active across stages of decomposition. However, only 15% of studies found in the literature pertain to aquatic organisms and the role they play in investigations, but insects that are strictly related to aquatic ecosystems have not been extensively explored.

Here we present a study evaluating the communities of invertebrates contributing to carrion decomposition in freshwater aquatic environments in Italy, during the years 2018 and 2019. Pig carcasses were placed in four different areas of a pond in Piola di Susano, Emilia Romagna, Italy, during two seasonal periods (spring-early summer and late summer-fall); invertebrates were sampled from the carcasses throughout decomposition until skeletonization. Results showed that there is a significant change in community composition across the stages of decomposition and that behaviors of particular taxa appear to be correlated to the decomposition stage.

Forensic Entomology; Decomposition; Aquatic Insects

I47   The Persistence of DNA in Water From an Actively Decomposing Cadaver for Human Identification

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Learning Overview: After attending this presentation, attendees will have a clear understanding of the possibility of collecting DNA from a decomposing human cadaver in fresh water in addition to DNA from the surrounding water for up to one month after the cadaver has been removed.

Impact Statement: This presentation will impact the forensic science community by bringing awareness to investigators and forensic laboratories that it may be possible to identify a victim that has been decomposing in standing water for a one-month period via collection and Short Tandem Repeat (STR) analysis of the contaminated water after the body had been removed.

Human environmental DNA (eDNA) has been recovered from environmental samples known as human genetic bycatch.[1] eDNA is a tool used to commonly track aquatic life but has been shown to yield high-quality human DNA.[1] Research on human genetic bycatch from eDNA is a widely understudied field but could have beneficial applications to the forensic community.

In the summer of 2023, a pilot study was conducted at the Southeast Texas Applied Forensic Science Facility, Sam Houston State University in Huntsville, TX, to determine whether sufficient amounts of DNA could be recovered from a decomposing body submerged in freshwater for a month using routine STR analysis. More interestingly, we examined whether the individual could also be positively identified from DNA recovered from water samples collected from the tank after the body had been removed.

One human cadaver was placed in a 228 x 85 x 57cm tub and filled with approximately 500L of freshwater. The female cadaver placed in the tub had been autopsied, with her right lower limb disarticulated prior to this study. Samples were taken three times a week from the thigh muscle (rectus femoris) and the surrounding water for 30 days. The cadaver was then removed from the tank, and water samples were continued to be collected three times a week for an additional 30 days.

Vacuum filtration with a Buchner funnel system and a nitrocellulose filter were used to collect DNA from 30mL of water sampled at each time point. The DNeasy® Blood and Tissue Kit protocol, Purification of total DNA from Animal Blood or Cells, was used for purification with some modification as outlined Djurhuus et al.[2] DNA was extracted from 10mg of muscle tissue using the EZ1® DNA Investigator kit on the EZ1® platform. Neat DNA extracts were then quantified via quantitative Polymerase Chain Reaction (qPCR) using the Quantifiler® Trio kit on the ABI® 7500 instrument. All samples with detectable amounts of DNA were then amplified using the VeriFiler® Plus PCR Amplification kit to generate STR profiles for analysis.

Results of this pilot study showed that quantifiable amounts of DNA were recovered from muscle tissue collected from a decomposing cadaver submerged in freshwater for up to one month. Full or partial STR profiles could be obtained through the length of the study; however, DNA degradation and loss of loci increased markedly over time. The ability to obtain enough DNA for even partial profiles became difficult after two weeks, and the vast majority of water samples failed to generate an STR profile after the cadaver had been removed from the tank.

References:
1.   Whitmore, L., McCauley, M., Farrell, J.A. et al. Inadvertent human genomic bycatch and intentional capture raise beneficial applications and ethical concerns with environmental DNA. *Nat Ecol Evol* 7, 873–888 (2023). https://doi.org/10.1038/s41559-023-02056-2.
2.   A. Djurhuus et al., Evaluation of Filtration and DNA Extraction Methods for Environmental DNA Biodiversity Assessments across Multiple Trophic Levels, *Front Mar Sci*, vol. 4, Oct. 2017, doi: 10.3389/fmars.2017.00314.

DNA Extraction; Taphonomy; Water

I48   Death From Angioedema With Anaphylaxis and COVID-19

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Learning Overview: The goals of this presentation are to: (1) describe the types of angioedema and if COVID-19 could cause angioedema; (2) inform attendees about the work-up of a decedent who died from angioedema in a postmortem setting; and (3) analyze the quality of the clinical outpatient management in the case being presented and if this could have been a potentially preventable death.

Impact Statement: The presentation will impact the forensic science community by demonstrating that COVID-19 can cause angioedema and screening for same as part of the medicolegal death investigation is warranted and by updating attendees on what should be done as part of the clinical evaluation of a patient with angioedema to guide them in the decision-making of whether to admit the patient for observation/airway management.

The decedent was a 58-year-old man who developed swelling of the face, neck, and throat resulting in difficulty swallowing. He went to his primary care doctor, who treated him with epinephrine, dexamethasone, diphenhydramine, and ranitidine. He remained at the doctor's office for an hour, with improvement of symptoms, and then went to the pharmacy. In the parking lot of the pharmacy, he developed difficulty breathing and swallowing and could not be resuscitated. He did not report any food or drug allergies and had not suffered from any insect bites.

His girlfriend told the Medical Examiner-Coroner (ME-C) investigator that approximately two weeks prior to his demise he was diagnosed with high blood pressure and started on an ACE inhibitor. In addition to facial swelling, he had difficulty swallowing that day. The investigator also noted red discoloration of the arms in addition to facial swelling. There was no history of itching or hives. His last meal was the night prior and included salmon and green beans.

Autopsy showed macroglossia (with edema) and edema of the lips, trachea, epiglottis, larynx, and bronchi. Histology of the epiglottis showed significant edema of the epiglottis, forming a blister-like structure, and edema of the aryepiglottic folds. The stomach contained 10mL of pink fluid, no food or pill particles. A urine screen was negative for drugs. Nasopharyngeal Swab Film Array Viral Panel studies revealed the detection of SARs Cov 2(COVID 19).

Angioedema is non-dependent, non-pitting edema at a variety of sites. Its forms can be divided into histamine-mediated and bradykinin-mediated types. Histamine-mediated forms can present similarly to anaphylaxis, while bradykinin-mediated angioedema is slower in onset, presents with greater face and oropharyngeal involvement, and has higher risk of progression.

A discussion analyzing the quality of the outpatient management in the case with a focus on acts of omission in clinical evaluation by the primary care doctor who discharged him home instead of admitting him to the hospital for airway management/observation, differential diagnosis, what additional work up was requested, and final conclusions will be presented.

After SARS-CoV-2 gains cellular entry by binding to ACE2, it results in down-regulation of surface ACE2, impairing its role in the breakdown of several substrates, including bradykinin metabolites. Vascular permeability is a proposed pathological feature of COVID-19, and the dry cough has been remarked to resemble the idiopathic cough seen in 10–15% of patients who receive angiotensin-converting enzyme (ACE) inhibitors. Both ACE inhibitor-induced cough and certain forms of angioedema are thought to be bradykinin dependent. Bradykinin and its metabolites are cleaved by a series of enzymes, including ACE and its homolog, ACE2. Preclinical models have suggested that bradykinin receptor signaling may be a factor in facilitating pulmonary injury and inflammation. Whether the decedent died with COVID-19 or from COVID 19 causing angioedema will also be discussed.[1-4]

References:
1. Brit Jeffrey Long, MD, Alex Koyfman, MD, Michael Gottlieb, MD. Evaluation and Management of Angioedema in the Emergency Department. *Western Journal of Emergency Medicine*, Volume 20, no. 4: July 2019,Pages 587 to 600.
2. Jonathan Bernstein et al. Angioedema in the emergency department: A practical guide to differential diagnosis and management. *International Journal Emergency Medicine.* 2017; 10: 15.
3. I Mohamad, MS Narayanan. Ludwig's angina -Double tongue appearance. *New England Journal of Medicine* 2019; 381:163-163.
4. Angioedema and COVID-19. *European Heart Journal* (Volume 41, Issue 34, 7 September 2020, Pages 3283–3284.

Asphyxiation; Evaluation; COVID-19

I49 **Deaths Due to COVID-19 Infection in Children and Young People Aged 0–19 at the Harris County Institute of Forensic Sciences in Houston, Texas**

Jennifer L. Ross*, Harris County Institute of Forensic Sciences, Spring, TX

Learning Overview: The goal of this presentation is to help attendees have a better understanding of the deaths in children and young people aged 0–19 years due to a COVID-19 infection at the medical examiner's office in Houston, TX, and to be able to characterize risk factors and gross and histologic findings in this population.

Impact Statement: This presentation will impact the forensic science community by providing information about those cases of COVID-19 infection in children and young people that did not die in the hospital, but presented to the medical examiner's office in Houston, TX.

In the United States, COVID-19 infection is rarely the primary cause of death in Children and Young People (CYP) aged 0-19 years.[1,2] Because COVID-19 deaths in CYP are rare, the mortality burden of COVID-19 in this population is best understood in the context of all other causes of death in CYP. Currently, there is a relative lack of information characterizing the CYP COVID-19 deaths examined at the Harris County Institute of Forensic Sciences (HCIFS).

retrospective study was conducted to review all deaths in CYP (age 0-19) with COVID-19 infection as a primary or contributory cause of death on autopsies performed by the medical examiner's office in Houston, TX, (one of the most populous cities in the United States) during a three-year period (2020 to 2022). Death due to COVID-19 infection was found in 0.54% of all CYP deaths examined at HCIFS in the year 2020, 1.03% of all CYP deaths examined at HCIFS in the year 2021, and 1.23% of all CYP deaths examined at HCIFS in the year 2022 (total of 0.0095% of all CYP deaths due to COVID-19 infection over this three-year period). A total of 11 cases were classified with COVID-19 infection as the primary or contributory cause of death in CYP during this three-year period. The demographics of the individuals, symptoms of infection, comorbidities and autopsy and histologic findings were reviewed. COVID-19-related fatalities primarily occurred in Hispanic male CYP, and the average age at death was 10 years (range: 1 month to 19 years). Obesity was defined as comorbidity in 3 of the 11 cases. Other contributing factors included asthma exacerbation (2 cases), co-existing pneumonia (2 cases), meningoencephalitis (1 case), viral myocarditis (1 case) and toxic megacolon (1 case).

The results of this study provide a better understanding of the deaths in children and young people aged 0-19 years due to COVID-19 infection that were examined at the medical examiner's office in this region, creating an opportunity to characterize risk factors in this population.

References:
1. S Flaxman; C Whittaker; E Semenova, et al. Assessment of COVID-19 as the Underlying Cause of Death Among Children and Young People Aged 0 to 19 Years in the US. *JAMA Network Open.* 2023;6(1):e2253590. doi:10.1001/jamanetworkopen.2022.53590.
2. *COVID-19 Mortality Among Children and Adolescents United States January 2020-September 2022.* www.covkidproject.org.

COVID-19; Cause of Death; Pediatric

I50 Drowning Associated With Carbon Monoxide Toxicity in the Setting of Recreational Boating

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Learning Overview: After attending this presentation, attendees will understand the importance of considering carbon monoxide toxicity as a potential risk factor contributing to accidental drowning associated with recreational boating.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of carbon monoxide testing in boating-associated drowning cases and its importance in public health and safety awareness surrounding recreational boating.

While drowning is a well-known risk associated with boating, carbon monoxide toxicity from boat exhaust is an often-overlooked risk that can be associated with this recreational activity. Dangerous levels of carbon monoxide are most commonly considered as a risk associated with enclosed spaces; however, open-air exposure during recreational boating is also an important means of toxic accumulation that is less well known to the general public. Engine exhaust accumulates at the aft region of the boat, either while idling or when the boat is in motion; thus, it is dangerous for passengers to sit in the far aft region of the boat. Recreational boating statistics from the United States Coast Guard listed 47 deaths associated with carbon monoxide toxicity (drowning unspecified) from 2013–2022.[1,2] This presentation strives to highlight the relevance of carbon monoxide testing in cases of drowning while boating. Presented here are two similar cases of open-air carbon monoxide exposure contributing to drowning during recreational boating.

A 15-year-old female was sitting on the back platform of a slowly moving wake boat. Witnesses onboard stated she appeared to be falling asleep. She removed her life jacket and moments later, fell off the platform and into the lake. Her body was later recovered after a search. Autopsy findings demonstrated fixed pink posterior lividity. Postmortem toxicology testing demonstrated a carboxyhemoglobin saturation concentration in femoral blood of 52%.

An 8-year-old male was last seen removing his life jacket while sitting on the back platform of an idling wake boat. The family later noticed that he was no longer on the platform, and his body was recovered from the lake. Autopsy findings demonstrated cherry-red coloration of the organs and soft tissues. Postmortem toxicology testing demonstrated a carboxyhemoglobin saturation in femoral blood of 51%.

References:
1. *2017 Recreational Boating Statistics.* U.S. Department of Homeland Security, U.S. Coast Guard, and Office of Auxiliary and Boating Safety. 11 May 2018, www.uscgboating.org/library/accident-statistics/Recreational-Boating-Statistics-2017.pdf.
2. *2022 Recreational Boating Statistics.* U.S. Department of Homeland Security, U.S. Coast Guard, and Office of Auxiliary and Boating Safety. 25 May 2023, www.uscgboating.org/library/accident-statistics/Recreational-Boating-Statistics-2022.pdf.

Carbon Monoxide; Recreational Boating; Drowning

I51   A Cornucopia of Death by Gas: Fatalities Involving Organic and Inorganic Gases

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Learning Overview: After attending this presentation, attendees will gain an understanding of the spectrum of fatalities caused by organic and inorganic gases, to include the circumstances, scene and autopsy findings, toxicological analysis, mechanisms of death, cause and manner of death, and epidemiological patterns by highlighting several case studies from residential and workplace settings.

Impact Statement: This presentation will impact the forensic science community by providing insight into fatalities causes by gases, highlighting scene investigation and multidisciplinary approaches, reviewing autopsy findings, providing an awareness of the limitations of toxicological testing, and assisting in determining cause and manner of death.

Forensic pathologists encounter a wide variety of fatalities associated with different organic and inorganic gases. These types of deaths can be challenging and often rely heavily on thorough investigative information as many gases are not able to be detected in postmortem specimens or may not be tested for and missed entirely, resulting in inaccurate causes of death. Fatalities occur in a wide variety of settings to include residential or industrial scenes.

The New York City Office of Chief Medical Examiner and Harris County Institute of Forensic Sciences electronic databases were searched for all fatalities where the cause of death was due to inhalation of a gas during the past ten years, excluding gases related to combustion, such as carbon monoxide. A total of 281 cases were identified—188 in New York City and 95 in Houston.

Of these cases, 211 were male and 70 were female. The ages ranged from 17 to 95 years (average age: 42 years). 200 were White, 27 were Hispanic, 25 were Asian, 20 were Black, and 9 were unknown. The manner of death was Suicide in 171 cases, Accident in 109 cases, and Natural in one case (complications of chronic substance abuse). The gases attributed to cause of death were helium (105), difluoroethane (75), nitrogen (47), chloroethane (23), argon (10), nitrous oxide (7), propane (5), carbon dioxide (3), butane (2), methane (2), difluorochloromethane (1), chlorine (1), hydrogen sulfide (1), and unknown gas (1). Four cases also attributed drowning to the cause of death. There were two sets of companion suicide cases, and two cases had two different gases.

Many of the fatalities described are associated with intentional inhalation of a gas. There is readily available information to those seeking a suicide method involving gases, and the inert gases are easy to obtain, such as helium from a party store. Other deaths were due to accidental interactions with gases with a lethal outcome. Some deaths occurred in the workplace, such as a welder using argon and a coffee worker extracting caffeine using carbon dioxide. Volatile gases are a source of substance abuse and accidental death, due to their ability to induce psychoactive effects, and are easily obtainable. They are also used in autoerotic activities and were associated with one death that also cited airway obstruction due to a sexual device as part of the cause of death and in one death at a spa in a flotation tank. Two propane fatalities involved autoerotic activities.

Death by inhalation of gas generally occurs by two mechanisms: asphyxia due to displacement of oxygen or depression of the central nervous system. Inhalation of most non-oxygen elemental gases (except for halogens such as chlorine) and volatile saturated organic compounds (alkanes) produce hypoxia; however, with exhalation of carbon dioxide continuing, there is no stimulation of the respiratory center and the damaging effects of asphyxia occur rapidly, beginning with the central nervous system. Inhalation of halogenated organic compounds at high concentrations results in depression of the central nervous system and often other side effects. Other gases such as nitrous oxide, butane, and hydrogen sulfide have their own unique pathophysiological effects on the body.[1-15]

Toxicological testing is challenging and is not readily available. Reference laboratories offer testing on volatile compounds, such as difluoroethane. Theoretical scientific methods exist to test for atmospheric gases such as carbon dioxide.[7,10] However, the most useful tool is most often a thorough scene investigation, which may require a multidisciplinary approach, such as the Occupational Safety and Health Administration, emergency services (police and fire), and hospital agencies.[15]

References:
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Asphyxiation; Suicide; Scene Investigation

I52 The Postmortem Utility of Neuron-Specific Enolase and S100B for Differentiating Traumatic Brain Injury From Other Causes of Death

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Learning Overview: After attending this presentation, attendees will understand the role of the two biochemical markers, Neuron-Specific Enolase (NSE) and S100B in Traumatic Brain Injury (TBI) fatalities and whether the postmortem serum and Cerebrospinal Fluid (CSF) samples of these two biochemical markers can be utilized in differentiating TBI fatalities from other causes of death.

Impact Statement: This presentation will impact the forensic science community via discussion about the utility of the postmortem serum and CSF samples of these two biochemical markers and their ability to differentiate TBI fatalities from other causes of death.

It can be difficult to attribute TBI as the contributing cause of death where macroscopic symptoms like hemorrhage, laceration, and contusions are absent.[1] Thus, the application of biochemical markers to aid in its identification in tandem with the conventional postmortem examination may be of great interest and a promising field for forensic research and exploration. NSE and S100B were chosen because they were established to be neuron and neuroendocrine cells-specific and two of the well-studied antemortem neuro biochemical markers for the determination of TBI, with comparatively more specificity to neuronal tissue and commercial availability of the ELISA kit.[1-3] The aim of this study was to determine whether postmortem serum levels of S100B and CSF levels of S100B and NSE were associated with TBI and to examine how these levels were associated with survival and Postmortem Interval (PMI).

Autopsies with only TBI, following an alleged history of road traffic accident, assault, accidental fall, fall from height, fall of objects overhead, and any other form were included in the case group. Autopsies with only Isolated Trunk Trauma (ITT) or Mechanical Asphyxia (MA) or Myocardial Infarction (MI) were included in the control group. None of the controls showed any signs of head injury. The sample size was estimated using the method of comparison of two independent means with an expected minimum difference in the markers between TBI and other causes of death groups as 100 with an SD of 200 at 5% level significance and 80% power, which amounted to 63 cases in each group.

This study showed that serum S100B and CSF NSE were able to differentiate between deaths due to TBI from other causes of death, whereas CSF S100B was not able to do so. The markers, however, did not show any association with PMI. There was no association between the survival time and levels of the markers in the TBI group. Serum S100B was a better predictive marker with a sensitivity of 64.4%, specificity of 66.1%, and accuracy of 65.25%, at a cut-off value of 215.98 ng/L. CSF NSE was also able to differentiate the two groups with a sensitivity and specificity of 61%, and accuracy of 60.31%, at a cut-off value of 1.3505ng/mL.

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Traumatic Brain Injury; Neuron Specific Enolase; S100B

I53 The Clarksburg Insulin Murders

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Learning Overview: After attending this presentation, attendees will be aware of the background, circumstances, investigation, and successful prosecution of Reta Mays, a nursing assistant who was successfully convicted of homicidally administering unprescribed insulin to hospitalized patients. A systemic methodological approach to the investigation, autopsy, ancillary testing, and prosecution will be presented to teach the attendees best practices on how to approach the investigation and prosecution of these cases. A current update as to the postmortem detection of insulin and insulin analogs will be provided.

Impact Statement: This presentation will impact the forensic science community by presenting a case series of a successful investigation and prosecution of a nursing assistant who was convicted of homicidally administering unprescribed insulin to hospitalized patients. The best practices regarding the investigation, jurisdictional determination, autopsy, ancillary testing, and prosecution will be discussed.

The use of insulin as an instrument of homicide has been historically incredibly difficult to identify from a medical examiner and toxicologic perspective. Investigating cases of weaponized insulin in a health care setting present very unique investigative, forensic, toxicologic, and prosecutorial challenges.

This case series represents the investigation of a nursing assistant named Reta Mays who caused the deaths of at least seven patients at a hospital in West Virginia. This case was a comprehensive team effort involving over three years of persistent investigation from the Veterans Administration Office of the Inspector General, a team of forensic pathologists and investigators from the Armed Forces Medical Examiner System, multiple expert consultants, and a relentless United States Assistant District Attorney. The result was a successful prosecution of a series of homicides by use of the exogenous administration of unprescribed insulin.

The purpose of this presentation is to discuss the traditional and novel approaches to investigating insulin-suspected homicides with the purpose of educating on the pathophysiology of insulin, how to suspect potential insulin-related deaths, the importance and causes of refractory hypoglycemia, and several investigative approaches to suspected insulin homicides. The details of this case series will be discussed, focusing on the investigative clues, a medical examiner approach, toxicologic testing, and histology/immunohistochemistry. An up-to-date brief regarding the current state and capabilities of postmortem testing for insulin and insulin analogs will be provided.

The goal of this presentation is to provide a "roadmap" for the clues from a criminal investigation perspective, what to look for in autopsy, how to approach postmortem testing, and how to best incorporate immunohistochemistry as an adjunct to routine histologic testing. Insulin homicides are among the most difficult cases to investigate and prosecute due to the lack of the proverbial "smoking gun." The combination of thorough investigation, sound forensic practice, and utmost teamwork were all essential to providing closure to the families and resulted in sentencing of "the Monster that no one saw coming."

Homicide; Death Investigation; Microscopy

I54   A Complex Suicide With Over 250 Sharp Force Injuries and Falling From Height

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Learning Overview: After attending this presentation, attendees will understand the difficulties of distinguishing suicide from homicide in complex suicidal cases with multiple sharp force injuries.

Impact Statement: This presentation will impact the forensic science community by presenting an uncommon case of over 250 sharp force injuries and falling from height within a single suicidal act. This presentation will demonstrate the importance of accurate investigation on scene and at autopsy and the significance and challenges of correct contextualization.

Background: In 2021, a total of 9,215 suicides were committed in Germany, 398 of which were carried out by sharp force.[1,2] Self-inflicted multiple sharp force injuries, especially such a high number, are very uncommon and the case could be misinterpreted as homicide.[3]

Case Report: The 57-year-old left-handed woman was observed falling out of the bathroom window of the second floor by her husband coming home. A single-edged knife (length ca.14cm, width up to 2cm) was found on a table on the first floor. At the investigation on the scene, multiple, seemingly calm, bloodstain patterns were presented, reconstructing a trail from the first floor into the bathroom on the second floor.

The external examination demonstrated multiple injuries due to sharp force on throat (at least 41), right forearm (89), thorax and abdomen (120), part of which penetrated her clothes. Furthermore, a laceration of her scalp on the right side of her head and a fractured right forearm were found. The autopsy revealed multiple, predominantly superficial, injuries, forming groups of similar direction with partially parallel orientation, all of which were accessible for the left-handed woman.

The injuries had a maximum length of 6cm and a maximal depth of 7.5cm, three penetrating her abdominal cavity and superficially stabbing her liver. No signs of potentially deadly blood loss were found. Furthermore, a severe head-brain-trauma, characterized by a skull and skull-base fracture, aspiration of blood, characteristic signs of increased intracranial pressure and subarachnoidal hemorrhage, especially around the brainstem, was found, defining the cause of death. Toxicological analyses were negative for alcohol and other psychoactive substances.

Discussion: The self-infliction of over 250 distinguishable sharp force injuries can be seen as a forensic rarity. There are only a few published suicide cases with such a high number of sharp force injuries. Marx reported a case of 187 injuries and reviewed cases with approximately 285 and 300 injuries.[4] While multiple sharp force injuries and penetration of clothing are atypical for suicide, the morphological characteristics of the cuts and stab wounds are consistent with a suicidal act and seem to match the knife found. None of the autopsy findings indicated force due to another, unauthorized person.

In conclusion with the findings at the scene of death, including the calm bloodstain pattern, a suicide is most probable. In reconstruction of the progression of events, it is probable that the woman was interrupted by her husband while trying to commit suicide by sharp force, therefore changing her method and jumping out of the window, resulting in a complex suicide. Consequently, she died due to the more rapid effect of her head-brain-trauma in comparison to her potentially deadly stab wounds.

Conclusion: Cases with multiple self-inflicted sharp force injuries are challenging for the forensic pathologist when trying to differentiate between suicide and homicide. This case report demonstrates the importance of clearly defined characteristics for identifying suicidal deaths. Furthermore, it points toward the significance of the correct contextualization by the forensic pathologist.

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Suicide; Sharp Force; Fall From Height

I55   Medicolegal Pediatric Natural Deaths: Causes of Death and Demographics

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Learning Overview: The goal of this presentation is to show that: (1) determining natural causes of death in pediatric populations is an important part of forensic pathology practice and the public health record; (2) within our data set, there were only minimal differences in the causes of death before COVID-19 and during the COVID-19 pandemic; (3) age cohorts show differing rates of causes of death; and (4) demographic information showed increased representation of some racial/ethnic groups and decreased representation of other racial/ethnic groups in our data set compared to the demographic profile for the local population.

Impact Statement: This presentation will impact the forensic science community by encouraging a thorough review of pediatric cases to ensure accurate cause and manner of death determination and providing data (causes of death and demographics) on a cohort of medicolegal pediatric cases certified with natural manner of death following medicolegal investigation and complete autopsy from one of the most populous counties in the United States.

Determining cause and manner of death is the primary purpose of death investigation and autopsy. Proper death certification has far-reaching impacts on public health research and policy. Multiple published studies discuss cause and manner of death certification trends at varying levels of specificity, geographic region/extent, and sample sizes. These studies have shown a general downward trend in deaths due to natural causes but have highlighted an opportunity for genetic testing in efforts to prevent subsequent deaths in siblings and are therefore important deaths to continue to characterize.[1-3] This retrospective study examined pediatric (<18 years old) decedents certified as natural manner over the years 2017–2021 at the Harris County Institute of Forensic Sciences (HCIFS) in Houston, TX, following medicolegal investigation and complete autopsy. The HCIFS has a unique relationship with the neighboring children's hospital; thus, many of these cases were also reviewed by a board-certified pediatric pathologist with autopsy expertise. Overall, 221 cases (out of 1,356 pediatric deaths, 16.3%) were identified and aggregated. Cause of death, the pre-COVID-19 pandemic and mid-COVID-19 pandemic time period, and demographics (distributions by age and race/ethnicity) were descriptively analyzed.

Causes of death were categorized into complications of prematurity, congenital and genetic anomalies, infectious disease, neoplasms, seizure-related, Sudden Infant Death Syndrome (SIDS) (cases from 2017–2019, prior to currently used terminology of unexplained sudden death and consequent undermined rather than natural manners of death), and other. Overall, infectious diseases were the most often determined cause of death, with congenital and genetic conditions as the second most common. The most common causes of each death within each category were compiled.

The proportions of the cause of death categories were very similar in the pre-COVID-19 pandemic (2017–2019) and COVID-19 pandemic (2020–2021) time period. The proportion of deaths from infectious diseases decreased after the start of the COVID-19 pandemic in the United States. Only five deaths were fully or partially attributed to COVID-19 or subsequent complications, all of which had pre-disposing factors (e.g., obesity, asthma, prematurity).

Organizing the data by age group [Fetal, Infant (< year), Toddler (1–3 years), Preschool (3–5 years), Childhood (5–11 years), and Teenager (12–17 years)] shows changes in trends as decedents age, with findings consistent with the literature. The infant age group showed the highest number of deaths. As expected, deaths from sequela of prematurity occur only in the fetal and infant groups, while SIDS cases only occur in infants. Deaths from congenital causes show a bimodal distribution with the very young (infants) and oldest (teenagers) groups showing the two peaks. Similarly, deaths from infectious causes show a bimodal distribution with peaks in the infants (prior to being fully vaccinated) and childhood (school age) age groups. Within our dataset, seizure-related deaths and "other" deaths occur at higher frequencies with increasing age.

Representation of racial/ethnic groups among all HCIFS cases—adult and pediatric, including all manners of death—shows overrepresentation of Black and White decedents compared to the demographics of Harris County documented in the United States Census.[4] Among pediatric decedents certified with a natural manner of death, Black individuals are the only racial group overrepresented, with a greater discrepancy in the pediatric data than in the overall data. These disparities may represent, at least in part, underlying socioeconomic issues like poverty and/or differential access to health care services or implicit racial/ethnic bias among health care professionals.[5,6]

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Autopsy; Cause of Death; Pediatric

I56   An Unusual Form of Sudden Infant Death in the Arctic

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Learning Overview: This presentation will provide attendees with information on an unusual form of asphyxia in infants carried inside heavy winter clothing worn by their parental caregivers during transport on a non-enclosed vehicle in cold arctic conditions.

Impact Statement: This presentation will impact the forensic science comm unity by providing the forensic pathologist with tools to identify a cause of unusual sudden unexpected death of an infant outside of bedsharing, unsafe sleeping surface use, or other domestic sleeping scenarios.

Sudden unexpected infant death, unlike unexplained sudden death in infancy (also known as sudden infant death syndrome) is any sudden or unexpected death of an infant under one year that may have an explained cause discovered through autopsy and scene investigation. Most unexpected deaths in infancy are sleep-related, with and without hazards such as co-sleeping or soft bedding. Some deaths occur in unusual situations that can cause asphyxia. Situations that can lead to suffocation or strangulation by covering the airways include an unsafe sleeping surface (adult bed), objects around the infant, or other unusual situations such as active suffocation by the caregiver, other hazards in the home or in the natural environment.[1-5]

This presentation will introduce an unusual form of sudden death by suffocation unique to people living in cold environments. Two cases of sudden unexpected infant death were reported in Alaska involving the transportation of an infant contained in heavy winter clothing worn by the parental caregiver. One was a 5-month-old infant on the back of the caregiver contained within a heavy down jacket or parka while riding a snowmachine (snowmobile). The infant was facing forward with the face toward the back of the caregiver. The other was a 7-month-old facing the parental caregiver with his face into the chest of the caregiver while riding an all-terrain vehicle in cold weather. Another older child sibling was riding in front of the second infant and the caregiver. Both infants were discovered unresponsive when they were pulled out of the clothing. Neither infant was resuscitated in the initial attempt upon arrival of first responders. Autopsies of the infants were negative for injury or disease that cause death. In the absence of anatomic findings, toxicological studies and metabolic screening, the cause of death in these infants was reported as asphyxia, due to the individual enclosed in a small space created by thick, impermeable winter clothing with the face against the body of the caregiver. The manner was accidental in each case. Due to this unusual nature of these infant deaths, these cases are presented to provide information for this type of infant asphyxia in the outdoor setting for people who live in a similar climate setting as seen in Alaska.

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Asphyxia; Winter Clothing; Arctic

I57   Child Abuse Diagnostic Work-Up: Let's Put on Our Thinking Caps

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Learning Overview: The goal of this presentation is to discuss management matters of suspected Child Abuse (CA) through a clinical-based approach in accordance with the most recent and relevant scientific guidelines. Reported here is a clinical case characterized by the coexistence of physical abuse and neglect, with a positive toxicological test for cocaine, and a difficult differential diagnosis between maltreatment and iatrogenic damage, regarding the etiology of bone fractures.

Impact Statement: This presentation will impact the forensic science community by presenting a strategy that forensic pathologists should adopt before coming to a conclusion of CA in line with the sector guidelines. A precise methodological order is provided in the diagnostic assessment to guarantee justice to the most fragile categories.

CA is a recognized common public health problem that can be manifested in many ways. The recognition of CA in a setting of clinical practice is characterized by a challenging differential diagnosis between inflicted and non-inflicted injury. There is evidence that the physicians often miss opportunities of identification and intervention; the diagnostic difficulty also lies in non-uniform diagnostic criteria as there is currently no systematic standardization of CA diagnostic work-up.[1,2].

We report the case of a 8-year-old little girl, already affected with West's syndrome, an infantile myoclonic encephalopathy, in treatment with sodium valproate. She presented to medical doctors' attention for her rather poor psychophysical condition. She was not schooled and not subjected to compulsory vaccination prophylaxis. Her parents were illicit drug users.

The physical examination showed multiple and polyfocal bone fractures, so proving that sodium valproate (responsible for vitamin D deficiency and a reduction in bone mineral density) was not the cause of them was required.[3] The hair analysis showed positivity both for cocaine and benzoylecgonine over the entire sample length, meaning that the child tested positive for cocaine intake/administration at least twice in the previous year.

This case shows a pathological picture expressive of physical abuse and neglect, due to a lack of attention to medical health care by those exercising parental responsibility. Caregivers who are abusers may have impaired judgment, being unable to provide consistent care and supervision to their children.

Members of the community of forensic pathologists need to understand these risks because they are in the unique position of coordinating immediate and long-term treatment to victimized children; their interpretation of the child's injuries allow for proper investigation, decision-making, and protection of the child, eventually alerting the competent judicial authority and providing court testimony when necessary.

The complexity of this case and the number of investigations it has requested are a starting point to suggest the sequence of clinical investigations to be performed in case of suspicion of CA.

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Child Abuse; Diagnosis; Cocaine

I58 Suicides in Childhood and Adolescence: A Ten-Year Study From Jefferson County, Alabama

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Learning Overview: After attending this presentation, attendees will have gained information on: (1) childhood and adolescent suicide trends in recent years; (2) common personal, circumstantial, and environmental features of these incidents; and (3) the interaction of environmental and individual factors leading to suicidal behavior in the young.

Impact Statement: This presentation will impact the forensic science community by giving a comprehensive picture of suicide incidents in one county, contributing to awareness of the problem, and suggesting public health strategies to fight the phenomenon.

Suicide is an outcome provoked by a combination of biological, psychological, and environmental factors and the adverse effects of those factors on an individual's perceptions. All age groups are exposed to different psychological or physical difficulties and develop their own strategies for adaptation. Understanding the challenges, the individual adaptability, and the environmental features surrounding youth suicides is of utmost importance to establish effective preventive strategies.[1] The investigation of suicides in young people is often complicated due to the difficulty of some young people to fully understanding the consequences of their actions in addition to the domestic conflictual situation often surrounding these events.[2] This study aims to highlight the incidence of suicides in the young, analyze factors that could influence the death investigation process, recognize risky situations, and identify possible preventative measures.

The Jefferson County Coroner/Medical Examiner's Office database was queried for suicides in decedents <20 years old and occurring between 2013 and 2022 to assess for any changing patterns or trends. Each case was reviewed for demographic information, day and time of death, place of death, medical history, previous attempts or ideation, method of suicide, presence of suicide notes, presence of trigger events, the implement used, and postmortem toxicological results. A total of 40 incidents (a minimum of 3 in 2013 to a maximum of 7 in 2019) were classified as suicide in 2013–2022 in decedents aged 9 to 20 years old. Most of the decedents were male (85%) and over 16 years old (72.5%). A psychiatric condition was reported in 37.5% of cases, 10% had a documented medical condition, 30% had reported suicide attempts or ideation, and 2.5% had a history of drug abuse. A minority of decedents left a note of intent (17.5%). The fall/autumn season had a higher incidence of suicides, and most occurred in the evening hours (6:00 p.m.–12:00 a.m.). Regarding the place of death, 80% of the subjects took their life in their own residence or an acquaintance/relative's home. In 55% of cases, a precipitating event could be identified, of which a conflict with others (boyfriend or girlfriend or parents) or school issues were the most common. Gun-related and hanging were the most common methods, counting for 95% of cases (n=38). Toxicological data showed a detectable blood ethanol concentration in 22.5% of cases, and only one case tested positive for drugs (benzodiazepines).

Data provided by this retrospective study showed several specific risk factors among suicides in young people, such as male gender, age >16 years old, and a conflict situation. Other common findings in these events were the place of death, season, and evening hours. Although a concerning trend of increased suicides in this age group has been seen worldwide, rates remained relatively stable in Jefferson County (outside of 2019) over the years analyzed.[3] Reducing risk factors, as well as reinforcing support systems and limiting societal stressors, can play an essential role in mitigating suicides in the young.

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Suicide; Adolescent; Death Investigation

I59 Changes to the Certification of Sudden Unexplained Deaths in Infants From 2012 to 2023

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Learning Overview: After attending this presentation, attendees will understand the importance of a standardized approach to certification of unexplained sudden deaths in infant deaths to better understand the impact on vital health statistics.

Impact Statement: This presentation will impact the forensic science community by sharing how a change in practice to align with national organizational recommendations can improve consistency and data quality to better inform researchers, physicians, public health officials, and policy makers of the circumstances, risk factors, and autopsy findings in infant deaths.

The Sudden Unexpected Infant Death (SUID) rate has been stagnant since 1999 despite continued efforts of prevention.[1] This data is compiled through analysis of death certificates; however, the practices of death certifiers and the ability to analyze and code complicated cause of death statements is challenging. Cause of Death (COD) statements are coded using the International Classification of Diseases Tenth Revision (ICD-10) system and there are three categories most often assigned to deaths that fall into the SUID category: Ill-defined/Unknown (R99), Accidental Suffocation and Strangulation in Bed (ASSB; W75), or Sudden Infant Death Syndrome (SIDS; R95). In 2019, the National Association of Medical Examiners (NAME) Panel on Sudden Unexpected Death in Pediatrics released procedural guidance for the investigation, autopsy, and certification of deaths that remain "unexplained" in this age group.[2] This study will look at how this change in practice impacted the mortality data in one county after adoption of these recommendations.

A total of 186 infant deaths (less that 1 year of age) occurring between 2012 and 2023 was identified through a query of the case management database. The COD, Manners Of Death (MOD), autopsy findings, circumstances, and history were collected for each case. Extrinsic factors (e.g., bed-sharing, unsafe sleep surface, prone positioning) and intrinsic factors (e.g., prematurity, natural disease, birth anomalies) were collected through the investigation and/or autopsy report. Using published keywords and the current practices for ICD-10 coding described in chapter 13 of *SIDS Sudden Infant and Childhood Death: The Past, the Present and the Future*, a strategy was applied to assign ICD-10 codes to each COD statement before (n = 137) and after (n = 49) implementing new certification procedures (in 2020).[3] The COD and MOD varied based on certifier where prior to 2020, 71 deaths were classified as "accidents" and 66 (48%) as "undetermined" when applying ICD-10 codification to this group, 72 were coded as ASSB (53%) 40 as R99 (29%), and 25 as SIDS (18%). In contrast, since 2020, 35 (73%) of deaths were "undetermined" and 13 (27%) were "accidents" for the MOD; the majority (67%) were coded as SIDS, 6% as R99, and 27% as ASSB largely driven by use of a doll-reenactment.

Despite the challenges with a designation of SIDS deaths, the adoption of consistent procedures yielded better data and decreased the percentage of cases "lost" in the R99 (ill-defined/unknown) category. This may aid epidemiologists and researchers to better track and study SUID deaths and alert them to the need for primary data around intrinsic and extrinsic factors present in each case, allowing for more detailed tracking and analysis of risk factors and comorbidities.

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Infant Death; Certification; SIDS

I60   A Histological Examination of the Uterus to Confirm Early Pregnancy in Female Homicide Victims

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Learning Overview: After attending this presentation, attendees will understand the utility of a histologic examination of the uterus and endometrium to confirm early pregnancy in female homicide victims.

Impact Statement: This presentation will impact the forensic science community by outlining a method to confirm gestational status through histologic examination of the uterus in female homicide victims who have tested positive for pregnancy using over-the-counter pregnancy tests. This research is part of a larger protocol for the autopsy of a pregnant woman. The goal of the project is to provide practicing forensics pathologists with a practical guide to the postmortem examination of the gravid woman, particularly those who have died by violence. This portion of the protocol attempts to offer pathologists a straightforward method to confirm early pregnancy through histological examination of the uterus. Although this project was initially conceived as a method to detect pregnancy in deaths due to violence, the recent Supreme Court decision to overturn *Roe v. Wade* makes it likely that maternal mortality due to all causes, including violence, are likely to increase, making the findings applicable to a potentially greater number of cases.[1]

Pregnancy-associated deaths—deaths caused not by the pathologies of gestation, but instead due to violence temporally associated with pregnancy—are a significant source of maternal mortality. Research has found that as many as 20% of deaths during pregnancy were at the hands of another, making homicide the leading manner of death for expectant mothers.[2] Previous work by these researchers has demonstrated that commercially available, over-the-counter urine pregnancy tests will detect early pregnancy before gestation is anatomically evident; furthermore, postmortem urine, vitreous fluid, and decompositional fluid are acceptable samples for testing.

We purport that histological examination of the uterus validates the use of urine pregnancy test sticks to detect early pregnancy. Because at-home pregnancy tests are not designed for postmortem use or on body fluids other than urine, additional validation of the decedent's gestational status is prudent, particularly in homicide cases in which the decedent's pregnancy status potentially informs motive. Products of conception are not grossly evident until 6 to 8 weeks of gestation. However, changes to the endometrium that are indicative of pregnancy, like Arias-Stella change (hypertrophy and vacuolization of glandular epithelial cells, associated with marked nuclear pleomorphism, enlargement, and hyperchromasia) and decidualization (the transformation of endometrial cells into a polygonal epithelioid morphology to facilitate implantation), occur far earlier and are well-characterized.

This study employed tissue samples derived from autopsy associated with cases in which early pregnancy was detected using over-the-counter test sticks. The study found histological examination of uterine tissue corresponds with and validates histological detection of early pregnancy in postmortem tissue. Although forensic pathologists are infrequently called upon to read human endometrial biopsies, the changes associated with pregnancy are sufficiently marked enough to make interpretation straightforward.

This study shows the value and validity of postmortem pregnancy testing using commercially available test sticks on urine, vitreous fluid, and decompositional fluid coupled with histological examination of the uterus to detect early pregnancy in female decedents. The method is economical, easily interpretable, quick, and technically simple to perform.

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Pregnancy; Homicide; Autopsy

I61   Multiple Shots of Different Firearms in a Charred Corpse: A Case Report

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Learning Overview: After attending this presentation, attendees will better understand the importance of gathering all the available evidence, even though it seems very difficult, if not almost impossible, in order to reach the solution of the case with a multidisciplinary approach.

Impact Statement: This presentation will have an impact on the forensic scientific community by showing how it was possible to define not only the identity, but also the causes of death in a corpse despite its partial destruction.

Recovering a drawn body from exposure to flame often presents a challenge to the forensic pathologist who is expected to determine the identity of the victim, the presence or absence of essential signs that could indicate whether the deceased was alive or not when the fire broke out, the cause of death, potential poisoning or intoxication, the possibility of a third party intervention, and of potential criminal involvement.[1] Moreover, the direct action of heat can alter the characteristics of some thanatological findings; the new findings can be similar to other type of traumatic lesions; for example, skull fractures or the presence of extradural blood.[2]

In this case report are described the steps realized to identify a charred body found in a completely burned car and to define its cause of death. At first examination, the forensic pathologist detected a deeply charred corpse, lying prone in the trunk of the car, in an isolated country place. Due to the extreme fragility of the burnt skeleton with loss of bone fragments, including cranial ones, the whole vehicle was transported to a sheltered place. After cutting the car apart, the skeleton was gently removed and underwent a total body Computed Tomography (CT) scan that showed several foreign bodies compatible with firearm bullets at the base of the skull and in the thorax-abdomen. At the cadaveric section, a bullet was found into the thorax wall; the right lung with parenchymal destruction, multiple metal pellets and a retained wad, as well as the stomach, the left kidney, and the aorta were injured by a firearm; three wads and numerous metal pellets were found in the abdomen. Histologically, there were no carbonaceous residues in the airways and in the lung; also the carboxyhemoglobin level was very low (3.6%), consistent with postmortem carbonization. No exogenous substances ware found at toxicological exams. The cause of death was identified as a hemorrhagic shock due to multiple firearm shots. The genetic investigations, integrating the circumstantial data, allowed identification of the missing man.

In conclusion, despite the intense carbonization of the corpse, a forensic pathology investigation with a multidisciplinary approach contributed significantly to the solution of a murder case.

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Gunshot Residue; Charred Body; Interdisciplinary

I62 A Macro and Micro Time-Dependent Washer Woman Wrinkling and Reversibility Trivialized in a Bathtub Drowning: A Case Presentation

Pete Speth*, Forensic Consultant, Wenonah, NJ

Learning Overview: After attending this presentation, attendees will understand the importance of looking at ALL of the scientific evidence with an open mind in controversial cases, including evidence that may seem to be of secondary importance.

Impact Statement: This presentation will impact the forensic science community by providing an example of circumstantial confirmatory bias, influenced by partial interpretation of controversial autopsy findings. The final analysis affords attendees the opportunity to go back and review those choices with the benefit of hindsight.

A 40-year-old divorced female was found by her erstwhile boyfriend in a fetal position, drowned, in her bathtub filled with cold running water, wearing only a pair of panties. Her face was turned up out of the water with foam in the nares. It is uncertain whether her boyfriend turned off the hot water faucet (running cold by then) or the cold water faucet. A photo album of one of her four children was under her feet in the water, a kitchen paring knife was under her torso, wet newspapers were in the tub and on the edge of the tub, and a focally bloodstained tank-top was neatly folded over the edge of the tub. Bloodstains, smears, and splashes (but almost no castoff blood) were visible on the fitted sheet and comforter on her bed. There were bloodstains on the bedroom floor, hallway, and bathroom floor, and on the sink and far wall over the tub, the latter consistent with a bloody handprint.

Toxicology was positive for recent alprazolam administration. It was at the toxic level in the blood, but at the lowest lethal level (no pills in the stomach). No prescription container was found. Anti-inflammatory pills were poured out on the bedroom floor next to a chair pulled up to the closet.

The ex-husband became the prime suspect and was indicted. He initially stated that he could not remember the events, then provided a delusional story. Very recently he had neglected his hygiene, didn't change his clothes, and became uncharacteristically negligent in his work. Investigation determined that he had recently taken trazodone, escitalopram, and/or citalopram (and alprazolam??) for depression/anxiety prior to the drowned victim being found. His life had become very stressed due to ongoing and pending child custody battles with the victim, and she had a history of allegedly being abusive and also having possibly had past suicidal ideation. No note by the victim and no known suicidal ideation preceded this event.

A "battle of the experts" ensued at trial, to wit:

- The attribution of three superficial sliced/chopped wounds on the left wrist and an incised wound on the left lower leg of the drowning ex-wife (adult Non-Suicidal Self-Injury [NSSI] vs. a homicide staged to look like a suicide?).

- The attribution of the thyrohyoid muscle and retropharyngeal hemorrhages in the presence of Tardeau and hypostatic hemorrhages in the drowning victim (strangulation vs. artifact?).

- The opinions as to who administered the alprazolam and how it was administered?

- The interpretation of the bloodstains on the fitted bed sheet and comforter?

- The determination of the time since death/submersion when found by boyfriend?

- The attribution of the victim's and ex-husband's alleged motivations (suicide, NSSI, or homicide?).

- The manner of death (accident, suicide; homicide, or homicide staged to look like suicide?).

The ex-husband was found guilty of murder in a jury trial. An understanding of the significance of the presence or absence of washer woman wrinkling under the stipulated freshwater temperature and known timeframe of the circumstances was not appreciated at trial.

This presenter will show that the ex-husband could not have committed the crime. This is based on 1980s German experiments and associated German scholarly articles regarding time-dependent macro and histopathological characteristics of postmortem washer woman wrinkling in fresh and salt water at various temperatures. Those experiments also determined the maximal time that the respectively evolved wrinkling is still reversible when removed from the water.[1-11]

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Drowning; Neck Injury; Suicide

I63 Complete Laryngotracheal Separation Without Decapitation in a Case of Hanging: An Uncommon Phenomenon

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Learning Overview: After attending this presentation, attendees will be able to recognize an uncommon phenomenon of laryngotracheal separation in a case of complete hanging with a long drop and its possible mechanism of action.

Impact Statement: This presentation will impact the forensic science community by creating awareness of findings such as laryngotracheal separation in cases of complete hanging, which may be of important medicolegal significance. This presentation highlights the method of causation and manner that has very little mention in the available literature.

Complete or incomplete decapitation is an unusual complication of suicidal hanging. Incomplete decapitation due to hanging with soft tissue injuries, spinal cord dissection between C2 and C3, fracture of the hyoid bone, and injuries of cartilage are rare.[1,2] The comprehensive literature review of incomplete or complete decapitation by suicidal hanging emphasizes the importance of investigation of biomechanical process. Previous observations confirmed that body weight and falling distance were the most important factors in the pathomechanism of decapitation. In the hanging process, energy can be stored by changing the position of the human body in the gravitational field, by changing the shape of the hanging rope, and by changing the motion of the hanging body. The occurrence of complete or incomplete decapitation can increase by the increasing energy stored as potential energy at the starting position and the characteristics of the rope extended by the hanging body.

In present case a dead body of 62-year-old male was found suspended through a tree branch at about 19 feet from ground level. The upper point of suspension was firmly tied by the ligature material with fixed knot. The length of ligature material tied from branch to lower point of suspension was 13.5 feet. The person was missing for one day before the incident since evening. The body was first seen by the devotee of the adjacent temple who informed the police. It was brought to our hospital for postmortem examination. Autopsy showed that the deceased was well built and well nourished with a body length of 158cm and weighed 68kg. His tongue was clinched in between his teeth. The dead body showed rigor mortis and was well marked in the whole body. Gloves and stocking lividity was seen. The ligature material was nylon rope present *in situ* around the neck with one single loop and a running noose and slip knot situated over the nape of the neck. The length of the loop was 25cm, the circumference was 3.5cm, and the two free ends of the rope were 18cm and 407cm long. Bloodstains were present over the loop in places. The skin over the anterior aspect of the neck showed skin-deep lacerations due to the ligature material. The right sternomastoid muscle showed lacerations corresponding to the ligature mark. The larynx was transected at the supraglottic region. The thyroid cartilage showed a fracture of lamina in midline of length 2cm. The greater cornu of hyoid bone on the left side was fractured. The cervical spine was fractured at the level of C2 and C3 vertebrae with corresponding cord contusion and extravasation of blood in the surrounding area.

Such findings can be an addition for commenting on the antemortem nature of such cases together with the other established findings. Also, such findings can become significant if there are minimal or no findings available. In difficult cases where strangulation is suspected as well as other potential medicolegal problems with trauma involving the neck organs, a detailed inspection of these structures may provide invaluable information as to the mechanism of injury.

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Asphyxiation; Hanging; Medicolegal Death Investigation

I64 One House, Two Bodies: A Case Series and Considerations for Investigating Companion "Double Deaths," Including Challenges in the "Philemon and Baucis" Syndrome

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Learning Overview: The goal of this presentation is to teach attendees about the complexities of investigating companion "double deaths," in which two bodies are discovered at the same death scene and neither body shows overt trauma. Medicolegal death investigators and forensic pathologists will learn the "differential diagnosis" for these cases, which helps guide clarification of the circumstances, autopsy procedures, toxicology testing, and death certification.

Impact Statement: Occasionally, medical examiner and coroner offices face the challenge of investigating a companion case characterized by two decedents' bodies discovered at the same death scene. Especially if there are no overt traumatic injuries, questions arise as to what event or sequence of events would result in two individuals being dead in the same place, without one seeking help or being able or willing to report on the death of the other. Despite the inherently challenging nature of investigating these deaths, the forensic science literature on this topic appears to be sparse, with only a few published cases and a small case series from Europe. This presentation will impact the forensic science community by significantly bolstering what is known about these deaths by reporting the largest case series to date, including an interesting variety of cause and manner combinations. A framework will be outlined for investigators and forensic pathologists to consider when they encounter these difficult companion cases.

The discovery of two decedents at the same scene poses a challenge for death investigation, particularly if there is no overt trauma. Adjacent bodies could represent double homicide, double suicide, double accidental intoxication, environmental exposure, or something else that demands explanation above and beyond what is normally considered at a scene with only one decedent.

The term "Philemon and Baucis death" has been used in the literature to designate the specific scenario in which someone dies as a reaction to the death of another—in other words, a sudden cardiac death precipitated by discovering an emotionally close person's dead body. To the authors' knowledge, there have only been a few published cases and small case series reporting suspected "Philemon and Baucis" deaths, all of which come from Europe.[1-4] While sparse, the literature does appear to suggest some common features of this death scenario, including social isolation, co-dependent or "symbiotic" relationships, history of mental illness in one or both decedents, older individuals with cardiovascular pathology, and significant decompositional changes.

We discuss a series of 37 companion cases from the State of Connecticut Office of the Chief Medical Examiner over a ten-year period from 2013–2023. These include 3 cases with features typical of the described "Philemon and Baucis" death, 8 companion cases where one decedent was the functional caregiver of the other decedent, and 21 cases of double intoxication (including 3 carbon monoxide exposures). Five additional companions highlight the diversity of possibilities for manner determination in these scenarios, including a "homicide followed by suicide" case superficially mimicking a "Philemon and Baucis" death.

The challenges of these cases may include the often inherently limited investigation both circumstantially and anatomically, the question of determining the order in which the individuals died, and the difficulty of drawing a conclusion of "double natural" death. Establishing a caregiving relationship between the decedents may significantly enlighten and inform the investigation. Two bodies at one scene without overt trauma should elicit a "differential diagnosis" in the investigator's mind, which helps guide clarification of the circumstances, autopsy procedures, toxicology testing, and ultimately death certification.

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Scene Investigation; Manner of Death; Autopsy

I65   A Diamond in the Rough—Myocardial Infarction as a Complication of "Near Hanging" in an Adolescent: An Exceptionally Rare-Find Case Report

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Learning Overview: After attending this presentation, attendees will be informed regarding a case of myocardial infarction as a complication of hanging of a 17-year-old boy. This kind of evolution of "near hanging" is extremely rare, with hardly any examples in existing literature concerning the subject, making it a learning opportunity of great importance.

Impact Statement: This presentation will impact the forensic science community by emphasizing the importance of meticulously conducted autopsy, overlapping its main objectives, like cause and manner of death, underlining not to take any of it for granted or jump to conclusions early, even if a case at hand may be seemingly understandable.

"Near hanging" is a term used to refer to situations in which individuals who attempted suicide by hanging and survived at least long enough to be taken to the hospital. It is an infrequent cause of trauma, with an optimal clinical approach still to be defined and remaining controversial, due to its rather small sample size.[1,2] In the cases culminating in victims' deaths, according to available studies, cause of death associated with near hanging would mainly concern the central nervous system (anoxic encephalopathy) and lungs (pneumonia and/or pulmonary oedema).[3,4]

This case concerns a 17-year-old boy, found by his grandmother, hanged by the neck. Paramedics were called to the scene, found the boy in cardiac arrest, and immediately initiated advanced life support algorithm, including intubation and restoring circulation after approximately ten minutes. The victim was then taken to the nearest hospital and admitted to the Pediatric Intensive Care Unit, where he presented himself with fixed mydriasis. He was submitted to a head computerized tomography, which did not detect any alterations. On the next day, the exam was repeated and this time revealed extensive alterations compatible with cerebral anoxia. He remained completely unresponsive, with a Glasgow Coma Scale score of 3, with serial Doppler ultrasounds and electroencephalograms conducted, not reaching the criteria of brain inactivity, nonetheless, with poor prognosis. He had daily echocardiograms. On day 5, a severe global systolic disfunction of the left ventricle, associated with QT alterations in his electrocardiography, was detected. He was pronounced brain dead later that day and most of his organs were collected for donation.

During the postmortem exam, we would detect findings compatible with hanging, such as anterior cervical abrasion and its corresponding subcutaneous tissue congestion (the hanging mark), and cerebral oedema and softening. However, the most interesting and unusual finding was the "tigroid" appearance of the myocardium of the left ventricle. It was sampled, sent for microscopical examination, and diagnosed as acute myocardial infarction, confirming our suspicion.

In this case, the cause of death was classified as mechanical asphyxia due to fatal pressure on the neck, namely hanging, complicated by acute myocardial infarction, confirmed microscopically, and timed as occurring in the last 24 hours. As per the manner of death, forensic pathologists have defined it as undetermined, not having been provided with data convincing enough to affirmatively define it as either suicide, homicide, or accidental.

This communication is an important learning opportunity for the forensic pathology community, presenting a rare and previously practically undescribed complication of a "near hanging" case.

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Autopsy; Hanging; Myocardial Infarction

I66 A Macro and Micro Time-Dependent Washer Woman Wrinkling and Reversibility in a Bathtub Drowning Case: 20th-Century German Research Proves Innocence

Pete Speth*, Forensic Consultant, Wenonah, NJ

Learning Overview: After attending this presentation, attendees will know how to determine the time-since-submersion in non-decomposed bodies recovered from a watery environment based on the presence or reversibility of the wrinkling and the macro- and micro-pathology.

Impact Statement: This presentation will not only analyze the findings of the given case being presented, but will also provide attendees with translations of the relevant, previously untranslated German articles, which contain excellent illustrations.

As stated in the case presentation, the ex-husband was found guilty of murder in the drowning of his ex-wife in her bathtub. Not withstanding the disputed "battle of the experts," this presenter will demonstrate why the ex-husband could not have committed the crime. This is based on German experiments described in untranslated 1980s German scholarly literature regarding the time-dependent macro and histopathological characteristics of postmortem washer woman wrinkling in fresh and salt water at various temperatures. That literature also determined the maximum time of submersion at various temperatures that still allows for reversibility of the wrinkling when removed from the water.

Weber 1982: In his experiments utilizing well-preserved, early postmortem corpses, Weber submerged their feet in fresh water at 98.6ºF for 4hrs.[1] He then snap-froze toes in liquid nitrogen, tangentially sliced them in a cryostat, and stained the slides with Methylene blue. At that temperature after 4 hours, there was multicentric epidermolysis, development of subepithelial cavities, with sweat gland ducts torn off the basement membrane. He also found that this stage of irreversible damage corresponded with the macro findings of so-called "Runzelbildung" (squiggle- and circular-shaped wrinkles), which had important significance in subsequent experiments.

Weber 1984: In his experiments utilizing well-preserved, early postmortem corpses, Weber submerged their hands in fresh water at 98.6ºF.[2] He documented the macro changes at intervals out to 24 hours. Washer woman wrinkling was apparent already after ten minutes and reached the irreversible stage by six hours based on the macro and histopathological findings in his 1982 experiments.

Reh 1984: Reh, utilizing five early postmortem, well-preserved corpses, submerged their hands and feet in fresh water at temperatures ranging from 50ºF to 64ºF.[3] He documented the temperature-dependent developing macroscopic findings. Wrinkling was well-developed after 20 to 30 minutes and involved the entire fingers and toes by 50 to 60 minutes. Reh also documented recovery times in room air after removal from the water. This transpired slowly even after short immersions, and after immersion of only 35 minutes, wrinkling was still present after 60 minutes on the tips, albeit reduced. Wrinkling, present over the entirety of the fingers after 120 minutes of submersion at 55ºF, had not yet fully recovered after a subsequent 16-hour exposure to room air. Regarding how long washer woman wrinkling must develop before it no longer is reversible: based on his experience—"a duration of less than 5 hours."

Püschel 1985: Püschel submerged 24 well-preserved pairs of postmortem hands for 72 hours, whereby 8 pairs were submerged in 50ºF water, one from each pair in freshwater, the other in salt-water.[4] Similarly, he exposed 8 pairs in 68ºF water and 8 pairs in 86ºF water. He photographically documented and tabulated the development of macro-findings.

With regard to our case: Photos specifically of the fingers were not taken; reference to presence or absence of wrinkling is not stated in the autopsy report. A photograph of our victim 4 hours after removal from the 60º F water, demonstrated absence of wrinkling on the visible fingertips. Fingers, visible in photos the next morning at autopsy, displayed no wrinkling. Prosecution stipulated to no wrinkling. Based on Reh's conclusions, and when finding the range-of-irreversibility on Püschel's tabulation at 60ºF, the victim could not have been submerged for more than 5 to 6 hours and still have complete reversibility of wrinkling. The victim was last known alive 21 hours prior to being removed from the water. More notably, the ex-husband's activities are documented and stipulated by the prosecution during the 13 hours prior to the victim being removed from the water. Therefore, the convicted ex-husband could not have drowned the victim.

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Drowning; Time of Death; Method Development

I67   Deaths Involving Forklifts in Cook County, Illinois: A Retrospective Review

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Learning Overview: After attending this presentation, attendees will have better knowledge of the characteristics of deaths involving forklifts in a large metropolitan area to emphasize the non-rarity of these events and the potential impact on occupational safety.

Impact Statement: This presentation will impact the forensic science community by exploring demographic data, cause and manner of death, mechanism of death, and autopsy and toxicology findings in deaths involving forklifts in order to inform occupational safety and contribute to the prevention of these events.

Forklifts are powered industrial trucks that are commonly used to move heavy materials in a variety of industries, including warehousing, manufacturing, and construction.[1] While essential in many workplaces, forklifts can be extremely hazardous to those operating or working near them. According to the United States Bureau of Labor Statistics, there were 614 forklift-related fatalities from 2011 to 2017, with an average of nearly 88 deaths per year.[2]

This is a retrospective study aiming to characterize deaths involving forklifts. The Cook County Medical Examiner's Office database was searched to identify cases between 2015 and 2022 with "forklift" in the investigative report. No limits for age, sex, race, or cause of death were imposed. A total of 33 cases were identified. After reviewing the cases, 12 were excluded in which "forklift" was mentioned in the narrative but was not related to the death. This yielded 21 cases in which a forklift was being operated or was intimately involved in the death. Information regarding decedent demographics, cause and manner of death, mechanism of death, and autopsy and toxicology findings was obtained from the investigative reports, autopsy reports, and toxicological studies.

Of the 21 cases, there were 16 accidents, 4 natural events, and 1 suicide. The mean age of the decedents was 42 years (age range: 18–60), and 100% of the decedents were male. Of the 16 accidents, most causes of death were due to blunt force injury (15), and 1 was asphyxiation. The mechanism of death in these cases varied widely, including forklift vs. pedestrian (5), fall from a forklift (3), overturns (3), objects falling from the forklift (3), and compression/pinning (2). Eleven cases mentioned the word forklift on the death certificate.

This study shows that forklift-related fatalities are not exceedingly rare events. While a majority of deaths involving forklifts are accidents, it is interesting to note that both natural events and suicides have occurred. The data will be further analyzed in an attempt to establish any trends or characteristics that are unique to forklift-related fatalities. Knowing the features of these deaths may help the forensic community identify such cases, as well as providing important occupational safety information. Ultimately, the information obtained from this study can contribute to the prevention of forklift-related deaths and injuries in work settings. A thorough discussion of the results will be presented to attendees.

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Workplace; Occupational Incidents; Characteristics

I68   Myocardial Scarring and Interstitial Fibrosis: An Unknowable, Unpredictable, and Unavoidable Tragedy

Gerald Feigin*, Gloucester Camden Salem County Medical Examiner, Sewell, NJ; Priya Banerjee, Anchor Forensic Pathology LLC, Barrington, RI; Gil Sapir, Chicago, IL

Learning Overview: After attending this presentation, attendees will recognize the necessity of always examining the heart muscle in its entirety, especially where there is no obvious cause of death, including coronary artery disease, drugs, trauma, and pulmonary emboli.

Impact Statement: The forensic science community will be impacted through improved standards of autopsy practice by realizing cardiac scarring and interstitial fibrosis is an unexpected cause of death. This yields more accurate autopsy findings and a more accurate determination of the cause and manner of death.

This case concerns a 19-year Caucasian male who was participating in the "Crucible" to complete his Marine Corps basic training. He had no known significant past medical history nor ever had an Electrocardiogram (EKG) screening (including none performed as part of military enrollment physical). On the last day, he had been found deceased away from the main group after an unwitnessed collapse. It was reported that he was fatigued, sweating profusely, and with muscle soreness but without confusion in hours prior to being found. Aggressive resuscitation was performed in the field, but he was declared dead. No core body temperature was obtained. The drill instructor was prosecuted for negligent homicide.[1]

Jurisdiction was taken by the local coroner and an autopsy was performed. The examination showed no significant physical trauma or naturel disease. There were epicardial petechial hemorrhages, left adrenal gland hemorrhage, and pulmonary edema. Toxicology was negative for alcohol, drugs, and medications. Postmortem vitreous electrolytes showed a hyponatremia pattern. Cause of death - hyperthermia (heat stroke). Manner of death was Accident.

By family request, a second private autopsy was performed. The heart had very limited dissection from the prior examination. Subsequent additional dissection and increased sampling for histology revealed: the heart color was mottled red to brown, to pale; histology showed multiple scars; interstitial fibrosis (scar tissue between and around heart cells; hypertrophy (enlargement of cells) to a mild-moderate degree; binucleate heart muscle cells (two nuclei in a cell instead of one); and focal inflammation as well as rare dying heart cells.[2,3] Genetic DNA studies showed two mutations of uncertain significance.

The second autopsy with expanded tissue analysis was pivotal in finding the previously unknown cardiac disease. However, no definite etiology could be determined. It is recommended that extensive myocardial sampling should be undertaken regarding any possible hyperthermia death, and any death where no definitive gross or toxicologic cause of death is observed.[4]

When there is no obvious cause of death, including coronary artery disease, drugs, trauma, pulmonary emboli, always look at the heart muscle in its entirety.[5,6] The postmortem examination should include: collecting specimen from a minimum of 7–10 areas of the left ventricle focusing on any and every area that appears grossly different; preserve and properly fix extra myocardial tissue for future study; and use Masson's trichrome stain to exhibit scarring.[7] When possible, genetic testing also adds crucial information in possible causes and considerations for living relatives.

The Marine's death was an unknowable, unpredictable, and unavoidable tragedy due to the severe preexisting cardiac disease. Without the contradictory second autopsy, military and judicial injustice would have occurred.[8] Myocardial scarring and interstitial fibrosis distinguished this death from the assumed hyperthermia. Proper autopsy practice must include complete dissection of the heart and expanded histologic analysis to ensure rare conditions are explored. There are also clinical considerations for the living relatives who should be referred for cardiac evaluation to prevent death. Always thoroughly examine the heart.

References:
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2. De Jong, S., van Veen, T.A., van Rijen, H.V. and de Bakker, J.M., 2011. Fibrosis and cardiac arrhythmias. *Journal of cardiovascular pharmacology*, 57(6), pp.630-638.
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6. Follow the "Willie Sutton Rule": Go where the money is - the heart. Always, always look at the heart.(paraphrased)
7. Masson's trichrome stain is used to exhibit scarring in bright blue color and very fine detail.
8. *United States v. Steven Smiley*, supra, n.1, Court martial verdict of not guilty for: negligent homicide and lesser included charges (obstruction of justice; cruelty, oppression, or maltreatment of subordinates not guilty of dereliction of duty resulting in death). The Marine Corps has a historical conviction rate of 98% when a Marine goes to court martial. Jeff Schogol, "Parris Island drill instructor found not guilty of Marine's death during Crucible," *Task & Purpose*, July 28, 2023, https://taskandpurpose.com/news/parris-island-drill-instructor-found-not-guilty-of-marines-death-during-crucible/ (accessed July 29, 2023).

Myocardial Interstitial Fibrosis; Hyperthermia; Cardiac Fibrosis

I69   Death by Lung Hypoplasia as a Result of a Bilateral Congenital Diaphragmatic Hernia

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Learning Overview: After attending this presentation, attendees will understand the characteristic autopsy findings in cases of Congenital Diaphragmatic Hernia (CDH) and the importance of accurate investigation and forensic autopsy in unexplained infant death, especially when suspecting medical maltreatment.

Impact Statement: This presentation will impact the forensic science community by presenting an uncommon case of bilateral CDH; it will create greater awareness of the importance of implementing extensive prenatal diagnostic to reduce the number of infant deaths due to lung-hypoplasia as a result of CDH.

Background: CDH accounts for ca. 8% of all congenital anomalies. The incidence of CDH is at approximately 1 out of 2,000–3,000 infants. In ca. 85% of CDH cases, the herniation is left-sided, only in approximately 2% of the CDH-patients are the defects bilateral.[1] Around the eighth week post-conception, the embryological formation of the diaphragm ends.[2] Due to the diaphragmatic malformation, the abdominal organs herniate into the thoracic cavity, often resulting in lung hypoplasia, forming a life-threatening pathology, especially if not properly diagnosed.[3]

In Germany three prenatal sonographic screenings are performed (the first between the 8th to 12th week of pregnancy, the second between the 18th to 22nd week, and the third between the 28th to 32nd week). A screening of the fetal organs is an optional part of the second sonographic screening.[4]

Case Report: A 35-year-old primipara was admitted as an inpatient due to vaginal bleeding in the 41st week of pregnancy. The baseline fetal heart rate in the cardiotocography was tachycardic (160bpm); consequently, a caesarean section was performed. During the operation the heart rate dropped to 67 beats per minute, resulting in switching to an emergency cesarean section. After delivery, the infant was found lifeless with an Apgar-score of 1/0/0. Resuscitation was initiated immediately. In the 19th minute after birth, the infant was intubated. While intubating, the breathing sounds were significantly reduced and only audible with high ventilation pressure. After 30 minutes, the unsuccessful resuscitation was stopped.

The external examination of the infant showed an abnormally high amount of body hair (hypertrichosis). Next to this finding, no signs of underdevelopment or maldevelopment were found. The body was formed age appropriate. The autopsy revealed a bilateral CDH. The defects in the diaphragm measured 4.5cm on the left and 3.5cm on the right side. The edging of the defects was rounded and slightly swollen. On the left side the stomach, spleen, part of the small intestine, and part of the left hepatic lobe were translocated into the thoracic cavity. On the right side, part of the right hepatic lobe herniated through the defect. The liver showed two constrictions due to the diaphragmatic gap. Furthermore, a massive bilateral lung hypoplasia was found, defining the cause of death. Both lungs together weighed 5g, which is equivalent of the normal lung weight at the 19th to 20th week of pregnancy. The lung flotation test was positive. Therefore, no indication of failed ventilation was found.

Discussion: The autopsy revealed a rare case of bilateral CDH resulting in bilateral lung hypoplasia. At the moment of birth, there was no possibility for the infant to survive. The positive lung flotation test and a review of the hospital report showed no indication of medical maltreatment in the context of the birth. It is unknown which prenatal diagnostics the mother attended and if the detection of this serious malformation could have helped with the outcome. The hypertrichosis, in combination with the CDH, could possibly have been part of a syndromal disease.

Conclusion: Unexpected infant deaths due to bilateral CDH are uncommon. In this case, the possibility of medical maltreatment was ruled out through forensic autopsy. This case report demonstrates the importance of forensic autopsy in cases of unexpected infant death.

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Infant Death; Sudden Death; Autopsy

I70   Utilizing an In-House Forensic Epidemiologist in the Denver Office of the Medical Examiner

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Learning Overview: After attending this presentation, attendees will better understand what a forensic epidemiologist does and the significance of this role within a medical examiner's office.

Impact Statement: This presentation will impact the forensic science community by informing attendees how medical examiner and coroner offices can add a forensic epidemiologist to streamline their processes, recognize epidemiologic changes over time, provide data that may be used to impact policies and legislation, and aid in notifications about important health issues in a timely manner in hopes of preventing deaths.

The Denver Office of the Medical Examiner (DOME) employs a forensic epidemiologist, only one of a few in the country, in a grant-funded position. This work has been defined in different ways, but one definition stands out: "the use of epidemiologic methods as part of an ongoing investigation of a health problem."[1] The forensic epidemiologist at DOME pulls information from a web-based software case management system and utilizes a separate program to assimilate this information into unique reports (dashboards) that put the data into interactive graphs.

The dashboards are versatile and have many purposes. Some dashboards provide data to local government officials and ultimately may provide for policy making and legislation. Interactive dashboards are available on the DOME website for public viewing.[2] One can visualize trends of fatal intoxications, prevalence of different drugs over time, traffic fatalities, deaths among individuals experiencing homelessness, and more. In turn, the forensic epidemiologist has been able to rapidly synthesize beautiful graphic displays that are easily incorporated into the Annual Report for DOME, resulting in a much quicker and streamlined publication process and impressive work product.[3]

Many dashboards are utilized in the day-to-day operations of the Office and provide various individuals with vital information. There are specific dashboards for nearly all the sections, including pathologists, investigators, office management, family advocates, and autopsy technicians, each with information that is most important to them. For example, the investigators get notifications from a dashboard that tracks bodies still in the facility after 30 days and another dashboard that tracks cases in which next of kin have not been notified of a decedent's death, thus directing their attention to high-priority tasks. The pathologists use a database to see their total case numbers at-a-glance and very easily display them by various criteria such as case type, cause and manner of death, and even their report turnaround times.

The office has recently changed to in-house transport of decedents to the facility, and a dashboard is used to monitor multiple variables, such as the number of transports, who has done them, and when completed. This helps verify how this new protocol is progressing and if improvements are needed. Another dashboard, that is constantly updated, is available for the administrative team to track the status of death certificates within the jurisdiction.

The ability to quickly analyze the data generated by DOME is invaluable for the day-to-day practice, serves to identify areas that could use improvement, helps with tracking those improvements, and allows for quick discovery of trends that are of public health importance.

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Forensic Epidemiologist; Public Health; Data

I71   A Multidisciplinary Approach to the Canonical Recognition Activities Conducted on the Bone Remains Attributed to St. Hippolistus and the Martyrs Crescentius and Irenaeus (4th Century AD)

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Learning Overview: After attending this presentation, attendees will have significant insights into the technical-scientific contribution to the canonical recognition activities on relics and mortal remains associated with saints and martyrs.

Impact Statement: This presentation will impact the forensic science community by explaining the usefulness of a multidisciplinary approach to canonical recognition activities.

Relics and mortal remains associated with saints and martyrs have long been revered objects of religious significance. Canonical procedure dictates that these sacred artifacts undergo rigorous scientific investigation to verify their authenticity, preserve their integrity, and foster veneration. This study delves into the canonical reconnaissance operation carried out by the Diocese of Avellino (Italy) in preparation for the Hippolistian Jubilee, commemorating 1,720 years since the martyrdom of St. Hippolistus on May 1, 303 A.D. The investigation focused on bone remains attributed to St. Hippolistus and the child martyrs Crescentius and Irenaeus, which were preserved at the Specus Martyrum of Atripalda (ancient Abellinum, Italy).

The study was based on a comprehensive historical and canonical investigation, drawing from available sources such as the Martyrologium Hieronimianum and Passio Sancti Hippolysti (reissued in the Acta Sanctorum bollandiani–1776). According to these sources, St. Hippolistus, born in Antioch during the second decade of the third century AD, was martyred at an advanced age in Avellino (Italy) during the Diocletian persecution. The Passio of the child martyrs Crescentius and Irenaeus was intertwined with that of St. Hippolistus, with the two brothers reportedly martyred at approximately 6 and 10–12 years of age, respectively. Subsequent anthropological investigations were carried out to identify a male subject of Caucasoid origin aged around 77 years, as well as two subadult subjects.

Morphological anthropological analysis was conducted on the bone remains, involving progressive stages of inspection and description, including recognition, lateralization, and cataloging. The search for anthropomorphic and anthropometric features, along with radiographic investigations using plain Radiography/X-ray (RX), were also undertaken. Additionally, bone fragments were subjected to Carbon-14 (14C) dating analysis.

The canonic recognition investigations led to the reconstruction of three anthropological profiles, with insights into the population of origin (ancestry), sex determination, and estimated age at the time of death for each subject. Specifically, one subject (A) was identified as an adult-senile male of Caucasoid origin, showing pathological deformities suggestive of arthrosis. Meanwhile, two subadult subjects (B and C) were aged approximately 4–6 years and 14–16 years, respectively. Consequently, the anthropological evaluations strongly indicated a compatibility between the analyzed bone remains and the profiles attributed to St. Hippolistus and the martyrs Crescentius and Irenaeus.

The multidisciplinary approach reported above, consisting of different medicolegal, anthropological, odontological, and bioarchaeological aspects, represents an essential technical-scientific contribution to canonical recognition activities, useful in the definition of well-founded and shared operational and evaluative methodology.

Forensic Science; Religion; Human Remains

I72   A Death in the Setting of Diabetic Ketoacidosis and Concurrent SGLT2 Inhibitor Use

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Learning Overview: This presentation will describe a case of possible acute drug reaction. After attending this presentation, attendees will recognize the varied presentations of acute drug reactions at autopsy. Recognizing the constellation of findings early can greatly assist in obtaining timely laboratory studies to support the diagnosis.

Impact Statement: This presentation will impact the forensic science community by describing the autopsy findings that should suggest acute hypersensitivity reaction and summarize the severe reactions to SGLT2 inhibitor medications.

Type 2 diabetes mellitus (T2DM) is a form of diabetes that is characterized by high blood sugar, lack of insulin production, and insulin resistance. Chronic complications of T2DM include polyuria, heart disease, and neuropathy, while more acute complications include ketoacidosis and stroke. T2DM can be treated with exercise and dietary changes, though pharmaceuticals are frequently prescribed as part of an initial treatment regimen. First-line drugs include insulin and metformin, while second- and third-line agents include newer classes of medications such as glucagon-like peptide-1 (GLP-1) receptor agonists and sodium-glucose transport protein 2 (SGLT2) inhibitors. Apart from controlling blood sugar, SGLT2 inhibitors have been shown to provide significant cardiovascular benefit. This class of medications, however, has also been demonstrated to have a side-effect profile that includes urinary tract infections, ketoacidosis, necrotizing fasciitis, and hypersensitivity reactions.[1,2]

We report a case of a 43-year-old Caucasian male with a history of T2DM who was found deceased on his kitchen floor after leaving work early two days before with feelings of general malaise. Multiple empty beer cans and prescription medication containers, including metformin and an SGLT2 inhibitor (empagliflozin), were found at the scene. The postmortem examination was remarkable for massive pharyngolaryngeal edema with epiglottitis and acute esophageal necrosis. Analysis of vitreous fluid revealed a markedly elevated glucose with an elevated beta-hydroxybutyrate. A postmortem tryptase was not elevated, though this was performed beyond the acknowledged stability cutoffs. Histologic examination of the epiglottis and uvula revealed numerous degranulating mast cells and eosinophils.

Though uncontrolled diabetes can itself serve as an individual risk factor for epiglottitis and acute esophageal necrosis, it is the authors' belief that this constellation of findings in this decedent is better explained by the concurrent use of and reaction to an SGLT2 inhibitor in the setting of uncontrolled T2DM. This case highlights the importance of varied presentations of acute drug reactions at autopsy and will summarize the severe reactions to SGLT2 inhibitor medications.

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Autopsy; Histological; Drug Analysis

I73   **Death Caused by Wild Animals: _Ex Africa Semper Aliquid Novi_ (Out of Africa There Is Always Something New)**

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Learning Overview: This presentation will provide insight into deaths caused by wild animals in South Africa and may be of interest to all parties involved in death investigation, especially cases where man and nature interact.

Impact Statement: This presentation will impact the forensic science community by assisting death investigators and pathologists in other countries to understand the profile of deaths caused by wild animals.

Globally, thousands of human deaths are caused every year by (or attributed to) animals, in one way or another. Yet there appears to be a paucity of scientific literature reviewing this phenomenon. South Africa has been rated the third most biologically diverse country in the world (after Brazil and Indonesia), but more specifically, probably has the biggest number and spectrum of deadly predators and other dangerous beasts such as elephant, rhino, buffalo and hippo.

As is the case elsewhere in Africa, we see numerous "near miss" events and many human fatalities annually in South Africa, as a direct result of the proximity between man and beast, stemming from this economic symbiosis and, indeed, competition. Every day, those who visit as tourists, those who service this industry, and those who actively engage in the sector or are exposed on various other fronts (legitimate and indeed, illegitimate) are at risk. Unfortunately, we have a very poor grasp of the nature and magnitude of such fatal outcome events across the country as there is no cohesive or systematic official reporting of cases and media reports are erratic and of a regional nature.

This presentation seeks to provide a brief overview of the circumstances and settings under which such fatalities regularly take place in South Africa, with particular reference to incidents involving tourists, those who are involved in the broader wildlife industry, and those who may be affected or involved in more peripheral or indirect ways. A small series of case studies will be presented to reflect some of the diverse and unusual fatalities seen in relation to this growing and vital sector of the economy.

The benefit of medicolegal investigation of death in society should extend beyond the individual, case-based value add: forensic pathologists have greatly contributed to an understanding of the broader public health issues related to illicit drug use, interpersonal violence, suicide and newer or unusual illnesses (of which COVID-19 is a good example). It is proposed that developing countries in particular should make every effort to introduce efficient data gathering and reporting systems in respect of categories of death that may be of specific importance and relevance in those communities, including, for example, industry-specific fatalities in the mining, construction, or agrochemical sector and, indeed, wildlife tourism and related activities.

Autopsy; Death Investigation; Mauling

I74 Investigating Equine-Related and Bovine-Related Human Fatalities in Oklahoma

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Learning Overview: After attending this presentation, attendees will be informed about the patterns of demographics, injuries sustained, and locations of injury in equine-related and bovine-related human deaths. Attendees will also learn about the potential application of safety protocols to decrease these fatalities.

Impact Statement: This presentation will impact the forensic science community by identifying a rare phenomenon of fatalities in which the victims were often members of vulnerable populations. These cases showed discernible patterns in injury and injury location, which could help aid forensic pathologists and medicolegal death investigators in their investigations of large animal-related deaths.

Since the late 1990s, there have been a growing number of research efforts evaluating the patterns associated with equine-related injuries. Most of the data collected focuses on demographics, cause of injury, and sustained injuries of the individuals studied. Though there is a copious amount of published research discussing rates of injury and safety concerns in both equine and bovine activities, there is an impressive lack of research concerning mortality in these industries.[1-19] This study aims to identify patterns in the demographics, causes of sustained injury, location of injury, cause of death, and mechanisms of death in equine-related fatalities in comparison to bovine-related fatalities in Oklahoma.

Data for this study was collected by examining cases from the years 2000–2022 from the Oklahoma Office of the Chief Medical Examiner case database. Of the total cases, 80% of the decedents were male. A large majority of the equine-related fatality cases involved males under 18 years of age and between 60–69 years of age with sustained injuries of the head, neck, and thoracic regions while being mounted. These equine-related injuries were most often inflicted by being kicked or were a result of blunt force trauma upon impact with a hard surface. A majority of the bovine-related fatality cases involved males of the ages 60–69 and 70–79, with sustained injuries of the head, neck, and thoracic regions while being unmounted. These bovine-related injuries were most often inflicted by being butted, trampled, or resulted from blunt force trauma upon impact. Of the total cases examined, approximately 42% of the causes of death were Blunt Force Trauma of the Head/Neck and about 32% were Multiple Blunt Force Injuries. Only 10 mechanisms of death were discussed in the 71 cases analyzed. The lack of reporting for mechanism of death in both subsets may be attributed to the decedents not undergoing autopsy.

The findings in this study support the current literature, where there was a higher frequency of children in equine-related cases and a higher frequency of elder adults in bovine-related cases. There was also a similarity between current literature and the findings in this study, where equine-related cases most often occurred while mounted and bovine-related cases most often occurred while unmounted. In both equine- and bovine-related cases, injuries to the head/neck region and thoracic cavity were most prevalent.

Future research could determine whether other jurisdictions outside of Oklahoma experience similar patterns with equine- and bovine- related fatalities. A more detailed understanding of these patterns could inform safety protocol research, presenting public education and alternative approaches to preventative health measures targeted at decreasing equine- and bovine-related fatalities.

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Fatal Death; Injury; Head Trauma

I75   The Perils of Body Size Assumption in Subadult Sibling Death Identifications

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Learning Overview: The goals of this presentation are to: (1) outline multi-fatality incidents involving siblings; (2) define unique challenges involved with visual identifications in these situations; (3) discuss the body-size assumption; (4) discover the best scientific methods for identification in these instances; and (5) share casework that supports these principles.

Impact Statement: This presentation will impact the forensic science community by outlining the reasons to utilize scientific methods of positive identification as a best practice in cases of subadult sibling deaths versus only relying on the body-size assumption.

Multiple fatality incidents involving a family of victims are a periodic challenge for forensic authorities. Particular incidents where multiple child decedents are involved can be most stressful and time-dependent for autopsy, identification, and release. This can lead to expediting non-scientific identifications in an effort to appease surviving next of kin, the public, and media but is not the best practice in most of these situations. Often these incidents involve a housefire, Motor Vehicle Accidents (MVA) involving fragmentation, or other nidus that precludes reliable visual identification of the victims. Death or incapacitation of parents can also mean less reliable information regarding deceased children from distant next of kin who do not routinely have contact with the family.

It is no mystery that children that are even siblings may grow to have vastly different statures, body types, and body mass. Sometimes younger children surpass their elder siblings in body size at an early age. Conversely, close-in-age siblings and dizygotic twins that are expected to be similar are sometimes in fact quite different. Often the assumption of "bigger must be older" is erroneous and can lead to misidentification.

Fortunately, science works. In these sibling situations, DNA may not be the best first-choice due to the similarities in familial profiles and the problems associated with mixed reference samples between subadult co-habitants. Subadult fingerprint records rarely exist. When antemortem dental records exist for one or more of the siblings, this should be the first line of scientific identification through comparison and/or exclusion. Even when antemortem records are not available, a subadult decedent's age profile can be created through dental age estimation techniques. Dental age estimation is commonly known to be among the best methods for being the least affected by environmental and congenital factors. Because of this fact, detailed morphometric dental age estimation containing statistics can be used to either parse out the older/younger or verify same age siblings, regardless of body size.

This presentation will outline two incidents from the presenter's casework where the body size assumption led to initial confusion at the scene and morgue. However, "cooler heads" prevailed and the time to conduct scientific analysis of dental data was afforded, ending up with positive identifications that would have otherwise been false solely based on the body size assumption.

Victim Identification; Subadult; Physical Assessment

I76   The Prevalence of Ethanol and Drugs Detected in Road Traffic Deaths: A Ten-Year Survey From Jefferson County, Alabama

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Learning Overview: Attendees of this presentation will receive information on the epidemiology of deaths related to motor vehicle accidents and on the prevalence of ethanol and drugs detected in postmortem samples during the investigation of these incidents.

Impact Statement: This presentation will impact the forensic science community by displaying common features of road traffic deaths with drugs detected and sharing information that could aid in preventing and deterring the phenomenon.

Across the world, almost 3,700 people are killed each day in crashes involving motor vehicles, bicycles, or pedestrians, equating to 1.35 million people killed annually. Many risk factors have been identified in road traffic deaths, some preventable such as road users' behaviors.[1] Of these, ethanol- and drug-related impaired driving is the most perceived public health issue. A large body of research has demonstrated that driving with a Blood Alcohol Concentration (BAC) above the legal limit is significantly associated with fatal crash involvement and higher BACs are associated with increased accident consequences[2] Regarding drugs, the risk of accidents and subsequent death may vary depending on the type of drug used. Data from the World Health Organization shows a prevalence of drugs ranging from 8.8% to 33.5% in fatally injured drivers.[3] This study aims to analyze road traffic fatalities throughout Jefferson County, AL, and analyze the prevalence of ethanol and drugs detected. Additionally, after reviewing demographic data and case information, a realistic picture will be provided over a ten-year period of the drink- and drug-impaired driving problem in Jefferson County, AL, through fatal driving cases.

The Jefferson County Coroner/Medical Examiner's Office (JCCMEO) database was reviewed for all road traffic fatalities from 2013 to 2022. The University of Alabama at Birmingham (UAB) Forensic Toxicology Laboratory performed analyses in all cases. Samples were analyzed for ethanol and drugs of abuse (both illicit and prescription drugs were confirmed). Blood and vitreous ethanol results were obtained using headspace-gas chromatography with flame ionization detection. Drug screening was performed by an enzyme-multiplied immunoassay technique in urine with confirmation in preserved blood by gas chromatography/mass spectrometry.

Over the ten-year period, 1,110 motor vehicle-related deaths were investigated by the JCCMEO, with a mean of 111 deaths annually. In contrast to previous years, a 15.5% increase was observed in 2020–2022. A majority of decedents were male (72.5%) and involved people between 21 and 50 years (55.9%). Nearly 2/3 of the fatalities involved drivers (n=727 (65.5%)), whereas passenger and non-motorized road users (pedestrians and cyclists) accounted for 143 (12.9%) and 240 (21.6%) cases, respectively. Among drivers, 45.5% (n=331) would have been considered intoxicated with a mean BAC of 0.19g/dL (nearly 2.4 times the legal limit of 0.08g/dL). Drivers with ethanol and drugs detected had a mean BAC of 0.16g/dL, and of these, cocaine (44 cases), amphetamines (41 cases), and opiates (32 cases) were detected most frequently (either alone or in conjunction with each other). In non-motorized road users, 47.9% (n=115), had a mean BAC of 0.23g/dL when only ethanol was detected and a mean BAC of 0.15g/dL in cases with both ethanol and drugs detected. In these cases, cocaine (32 cases), amphetamines (16 cases,) and benzodiazepines (11 cases) were most frequently seen. Road traffic deaths in those with drugs and/or ethanol detected (considered "intoxicated" road users) occurred most frequently during evening and night hours (6:00 p.m.–6:00 a.m.) and on the weekend (Friday through Sunday), accounting for 67.5% and 54.5% of cases, respectively.

In the past three years (2020–2022), there has been an increase in the incidence of intoxicated subjects compared to the pre-pandemic period (from 44.13% to 49.09%). However, this difference was not significant ($P > 0.05$).

This study analyzing road traffic fatalities highlights the ongoing problem of alcohol and drug use before travel (either motorized or non-motorized), with 45% of the deaths in a ten-year period having one or more substances detected. Utilizing this information, including that most fatal events occurred overnight and on the weekends, may assist public health and law enforcement agencies in establishing preventative strategies and mobilizing deterrent measures during these time periods.

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Traffic Accident; Driving Under the Influence; Motor Vehicle

I77   The Graveyard Spiral and the Day the Music Died

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Learning Overview: Attendees of this presentation will learn how a sensory illusion arising in the inner ear can cause aircraft pilots to experience spatial disorientation and loss of altitude awareness, leading to the graveyard spiral and/or controlled flight into terrain.

Impact Statement: This presentation will assist the forensic community in understanding how aircraft accidents can occur when new and inexperienced pilots fly at night or into meteorological conditions where they can no longer see the horizon.

What do Patsy Cline, Buddy Holly, J. P. Richardson, Ritchie Valens, and John F. Kennedy, Jr. have in common? All five died in aircraft accidents due to controlled flight into the terrain when the pilots became spatially disoriented and put the aircraft into a "graveyard spiral." A graveyard spiral is a tight-banked, diving turn that can end in aircraft structural failure or high-speed impact with the ground. It results from a sensory illusion in the inner ear where the pilot experiences spatial disorientation and loss of aircraft attitude awareness. It is most common at night or in poor weather where no horizon exists to provide visual correction for misleading inner-ear signals. The pilot mistakenly perceives the aircraft as in wings-level flight when the plane is actually in a banking turn. The altimeter indicates an ongoing drop in altitude, and the pilot will try to correct the loss of altitude by pulling back on the control yoke. This adjustment will tighten the turn's radius and quicken the descent rate until the pilot sees the error or contact with the terrain occurs.

The critical elements for graveyard spirals are that the pilot cannot see the horizon and does not take notice of or trust the cockpit instruments. Graveyard spiral accidents commonly start with a prolonged entry into a banked turn. Because the turn happens so slowly, the fluid in the pilot's inner ear creates little to no friction, and they do not feel like they are turning. The pilot's body tells them that they are still straight and level. If they mistrust their instruments, especially the attitude indicator, the problem may quickly worsen.

There are two possible outcomes in this situation. (1) They return the wings to level, but because the fluid in their inner ears was at rest during the turn, they now feel like they are banking in the opposite direction. This overwhelming sensation makes them believe their attitude indicator is incorrect, and they return to the original turn, spiraling until they hit the ground. (2) They are so convinced that the wings are level that the pilot ignores the attitude indicator, and they continue the original turn until they hit the ground

The graveyard spiral most commonly occurs when inexperienced pilots without instrument ratings fly at night or into instrument meteorological conditions and can no longer see the horizon. These pilots are accustomed to flying by the seat of their pants, and because of inner ear sensory illusions, they may fail to recognize or react to their instrument readings.

Accident; Crash; Unexpected Death

I78 I Didn't Want to Kill Him! The Role of Cocaine in Pseudoasphyxia by Compression of the Carotid Sinus

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Learning Overview: Death from compression of the carotid glomus during neck grasping is a somewhat debated cause of death in the scientific world. Many studies have been presented supporting this etiology and others refuting it. The goal of this presentation is to present a rare case of death due to carotid sinus compression in a subject with acute cocaine intoxication during a choking attempt.

Impact Statement: This presentation will impact the forensic science community by discussing the importance of taking into consideration carotid sinus compression as a cause of death in the presence of neuro stimulants such as cocaine in cases of a struggle. This presentation seeks to show to forensic pathologist colleagues the practical steps (from autopsy to toxicological examination) and the scientific reasons that allowed resolution of a peculiar case of pseudoasphyxia by compression of the carotid sinus in an acute cocaine intoxication state.

A 20-year-old boy was found dead, naked wearing only his socks, in a ditch during a night in November. Numerous scratches were found on the neck and below the right earlobe was a small bruise. The corpse was examined externally, revealing two additional ecchymotic areas at the V metacarpal of the right hand. The socks were broken, and the heels were grazed. On the same day, a transgender prostitute showing multiple bruises on his face and costal fractures presented himself to the police, denouncing an assault. The autopsy examination of the boy's body showed total indemnity of the neck muscles. However, at the level of the right carotid sinus, a modest area of hematic infiltration was evident, corresponding to the ecchymosis shown on external examination.

Histological examinations showed the presence of erythrocytic infiltrates at the carotid glomus and peri-carotid tissues. Finally, concurrent toxicological examinations performed on blood and urine—corrected for weight and age—showed that at the time of death, the boy was in a state of acute cocaine intoxication (331.56ng/ml in blood, 4567.54ng/ml in urine). The set of necropsy analysis led to confirmation that the boy's death had occurred due to a sudden compression on the carotid sinus, whose state of hyperexcitability had been favored by the intake of cocaine, a neuro stimulant substance. Moreover, the absence of cervical muscle infiltration allowed exclusion of airway and vascular compression as the cause of death.

In the face of overwhelming autopsy evidence, the transgender man who showed up at the police station confessed: during a sexual act they had in the car after taking drugs together, the boy began to hit him violently in the face. The man, to free himself, tried several times to grab him on the neck. When he succeeded, the boy suddenly collapsed lifeless. Caught up in the excitement, the man hid him in the ditch. Death by compression of the carotid sinus during neck grabbing has been widely described in the literature, especially in strangulation cases.[1-3] Indeed, some individuals have abnormal carotid sinus sensitivity, a well-known condition called "carotid sinus syndrome."[4] However, further works have shown that even in the case of compressions longer than 30 seconds, they can induce no changes in the electrical conduction of the heart other than brief stages of bradycardia.[5]

In the present case, the influence of cocaine on nerve terminals was crucial in determining death. In fact, cocaine can induce peripheral blockade of norepinephrine reuptake, with concomitant baroreceptor inhibition and increased sympathetic nerve discharge.[6] The imposing vasoconstrictive stimulus induced by adrenaline and norepinephrine combined with its direct action on the carotid chemoceptive system resulted in a violent increase in the discharge rate of carotid sensors.[7] Moreover, additional elements that can increase the degree of carotid sinus response include environmental stressors such as low temperatures, the subject's state of nudity, and the excitement due to the struggle.[8] Thus, in this case, the carotid discharge on cardiac tissue was complemented by the direct excitatory stimulus induced by cocaine, resulting in a strong pro-arrhythmic input and subsequent cardiac arrest. In addition, stressogenic stimuli such as low temperatures and the boy's nakedness played a crucial role in determining the proarrhythmic stimulus at the cardiac level. Therefore, in case of suspected strangulation, the assessment of cocaine levels, associated with macro and microscopic analysis of the carotid sinus, becomes a pivotal point for the determination of the mechanism of death.

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Cocaine; Homicide; Sudden Death

I79   Not So Much the "Spice" of Life: Identifying a Source for a Series of MDMB-4en-PINACA Deaths

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Learning Overview: The goal of this presentation is to describe a series of seven deaths associated with a single source of MDMB-4en-PINACA, occurring over a three-month period (March–May 2023) at the Tarrant County Medical Examiner's office in Fort Worth, TX. Utilizing case investigation, postmortem examination, and forensic toxicology data, attendees will gain an understanding into the factors that helped identify the single source for this fatal product involved with these deaths. After attending this presentation, attendees will have gained insight into the mechanism and manner of death resulting from MDMB-4en-PINACA toxicity, as well as the importance of thorough investigation into seemingly unrelated cases. Attendees will also have further knowledge into the detection of both MDMB-4en-PINACA and butanoic acid metabolites in postmortem samples.

Impact Statement: This presentation will impact the forensic science community by showing how the importance of a collaborative investigation, coupled with available laboratory data, can provide an effective means for identifying sources of illicit drugs. This presentation will also reinforce the impact all elements of the forensics team have in contributing to the investigation of these deaths when a common drug source is involved. Pertinent postmortem findings will also be presented as related to published findings in these types of deaths.

Synthetic cannabinoids are a relatively new collection of designer drugs that have become increasingly popular within the past decade. MDMB-4en-PINACA is a newer synthetic cannabinoid, with one of the first reports on its metabolism appearing in the literature in 2019.[1] First marketed as "herbal incense" products, the drug's effects are similar to delta-9-Tetrahydrocannabinol (THC). In the United States, the Drug Enforcement Agency has temporarily placed the drug into Schedule I status on May 4, 2023, with at least one state classifying it as Schedule I.[2]

As emerging illicit compounds, synthetic cannabinoids primarily act as agonists of cannabinoid receptors and these substances pharmacokinetically behave in a manner similar to THC. Additionally, once consumed, their effects mimic natural cannabinoids. However, like other illicit substances, their potency and toxicological effects may vary greatly depending on the source of the drug. Toxicity can exhibit a spectrum of effects in a multitude of organ systems. Psychologic effects can range from slurred speech or impaired motor function to psychoses.[3-5] Severe physiologic effects include hypertension, hyperthermia, tachycardia, seizures, stroke, pulmonary hemorrhage, renal damage, and cardiomyopathy; it has also been hypothesized that some deaths are a result of arrythmia.[6-8] In our series, we will present pertinent autopsy findings with respect to different organ systems with a focus on cardiovascular findings.

In order to better determine if a person expired due to a drug toxicity, postmortem toxicology is essential in any case where drug abuse is suspected. The detection of MDMB-4en-PINACA can be found in blood, urine, and hair utilizing such methods as pooled Human Liver Microsomes (HLMs) assay and Liquid Chromatography/High-Resolution Mass Spectrometry (LC/HRMS).[9,10] In our cases, Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) was used to detect the parent substance and/or metabolite, which will not cross-react with the THC Enzyme-Linked Immuno-Sorbent Assay (ELISA) methodology.

At the conclusion of this presentation, attendees will have a general understanding of the pathological findings (or lack thereof) in MDMB-4en-PINACA cases based on our series. They will also be familiar with toxicology findings and how a collaborative effort was able to determine a source of the drug.

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MDMB-4en-PINACA; Toxicity; Case Series

I80   **Elevated Beta-Hydroxybutyrate Within a Pediatric Population and Its Associated Pathology: A Retrospective Case Series**

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Learning Overview: After attending this presentation, attendees will be able to recognize the different pathologic processes that can lead to an elevated postmortem vitreous and/or blood Beta-Hydroxybutyrate (BHB) level within a pediatric forensic autopsy population and its associated histologic findings.

Impact Statement: This presentation will impact the forensic science community by demonstrating what types of cases may have ketoacidosis at autopsy in the pediatric population and how to diagnose ketoacidosis using body fluids for biochemical analysis and histologic changes in the major organs.

Elevated BHB is used as a marker for ketoacidosis. Postmortem values for the diagnosis of fatal ketoacidosis and the associated clinical and histologic findings are limited in the pediatric population.

A retrospective case series of pediatric autopsy records within the Eastern Ontario Regional Forensic Pathology Unit were analyzed. Deaths from January 1, 2010, to December 31, 2022, of children under the age of 18 years with an elevated BHB level (either vitreous concentration >2mmol/L, or blood concentration >200mg/L) were examined. Autopsy reports were reviewed to obtain demographic information, clinical symptoms, and causes of death. Histology of the heart, lung, liver, and kidneys were reviewed by two pathologists blinded to the BHB levels. Consensus was obtained for scoring histologic changes in these tissues. Statistical analyses were performed to look for correlation between histologic changes and BHB levels.

During the study period, 512 pediatric autopsies were conducted of which 20 cases (3.9%) met the inclusion criteria. The age ranged from stillborn to 17 years, with a mean age of 6.28 years and a median age of 3.5 years. Thirteen were male. The mean concentration of vitreous BHB was 3.9mmol/L (median 2.69; range 2.0–11.86mmol/L); n=17 and the blood BHB had a mean of 416mg/L (median 291; range from <50mg/L to 1,188mg/L; n= 9). Death was attributed to diabetic ketoacidosis in only three cases, one of which was related to maternal diabetic ketoacidosis in a case of intrauterine death. The most common cause of death with an elevated BHB was infection (n=7). Elevated BHB levels were also seen with trauma, congenital heart disease, drug toxicity, malignancy, an unspecified inborn error of metabolism, and undetermined cause of death. A strong linear relationship was found between vitreous and blood BHB ($p<0.0001$), confirming both fluids can be used for identification of ketoacidosis.

Clinically, 55% of children with an elevated BHB had reported infectious symptoms but only 20% showed evidence of infection histologically. Histologic changes in the liver were the most common microscopic finding. Changes, however, were seen in all tissues examined in this pediatric population with hepatic steatosis (17 cases), glycogenated hepatocyte nuclei (5 cases), renal tubular vacuoles (7 cases), and heart vacuoles (9 cases) observed. The only statistically significant correlation was found between vitreous BHB and renal tubular vacuoles ($p=0.014$).

Overall, elevated BHB levels were identified in blood and/or vitreous in fatal diabetic and non-diabetic cases, including infection. Diabetic ketoacidosis had the highest level of BHB (11.86mmol/L), but over half of the cases studied demonstrated a vitreous BHB greater than 2.5mmol/L. Microscopic changes were seen in the heart, liver, and kidneys, including non-diabetic deaths within this study.

Autopsy; Pediatric; Metabolic

I81 Pediatric Fatalities Due to Methadone Toxicity in Cook County, Illinois: Considerations on the Manner of Death

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Learning Overview: After attending this presentation, attendees will have better knowledge of the characteristics of methadone-related toxicity in children and the various available tests that can be conducted to provide evidence to support the manner of death.

Impact Statement: This presentation will impact the forensic science community by presenting several cases of methadone toxicity in children and adolescents, drawing attention to the recent increase in the number of such cases. Furthermore, this presentation will provide practical examples and suggestions on how the forensic pathologist can effectively handle and investigate these cases to accurately determine the manner of death.

Methadone, a synthetic opioid used for pain management and opioid addiction treatment, has been associated with a recent increase of pediatric fatalities in Cook County, IL. There is no univocal consensus on how to determine the manner of death in such cases, and policies vary based on the medical examiner/coroner office. Deaths due to methadone toxicity in the pediatric population can be related to accidental ingestion, but voluntary administration of methadone, especially in very young or vulnerable subjects, cannot be excluded. When there is no conclusive evidence, the manner of death may be certified "undetermined."

In this retrospective analysis, a comprehensive examination was conducted on pediatric fatalities associated with methadone toxicity at the Cook County Medical Examiner's Office in Chicago, IL, between January 2015 and July 2023. Only subjects between 0 and 18 years were included. No other limits were imposed. Demographic data, medical histories, toxicology reports, autopsy reports, investigative and police reports, and circumstances surrounding each incident were evaluated to identify common trends and risk factors.

A thorough medicolegal death investigation approach has been used after the increased trend of childhood deaths related to methadone. This involved the collection of multiple specimens, including hair, at the time of autopsy. When toxicology analyses detected methadone and its metabolite 2-Ethylene-1,5-Dimethyl-3,3-Diphenylpyrrolidine (EDDP) in blood, additional tests on other matrices were performed to confirm the results. Hair samples were also tested to evaluate for chronic vs. single administration. The evidence of repeated administration of methadone helped in determining the manner of death.

Educating caregivers and health care providers about proper storage and disposal of medications is of utmost importance in reducing accidental exposures. Furthermore, health care professionals must exercise caution while prescribing methadone to adults in households with children, considering the potential for diversion and child access. A history of previous Department of Children and Family Services (DCFS) involvement should be considered a critical red flag for possible voluntary administration of methadone to vulnerable children.

Several cases will be presented to the attendees, and comments and suggestions on how to effectively handle and investigate these cases to accurately determine the manner of death will be provided.

Forensic Science; Methadone; Pediatric

I82   Postmortem Interval Estimation Using MiRNAs: A Forensic Application

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Learning Overview: This presentation seeks to introduce a novel scientific approach for estimating Postmortem Interval (PMI) in cases with extended PMI periods, employing microRNA (miRNA) analysis. This presentation will showcase the significance of miRNA as a reliable and stable biomarker for estimating time since death in longer PMI. By attending this presentation, attendees will gain a comprehensive understanding of the vital role of miRNA-based PMI estimation in forensic investigations. Attendees will recognize the miRNA's robustness as a valuable source of potential evidence, capable of offering crucial insights into the chronological sequence of events surrounding a death. This presentation aims to empower forensic professionals with a new perspective on PMI estimation, elevating the accuracy and efficacy of investigations involving PMI estimation in advanced stages.

Impact Statement: This presentation holds the potential to create a substantial impact within the forensic science community by highlighting the necessity of sharing miRNA-based PMI estimation, as it is the most crucial question for medicolegal experts. Our pioneering study conducted in India, focusing on PMI estimation through human cardiac tissue samples, yielded promising and encouraging results. Through this collaborative knowledge exchange, forensic professionals will gain valuable insights into the efficacy and potential of miRNA as a reliable biomarker for estimating PMI in cases with extended PMI durations.

The PMI is the time between physiological death and the subsequent finding of a deceased body.[1] Estimating PMI in suspicious deaths is one of the most difficult problems in forensic medicine and pathology, especially in the advanced decomposition stages.[2] PMI frequently lasts from 3 days to 18 months after death at this stage.[3,4] Accurate determination of time since death holds vital importance in criminal investigations, enabling investigators to narrow down the list of potential suspects.[5]

In recent times, the utilization of miRNA profiling has gained attention in various forensic fields, particularly in PMI estimation. This study seeks to showcase the promising capabilities of miRNA in estimating PMI during the advanced decomposition stage. Owing to their compact size, miRNAs exhibit greater stability compared to RNA, though not as durable as DNA. The research explores the feasibility of miRNA analysis as a reliable and robust approach for accurately determining PMI in challenging forensic scenarios.[6]

For this study, miR-195, miR-206, and miR-378 were chosen as the target miRNAs, while miR-1 served as the reference miRNA, aiming to estimate PMIs in cases with longer time spans after death. Five grams of left ventricle tissue of hearts were collected from medicolegal autopsies. Deceased individuals of road traffic accidents (aged 18–32 years) were involved in the study. Subsequently, these tissue samples were subjected to various time intervals at room temperature (12, 24, 48, 72, 96, 120, 168, and 196 hours) to simulate longer PMIs and the stability and potential of the selected miRNAs for accurate PMI estimation was investigated.

The samples were subjected to a Trizol-based RNA isolation protocol to extract total RNA, followed by complementary DNA (cDNA) preparation. The cDNA was then amplified using specific miRNA sets in Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) for analyzing miRNA expression at various time intervals. The findings revealed a significant correlation between miRNA expression and increasing PMI, with their changes showing significance over time. Notably, the ΔCt values exhibited a significant decrease from 12 hours to 196 hours for all miRNAs. Among the selected miRNAs, miRNA-206 demonstrated significance at more time intervals compared to the other two miRNAs. On the other hand, miRNA-1 remained stable until 196 hours, indicating its potential as a reliable endogenous marker for PMI estimation. These results indicate the feasibility of miRNA-206 and miRNA-1 as valuable tools for accurately estimating PMI during longer PMIs.

In conclusion, miRNA holds promising potential as a tool for future PMI estimation, particularly in cases of long PMIs, when combined with other established techniques. Nevertheless, further validation of the study's findings is necessary to achieve more accurate and precise PMI estimations.[7]

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Forensic; Postmortem Interval; micro-RNA

I83   **Recombinase Polymerase Amplification (RPA) for the Identification of Canine and Chicken Blood Samples**

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Learning Overview: After attending this presentation, attendees will be informed about RPA, an isothermal DNA and RNA amplification technique that is of growing interest in forensics. This technique in the current study has been applied to animal abuse cases for the on-site/field detection and species-specific identification of canine and chicken blood.

Impact Statement: RPA as mentioned is of growing interest for point of care or field-based DNA detection strategies. The technique, being cost effective, able to be performed at a range of ambient temperatures, and provide results within minutes, makes it highly attractive in forensic science settings where laboratory access may be limited, or resources are low. This presentation will impact the forensic science community by demonstrating its efficacy in the detection of canine and chicken blood, but the technique and process can be used to identify DNA from any species. The capabilities and applications of RPA are expected to have significant impact in non-human forensic cases.

Dogfighting and cockfighting are illegal in all 50 states through the federal Animal Welfare Act, which was established in 1966. Nonetheless, these so called "blood sports" still take place at an alarming rate in the United States. It is estimated that for just dogfighting alone, there are over 40,000 people involved annually, and over 16,000 dogs are killed annually through dog fights. Fines for dogfighting can be up to $250,000, with spectators able to be fined $5,000. Jail time for organizing a fight can be up to five years in prison and is considered a felony. The impact of organized fighting rings is far reaching. In addition to the obvious animal abuse and brutality involved, they are considered gateway crimes. Other forms of criminal activity are almost always associated with animal fighting such as racketeering, illegal gambling, illicit drugs, and illegal firearms.

One of the main problems with prosecuting such crimes and animal abuse cases in general is the fact that the abuse or fighting is not often observed by law enforcement during the act. Circumstantial evidence, lack of non-human related forensic tools and expertise, and the fact that due to other crimes associated with animal crimes, they often will take the priority in investigations. Criminal charges involved in dog/cock fighting prosecutions often involve an aggregate of evidence and crimes. One agency, the American Society for the Prevention of Cruelty to Animals (ASPCA), provides support to law enforcement at crimes scenes involving animal abuse.

One of the issues often encountered during processing scenes is the identification of blood stains as belonging to chickens or canines. Traditionally, samples would have to be taken and sent to specialist labs to do species identification via expensive methods such as Polymerase Chain Reaction (PCR). To aid in investigations and crime scene processing, this current study explored the use of Recombinase Polymerase Amplification (RPA). This method is an isothermal amplification process for DNA and RNA. This means the need for expensive laboratory equipment such as thermocyclers is removed. The process occurs at low and constant temperatures, not requiring an initial denaturing step. These features make RPA an attractive technique for field-based forensics and low resource settings. We report on the successful development of an RPA-based assay for the detection of chicken and dog DNA to provide a novel tool for investigators in crimes involving these animals.

Animal Abuse; Bloodstains; DNA

I84 The Development of Real-Time and Digital PCR Kits for Simultaneous Quantification of Human and Bacterial DNA From Biological Evidence

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Learning Overview: The goal of this presentation is to inform attendees of the real-time Polymerase Chain Reaction (PCR) and digital PCR kits that simultaneously quantify bacterial DNA and human mitochondrial DNA (mtDNA) and were developed to perform Short Tandem Repeat (STR) profiling and microbiome analysis on forensic evidence such as saliva, skin cells, and other biological materials collected from crime scenes.

Impact Statement: This presentation will impact the forensic science community by informing attendees that for the analysis of trace amounts of forensic DNA evidence through microbiome analysis, a precise quantification process of bacterial DNA and human DNA is necessary from purified DNA. Furthermore, the simultaneous quantification kit for human and bacterial DNA can also be utilized effectively in quality control, such as confirming contamination of bacterial and human DNA in biopharmaceuticals and other industries.

Microbiome refers to the community of microorganisms present both inside and outside the human body.[1] Forensic microbiome is a discipline that utilizes this microbiome from a forensic standpoint, applying it to determine the suspect's gender, estimate the Postmortem Interval (PMI), or identify the cause of death.[2,3] However, there are currently no kits available for quantifying bacteria and human DNA from forensic evidence. Therefore, this research aimed to develop a human-bacterial DNA simultaneous quantitative kit for microbiome analysis and Short Tandem Repeat (STR) profiling of forensic evidence.

To achieve this, two methods were employed: TaqMan® probe-based real-time PCR, which allows real-time quantification with a standard curve, and droplet digital PCR (ddPCR), which offers even higher sensitivity without the need for a standard curve. The QuantStudio™ 3 Real Time PCR System and the QX200™ Droplet Digital™ PCR System were used as the quantitative Polymerase Chain Reaction (qPCR) and ddPCR equipment, respectively. For designing specific primers and probes for bacteria and human DNA, 16S rRNA reference sequences from 343 bacterial species were obtained from the National Center for Biotechnology Information (NCBI), and ClustelW and Primer-BLAST were utilized to design primers and probes targeting the 16S V7 region. For human DNA, which is prone to contamination or exists in trace amounts, the mitochondrial DNA, known to have a high copy number per cell, was selected, specifically the NADH-dehydrogenase subunit 5 (ND5) gene, which has unique sequences for different species.[4,5]

A total of 47 mtDNA reference sequences were obtained from the NCBI for species such as human, ovine, bovine, porcine, avians, canine, and feline, and primer and probe design was performed using ClustelW and Primer-BLAST. Specificity was confirmed through conventional PCR and electrophoresis, showing that the designed primers and probes were specifically bound to their targets. DNA Free-Multiplex Master Mix was used for developing the qPCR kit, and ddPCR Supermix for Probes was used for ddPCR, following the product manuals for the amplification conditions, except for the annealing temperature. Annealing temperature optimization experiments resulted in an optimal condition of 60℃ for both methods, and sensitivity experiments confirmed accurate quantification down to 2 picograms. These developed kits allow simultaneous quantification of bacterial DNA and human mtDNA, enabling the determination of the presence and ratio of bacteria and human DNA. As a result, efficient analysis of the microbiome of forensic evidence and the possibility of performing STR analysis can be established, leading to a positive impact on the forensic aspect of evidence analysis.

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Human mtDNA and Bacterial DNA; Real-Time PCR; Droplet Digital PCR

I85 A Retrospective Review of Genetic Testing and Its Impact on Cause of Death

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Learning Overview: The goal of this presentation is to utilize a retrospective review of genetics performed at the Tarrant County Medical Examiner's (TCME) Office to evaluate the impact on cause of death. Genetics yielded few results that influenced the cause of death; however, the information obtained may prove to be useful in the future as more variants are associated with diseases.

Impact Statement: The National Association of Medical Examiners recommends that DNA samples are retained for cases of sudden unexplained death of individuals under 40 years of age.[1] Retaining these samples can be particularly useful for cases of Sudden Unexplained Infant Deaths (SUID) and Sudden Unexplained Death in Childhood (SUDC) as some hypothesize that 25–35% of autopsy-negative sudden unexplained deaths and 10% of sudden infant death syndrome cases can be attributed to genetic mutations that lead to cardiac arrhythmias, such as long QT syndrome or Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT).[2] This presentation will impact the forensic science community by informing attendees that the goal of this study was to determine the value of genetic testing in a forensic autopsy setting.

At the TCME Office in Fort Worth, TX, samples from all infant deaths, other unexplained pediatric deaths, and unexplained deaths of young adults have been sent for genetic testing via Ambry Genetics CardioNext® panel, which tests 92 genes related to inherited cardiovascular disease.[3]

Between January 2020 and June 2023, 80 requisition forms were submitted to Ambry Genetics from TCME. Of those, 71 met inclusion criteria of the date of death being during the study period, results being on file, and not holding a "pending" status for cause of death. The included data covered cases with an age range of 0 months to 55 years. Pediatric deaths comprised 61% of cases; 31% of the dataset was deaths under the age of 1 year with 48% of the dataset being of Caucasian ethnicity, 31% Black, 18.3% Hispanic, 1.4% Asian, and 1.4% unknow;. 63% of cases were male and 37% were female.

Of the 71 cases, 18 returned negative CardioNext® panels. The remaining 53 cases revealed 106 detected mutations, including 3 that were found in a case that received an expanded neurodevelopment panel. Mutations reported as either "Pathogenic Mutation," "Likely Pathogenic," "Carrier State," or "Variant of Unknown Significance." Clinical significance as reported by Ambry Genetics was dependent on the volume of current literature available to associate each mutation with a pathogenic state.

Of 106 mutations, 100 were classified as variants of unknown significance. Of the 6 remaining mutations, 1 mutation was classified as likely pathogenic, 2 were detected in the carrier state, and 3 were detected as expressed traits. One of the 3 carrier states was discovered in the expanded neurodevelopment panel, and the likely pathogenic mutation was detected in a case that also harbored a pathogenic mutation.

The three pathogenic mutations were found in the genes RYR2, TTR, and COL3A1, which are associated with CPVT, transthyretin amyloidosis, and Ehlers Danlos Syndrome (EDS), respectively. The ages of the decedents were 4 years, 16 years, and 26 years, respectively. The cause of death assigned to the case with the mutation in RYR2 was determined to be "CPVT due to pathogenic mutation in the RYR2 gene." The cause of death assigned to the individual with the COL3A1 mutation was determined to be "Aortic Dissection with Hemothorax due to Vascular Ehlers-Danlos Syndrome." However, the cause and manner of the case with the TTR mutation have been recorded as "Undetermined" due to the inconsistency between the detected mutation and the histological findings and age of the decedent.

Overall cardiac-related deaths (other than myocarditis) were recorded in 7 pediatric and 16 adult deaths. One pediatric cardiac death was determined based on genetic testing and detection of a pathogenic mutation in the RYR2 gene. Adult cardiac deaths also included 1 that was determined based on genetic testing results in the COL3A1 gene; however, autopsy findings provided additional context for cause of death.

Among 71 total cases that received genetic testing, 15 (21%) resulted in an undetermined cause of death. Among the 43 pediatric deaths, 9 (21%) resulted in an undetermined cause of death; and 16 (37%) were listed as SUID, SUDC, or a related variation. One cause of death in the whole dataset was determined based on genetic testing alone.

In conclusion, 71 forensic autopsy cases received genetic testing to aid in determination of cause of death, returning 106 mutations in the Ambry Genetics CardioNext® panel. Of these mutations, 2 greatly impacted the final cause of death. Based on this data, this author would not recommend excessive use of genetic testing in the forensic autopsy setting but feels that further analysis with a larger dataset is warranted.

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Autopsy; Genetics; Sudden Cardiac Death

I86 The Impact of Mouthwash on Microbiome Associated With Human Saliva and Its Implication in a Bacterial Signature-Based Body Fluid Identification Method

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Learning Overview: After attending this presentation, attendees will understand the effect, or lack thereof, of mouthwash on bacterial abundance and diversity utilized in the bacterial signature-based body fluid identification method developed by Dr. Denise Wohlfahrt et al.[1]

Impact Statement: The presentation will impact the forensic science community by informing attendees of the possible impacts on a developing body fluid identification method and what further validation may be required before the method is available for casework.

Body Fluid Identification (BFID) is important in forensic investigations. Body fluid can be identified by catalytic, immunological, microscopic, microbiological, and molecular methods. Recent studies have shown that a bacterial signature-based BFID method can identify seven body fluids in a single test with an overall accuracy of more than 88%.[1] The bacterial signature-based BFID method requires several validation studies before it can be implemented in a crime lab setting.

Mouthwashes are commonly used to prevent oral disease and maintain oral health. The use of mouthwash can impact oral microbiome composition and hence can have an impact on the accuracy of saliva identification using bacterial signature-based methods. The main aim of this study is to determine the impact of mouthwash (Listerine® mouthwash) on oral microbiome and its impact on the accuracy of human saliva identification using a bacterial signature-based method.

Saliva samples were self-collected with cotton swabs immediately before Listerine® use (reference), immediately after (0 hour), 2 hours after, 8 hours after, and 24 hours after from ten subjects (n=50). Bacterial DNA was extracted using the QIAGEN® DNA Investigator kit and was quantified using a quantitative Polymerase Chain Reaction (qPCR) -based method. Variable region four (V4) of the 16S rDNA was amplified via a Polymerase Chain Reaction (PCR) method, purified with the AMPure® XP Kit, and sequenced with a dual-index high throughput sequencing strategy on the MiSeq® FGx™ sequencing platform using the MiSeq® reagent kit v2. No significant difference in bacterial DNA yield, bacterial diversity, and bacterial structure was observed between various treatments (i.e., the mouthwash treatment didn't alter oral bacterial signature significantly). Overall, *Streptococcus*, *Gemella*, *Haemophilus*, *Veillonella*, and *Rothia* were the top five bacterial genera associated with human saliva samples. The bacterial signature-based method for BFID accurately identified saliva in the samples with an overall accuracy of more than 90%.

In conclusion, this study shows that oral samples collected even after the use of mouthwash have no significant impact on the bacterial signature associated with oral samples, and the use of mouthwash did little to impact the accuracy of the bacterial signature-based method for BFID.

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Bacterial Signature; Mouthwash; 16s rDNA Sequencing

I87   The Forensic Application of Monoclonal Anti-Human Glycophorin A (Anti-GPA) Antibody in Samples From Decomposed Bodies to Establish Vitality of the Injuries: An Experimental Study

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Learning Overview: The goal of this presentation is to showcase an experimental study that aims to investigate anti-GPA antibody immunohistochemical staining in order to evaluate the vitality both of soft tissue injuries and bone fractures sampled from corpses in advanced decomposition states.

Impact Statement: This presentation will impact the forensic science community by demonstrating the importance of glycophorin analysis to evaluate the vitality of injuries in corpses found in advanced decomposition states. Particularly, it could be very helpful when there is no evidence of hemorrhagic infiltrations, both at macroscopical analysis and histological investigation.

Glycophorins are an important group of Red Blood Cell (RBC) transmembrane proteins. Monoclonal antibodies against GPA are employed in immunohistochemical staining during postmortem examination: Through this method, it is possible to point out the RBC presence in tissues. In the forensic field, the Anti-GPA monoclonal antibodies are commonly used in human blood detection, identifying RBC membrane antigen.[1] Moreover, monoclonal antibodies anti-GPA can be used in immunohistochemical staining at postmortem examination to point out RBC presence in tissues.[2] Thus, if in non-putrefied corpses, the macroscopic evidence of hemorrhagic tissue infiltration is commonly considered a macroscopic sign of the viability of a lesion, research using anti-GPA antibodies could allow the differential diagnosis between ante-mortem and post-mortem lesions.

A large number of immunohistochemical staining is frequently used in order to establish the vitality in skin lesions, such as anti-α-1chymotrypsin, anti-fibronectin, anti-TGF-α andTGF-ß1, anti-inflammatory cytokines, anti-TNFα, anti-adhesion molecules, and anti-tryptase.[3,4] However, these antibodies show reactivity on well-preserved tissues, while their use remains uncertain when the postmortem examination is performed in decomposed corpses or in human skeletal remains.

Six cases were selected, analyzing autopsies' documentation performed by our Institute of Legal Medicine using four samples of fractured bones and three samples of soft tissues. For the control case, the fracture region of the femur and two experimentally produced injuries (skin and soft tissues surrounding laryngeal area) was sampled.

The anti- GPA antibody immunohistochemistry was performed in all cases, showing interesting results. Monoclonal antibodies against GPA resulted positive in all analyzed cases, indicating the presence of RBC, and demonstrating the vitality at the moment of the lesion. Indeed, the results in the control case were negative.

The discussed data confirmed that the histopathological investigation should be combined with the immunohistochemical examination: Indeed, evaluating the vitality of an injury, immunohistochemical diagnosis can provide reliable information.[5] Mainly, this study highlighted the importance of the GPA technique both on bones and on soft tissue in order to collect information on RBC presence, collecting information about the vitality of the lesion in decomposed corpses.

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Forensic Science; Decomposition; Histological

I88 A Metagenomic Approach in Forensic Science for Human Identification Among a North Indian Population

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Learning Overview: The goal of this presentation is to take a look at the exciting potential of the human microbiome in forensic investigations, providing light on its value as a source of evidence. Attendees will obtain interesting knowledge of how the human microbiome and human identification have grown as an important part of forensic research. Current human identification approaches are based on different genetic traits. However, forensic microbiome study is a new and novel method with the potential to improve the accuracy and scope of human identification in forensic science.

Impact Statement: This presentation will make a significant impact on the forensic science community by highlighting the value of identifying individuals using human microbiomes as a potentially useful forensic approach when combined with genome sequencing technology and bioinformatics. This cutting-edge technology establishes individual identities by using the microbial populations that reside in and on bodies. This presentation will look at how microbial forensics may be used for human identification, serving as a boon for the criminal justice system. Our 16S metagenomics analysis of oral plaque has yielded promising initial outcomes for forensic human identification in India. This presentation will also be an asset for research in India. So far in India, the forensic department is not using this as a tool, and this presentation will be eye-opening for the government of India.

The forensic microbiome is a developing area that has the potential to be developed as a valuable tool in criminal investigation, with capabilities of identifying criminals and uncovering key information at crime scenes. It comprises a variety of techniques such as metagenomics for microbiome profiling and analyzing the abundance and diversity of any potential pathogens and community present in different samples obtained from the scene of the crime, such as soil, skin, blood, plaque, etc.

This study is one of a kind from India, in which we have summarized the potential of microbiome in individual identification. The forensic human microbiome study on the Indian population (unpublished) along with the previously published research highlights the utility of microbial forensics in crime scene investigation.[1-4] We will also discuss developing technology and future prospects as well as the problems and limitations of microbial forensics.

Forensic approaches have been widely accepted around the world for individual identification. So, far this approach is not used in India due to various limitations. This is a one-of-a-kind study, where we extracted DNA from 100 dental plaque samples of the North Indian population from India. The preliminary analysis of the microbiome was performed with Kraken2; the results obtained have suggested that the alpha and beta diversity in all the samples were found to be 197±28 and 223±30. The principal component analysis shows the separate clustering between vegetarian and non-vegetarian individuals; these clusters are not biased due to the male or female ratio. The genus found in the abundance include *Streptococcus, Actinomyces, Bacillus,* etc. Pathogens associated with dental caries like *Streptococcus pyrogens* and *Streptococcus mutans* (1.44 and 5.57K reads) was found in abundance in all the samples. Each and every individual shows the different architecture of the microbiome that in the future can be used for forensic identification.

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Forensic Analysis; Oral Microbiota; Identification

I89   An Extracorporeal Tissue Degradation Model to Investigate PMI Markers

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Learning Overview: The goal of this presentation is to discuss the challenges of establishing optimal tissue degradation models to investigate postmortem biomarkers (protein degradation) for the estimation of the Postmortem Interval (PMI) and to present our new approach to analyze human extracorporeal tissue in this context. Despite it being an artificial (unrealistic) system, this model allows targeted investigation of (case-specific) influencing factors to improve the reliability and applicability of PMI estimations.

Impact Statement: This presentation will impact the forensic science community by informing attendees that, with some restrictions that are yet a matter of research, a human extracorporeal tissue degradation model offers the potential to synthesize the advantages of standardization (such as animal models) and applicability (human tissue) and thus can provide the optimal basis for the investigation of influencing factors and establishment of reliable and broadly applicable models for tissue degradation-based PMI estimation.

A most precise estimation of the PMI is often of critical importance for criminal investigations and forensic case work. Compared to other evidence such as witness testimonies, digital traces, or scene evidence, biomedical markers can be considered largely unforgeable and therefore also withstand in court. Research on postmortem biomarkers that can be reliably applied for PMI estimations has thus been a central topic in forensic science for decades. Therefore, different model systems, all with respective advantages and weaknesses, have been used. While animal models allow investigations under predefined conditions using appropriate sample sizes, obtained data always requires extreme caution for human application. Data collected from humans in autopsy cases and/or body donors at taphonomy facilities on the other hand inevitably come with uncontrollable preconditions regarding individual, as well as environmental factors, impeding standardization.

We aim to establish an extracorporeal postmortem tissue decomposition model, combining the requirements of applicability and standardization. Therefore, we compared postmortem protein degradation in human skeletal muscle to excised tissue blocks from the same individuals. Over the course of ten days, samples were collected from bodies donated to the Forensic Research Outdoor Station (FROST) at North Michigan University, both from *in situ* as well as extracorporeally stored tissue on a daily basis. At the University of Salzburg, all samples were homogenized, proteins separated by electrophoresis, and visualized by immunolabeling to investigate and compare degradation patterns and dynamics.

Obtained data indicate that there are indeed differences between *in situ* and extracorporeal degradation that, however, are clearly affiliated with specific (differing) environmental influences. As such we found that, for example, substantial maggot activity significantly affected decomposition in the body donors in later phases. At the same time, the extracorporeal degradation model was largely unaffected, indicating more preserved (uninfluenced) protein degradation dynamics. Hence, this model can provide a valuable basis for targeted investigation of influencing factors such as storage temperature, maggot activity, etc. to increase our understanding of protein degradation in the context of potential case-specific conditions and allow more reliable PMI estimations on this behalf.

In a follow-up study (Holzer et. al, also presented at this conference) we applied this extracorporeal human muscle degradation model in that we collected tissue blocks from autopsy cases with very short PMIs and left them to decompose at different temperatures. Further experiments will include the influence of maggot activity and microbes." TO "In a follow-up study we applied this extracorporeal human muscle degradation model in that we collected tissue blocks from autopsy cases with very short PMIs and left them to decompose at different temperatures. Further experiments will include the influence of maggot activity and microbes.

Protein; Degradation; Postmortem Interval

I90   When Immunohistochemistry Acquitted the Defendant: A Particular Scuba-Diving Death

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Learning Overview: This presentation will utilize results of immunohistochemistry to demonstrate the importance of this analysis in determining the cause of death following a fatal diving accident. After attending this presentation, attendees will better understand the importance of considering this kind of evidence and that immunohistochemistry can be decisive in the solution of some cases.

Impact Statement: This presentation will impact the forensic science community by showing that a thorough and standardized autopsy examination should always be desirable in order for the prosecutor to pass judgment.

A 53-year-old female diver was involved in an accident during a recreational dive to a maximum depth of 39 meters. The subject had many diving certificates and a long experience behind her, although she had suspended her diving activity for some years before the accident. She restarted directly with a deep dive. During the dive, she communicated some problems with the Buoyancy Control Device (BCD), and the dive was interrupted for safety reasons. During the ascent, the diver was reported to have lost the regulator from her mouth several times. The guides accompanied her to the surface. Upon leaving the water, the subject was cyanotic, with evidence of froth at mouth and nose. The diver was pronounced dead shortly after. An autopsy was ordered by the local prosecutor. The examination, performed by a first pathologist, observed: epicardial fat hyper-representation; patent coronary, with a small eccentric plaque in the descending branch of the left coronary artery, reducing the lumen by about 20%; myocardium was overall normal; smooth and shiny aorta, with small diffuse lipid plaques; no subpleural petechiae; clear, overexpanded lungs and, copious edema and bilateral congestion; negative docimasic test; fine hemorrhagic punctuation on the upper III of the tracheal mucosa; the stomach did not contain water but a few ccs of food liquid; and marked renal congestion. Barotrauma was indicated as the cause of death. The result was not considered decisive by the judge, who asked for a second opinion to determine the cause of death and any liability of the guides. Documentation was carefully examined anew by expert dive pathologists, together with photographic images and dive profiles.

It was decided that the histological samples with immunohistochemistry for CD61, serum anti-glycophorin, and desmin be examined. This technique made it possible to confirm the recurrence of the emphysematous appearance of the lungs due to laceration of the alveolar septa, widespread presence of platelet aggregates, fibrin, and heterogeneous thrombosis of the pulmonary microcirculation. Nothing was found in other body districts, excluding CID diagnosis, and thus proposing that the micro-thrombosis was the consequence of a slowdown of the microcirculation, which is well correlated to a primitive failure of the cardiac function. Other histological findings supported this hypothesis: the wall of the right ventricle with important fat replacement, atheromatous degeneration of the coronary wall, and widespread chronic pulmonary inflammation foci. Immunohistochemistry together with the examination of the documentation let the pathologists to define the causes of death and its natural manner. Barotrauma was thus ruled out as the primary cause of death. The public prosecutor asked for the acquittal of the guides, which the judge upheld.

Accident; Drowning; Histological

I91   The Value and Versatility of Histology in the Forensic Autopsy: Changing Cases From Suspicious to Natural Causes

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Learning Overview: The goal of this presentation is to reiterate the importance of taking routine histological samples at autopsy. Even in a suspicious case or when the deceased is in a state of advanced decomposition, it can reveal pathology unseen by the naked eye.

Impact Statement: This presentation will impact the forensic community by reminding us why we have minimum standards for histological sampling. Even when thought to be of little use, it can often reveal the answer.

The Victorian Institute of Forensic Medicine (VIFM) is a purpose-built statutory authority situated in Melbourne, Australia, serving a population of over 6.5 million people and providing medicolegal death investigation services to the Coroners Court of Victoria. The latter is mandated by statute to investigate prescribed categories of reportable deaths and include those that are sudden, unexpected, violent, or unnatural.

Two unrelated autopsy cases, both initially presenting as suspicious deaths, are presented that highlight the need for histology in medicolegal death investigations. A postmortem Computed Tomography (CT) scan conducted prior to autopsy showed neither skeletal trauma nor a potential cause of death for either case. Postmortem toxicological analysis was non-contributory.

The first case is a woman in her fifties residing with her partner; there was an active intervention order against him following an assault. She was found deceased on the bedroom floor beside a broken wardrobe door, and her partner was intoxicated and aggressive toward first responders. Autopsy did not reveal any significant injuries, and there were a few pale lesions identified in both liver lobes. Histology revealed widespread metastatic adenocarcinoma throughout the lungs, bone marrow, and liver; further special stains and immunohistochemistry confirmed this to be metastatic breast carcinoma.

The second case is a 38-year-old man who had been feeling unwell for three weeks but had not sought medical help. Five days after his flat mates saw him stumbling down the stairs, they found him deceased in bed in a state of decomposition; there was a history of heart disease in the family. Autopsy was hampered by decomposition and was essentially negative other than a slightly enlarged heart. Histology, despite autolytic changes, revealed florid intravascular proliferation of a haemopoietic malignancy in all organs sampled. Basic B and T cell markers were unhelpful, likely due to the degree of autolysis, and no further typing was investigated.

Histological; Disease; Death Investigation

I92   Evaluating the Spatial Relationship Between Microhemorrhages and Axonal Injury

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Learning Overview: After attending this presentation, attendees will understand the relationship between radiologic diffuse axonal injury and microscopic findings of microhemorrhage. Attendees will better understand conclusions that may be drawn from relating imaging and gross and microscopic microhemorrhages (or their absence).

Impact Statement: Postmortem Magnetic Resonance Imaging (MRI) is an imaging modality that is not available everywhere, and the findings obtained in these cases will impact and be of interest to the forensic science community. Correlations between imaging and microscopic findings are underpublished.

Traumatic Brain Injury (TBI) represents a significant cause of morbidity and mortality in the United States with over 65,000 Americans dying from TBI-related injuries in 2020.[1] Diffuse Axonal Injury (DAI) is one manifestation of TBI caused by accelerative-decelerative forces inducing shearing injury within subcortical and deep white matter tracts.[2] In antemortem and postmortem settings, DAI is stratified into three degrees of severity: involvement of subcortical white matter (grade I), corpus callosum (stage II), and brainstem (stage III). Clinically, MRI, specifically Susceptibility-Weighted MRI (SWI), is used to identify white matter microhemorrhages, which are considered the radiographic hallmark of DAI.[3] Microhemorrhage extent is used in clinical prognostication and decision-making; in some forensic settings, gross identification of microhemorrhages may be considered evidence of DAI without histopathologic evaluation.

The dogma that radiographic and gross microhemorrhages correspond with DAI persists despite a lack of pathologic studies examining this relationship; furthermore, recent studies suggest no correlation. To investigate this, three brain donors with acute TBI (survival interval: 7-13 days) were selected for imaging and subsequent neuropathological evaluation. Coronal brain slices were co-registered against *ex vivo* SWI images to guide microhemorrhage sampling. All SWI-identified white matter microhemorrhages and select small hemorrhages surrounding large parenchymal bleeds (termed "peri-lesional microhemorrhages") were sampled. Slides stained with hematoxylin and eosin and Amyloid Precursor Protein (APP) an axonal injury marker, underwent whole-slide scanning (Leica® Aperio AT2) and subsequent layering and annotation using the Halo® image analysis platform. White matter microhemorrhages were histologically confirmed in 74% of SWI lesions (n = 39 slides). Of slides without histologic white matter microhemorrhages, 70% demonstrated congested vasculature or blood in Virchow-Robin spaces. APP immunostaining was identified within 1.0mm in 93% of microhemorrhages (n = 14, across five sections). This study further characterizes the correlation between radiographic or gross white matter microhemorrhages and histologically identified axonal injury.

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Brain; Imaging; Neuropathology

I93   The Role of Thiols in Putrefaction: An Analysis of Biochemical Interactions in Forensic Cases

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Learning Overview: After attending this presentation, attendees will understand the role that thiols have in putrefaction through the description of two forensic cases and a literature review with an analysis of postmortem biochemical mechanisms.

Impact Statement: This presentation will impact the forensic science community by demonstrating how, in the evaluation of the Postmortem Interval (PMI) in forensic cases with thiol inhalation, it is necessary to consider these elements as putrefaction accelerators.

Introduction: Thiols constitute a large group of sulfur-containing volatile organic compounds (alkanethols, thioalcohols, and mercaptans). They belong to organic acids with a slightly higher degree of acidity than, for example, alcohols and phenols. They are derivatives of hydrogen sulfide and are formed during the processes of decomposition and rotting of organic material, for example during or after the fermentation of grape must, from a chemical reaction with ethanol (food alcohol) or acetaldehyde. They have an intense smell, similar to that of leeks and herbs up to putrid, often disgusting (several thioalcohols are also found in the glandular secretions of skunks). Accurately establishing the PMI becomes more complicated in cases of rotting corpses. Putrefaction is a post-mortal phenomenon that consists in the decomposition of tissues by bacteria, primitively anaerobic from the intestinal flora, with the production of Hydrogen Sulphide (H_2S). This process evolves according to different stages and is influenced in particular by environmental factors, mainly by temperature.

Materials and methods: Two forensic cases subjected to autopsy at the Institute of Forensic Medicine of the Magna Graecia University of Catanzaro relating to deaths that occurred with the use of gas were analyzed. A review of the scientific literature was carried out through the analysis of the PubMed®, NCBI®, SCOPUS®, and Google® Scholar. A search was also carried out on substances with the SH group in common use.

Case series: In Case 1, a boy inhaled domestic liquefied petroleum gas for suicidal purposes. In Case 2, a woman suffering from psychotic disorder committed suicide by using a hose attached to a methane gas cylinder.

Results: In both cases reported, on external examination, the cadavers showed advanced putrefaction phenomena despite regular storage in a cold room for a few days. Autopsy showed signs of asphyxia, including foamy fungus, subpleural and subconjunctival petechiae, and pulmonary edema.

Discussion: Volatile Sulfur Compounds (VSC) are toxic to humans. In addition to H_2S, these gases may also contain Methanethiol (methyl mercaptan, CH_3SH, MT), Dimethyl Sulfide (CH_3SCH_3, DMS), Dimethyl Disulfide (CH_3SSCH_3, DMDS) and/or Dimethyl Trisulfide (DMTS). Generally, between the 18th and 36th hour after death, the first visible sign of putrefaction, the green spot, appears in the right iliac fossa. This sign is due to the reaction between the hemoglobin deriving from postmortem hemolysis and the H_2S that is produced by the anaerobic metabolism of the bacteria, abundantly present in that anatomical region. From this bond, sulfohaemoglobin, sulfomethaemoglobin, and other pigments are formed, responsible for the characteristic greenish color and the "marbled" appearance of the corpse. Furthermore, the H_2S produced by anaerobic bacteria enters the composition of the putrefactive gas and is among the compounds responsible for the pungent odor typical of decomposition processes.

From the review of the literature concerning cases of deaths from H_2S poisoning, it emerged that the corpses showed a more intense greenish coloration of the skin and internal organs. With reference to our cases, it can be noted that in both cases, the toxic effects of the VCS contained in the inhaled gas were evident. Furthermore, it should be noted that the putrefactive process took place much more rapidly, despite the correct conservation of the bodies. Therefore, we hypothesize that the exposure to high concentrations of substances containing H_2S that occurred before death is related to the more rapid onset of putrefactive phenomena (i.e., that even after 6-12 hours, the putrefactive process begins). We also believe that in proper forensic practice, this correlation should be given due consideration in relation to the PMI estimate if such intoxications are suspected.

Putrefaction; Forensic Biochemistry; Thiols

I94   Iatrogenic Injuries Due to Endoscopic Techniques: The Role of Medicolegal Analysis for Forensic Purposes

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Learning Overview: After attending this presentation, attendees will understand what an iatrogenic injury is and the correct methodology to follow to reduce the occurrence of such events.

Impact Statement: This presentation will impact the forensic science community, demonstrating the role of forensic medicine in the evaluation of iatrogenic injuries encountered in the operating room and particularly during the performance of routine endoscopic procedures.

Introduction: The term "iatrogenic damage" refers to an injury due to medical treatment or device. It could be also caused by negligence, imprudence, or inexperience of the health care personnel in carrying out the patient's treatment.

Case Series: Five forensic cases were analyzed. In Case 1, a boy arrived at the hospital following a road accident with multiple rib fractures and pneumothorax and died suddenly following completion of chest drainage. In Case 2, an elderly man suffering from abdominal pain and increased tumor indexes underwent colonoscopy, during which the operator interrupted the procedure due to hemorrhage. In Case 3, a woman underwent esophagogastroduodenoscopy for gastroesophageal reflux. The procedure was abruptly interrupted due to bleeding. In Case 4, a patient underwent a thermotherapy procedure for benign prostate adenoma. In Case 5, a patient underwent a hysteroscopy procedure for uterine myoma, which was stopped due to profuse bleeding.

Results: In Case 1, the autopsy revealed cardiogenic shock attributable to multiple pinpoint lesions penetrating the full thickness of the myocardium due to chest drainage perforation. In Case 2, the patient underwent immediate abdominal surgery for perforation of the transverse colon during colonoscopy and died after about 20 days due to septic complications. In Case 3, autopsy revealed mediastinitis due to full-thickness perforation of the cervical esophagus during gastroscopy. In Case 4, the patient lost ejaculatory function as a result of improper use of a water vapor thermotherapy procedure. In Case 5, the patient immediately underwent adnexectomy with surgical removal of an ovary for iatrogenic perforation during hysteroscopy. In all cases, a direct responsibility was identified on the operator who carried out the procedure related to improper use of the endoscopic instrument in the absence of particular technical difficulties in the case.

Discussion: Iatrogenic lesions represent predictable and preventable events that sometimes occur in clinical practice, both during emergency procedures and routine procedures. The literature suggests that the gastrointestinal and genitourinary tracts are the anatomical sites most frequently affected; more rarely, perforations of the airways and heart occur.[1,2] Some cases of undiagnosed iatrogenic perforations have been reported in the literature, which were identified only at autopsy.[3] In the reported cases, we evidence that only Case 1 concerned an emergency procedure. The other cases reported referred to injuries that happened during elective and routine operations, and they showed no particular technical difficulties or patient comorbidities.

Conclusions: The operating room is statistically one of the health environments with the highest levels of clinical risk. Also, routine endoscopic procedures may be frequently responsible for iatrogenic lesions, sometimes with fatal outcomes. In this context, therefore, it is a crucial the role of the coroner, not only for defining the cause of death, but also for determining the clinical or surgical context in which the event occurred, any comorbidities of the subject, whether the lesion was avoidable, if the intervention required the overcoming of exceptional difficulties, and, not least, the presence of concomitant pathological circumstances capable of determining, in the same places and times, the death of the patient. The autopsy is decisive for the exact definition of the etiology of the death, the avoidability of the lesion, and, no less, to prevent errors in other similar interventions.

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Autopsy; Cause of Death; Forensic Analysis

I95   Neonatal Traumatic Deaths: Differentiating Homicidal and Accidental Cases by a Multidisciplinary Approach

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Learning Overview: Neonatal traumatic injuries represent a challenge for forensic pathologists. Often, the relatives refer to accidental trauma or to unspecified disease. In these cases, the forensic investigation has to be carried on through a complete examination of circumstantial data and autopsy findings integrated with complete radiologic examination. The purpose of this presentation is to highlight the relevance of a multidisciplinary approach to allow an accurate identification of the causes of the injuries.

Impact Statement: This presentation will impact the forensic science community by highlighting the relevance of adopting a multidisciplinary approach in cases of neonatal injuries suspected to infanticide. The complete forensic analysis, although showing similar circumstances, allowed an autopsy finding to reach a different etiology of the injuries. It emphasizes the relevance of a multidisciplinary approach in which the forensic pathologist must be the main figure.

The infant homicide rate is underestimated because of potential undetected or misclassified cases. A study based on World Health Organization (WHO) data compared death rates among 23 developed countries for children under one year old, revealing a mean death rate of 2.3 ± 2.6 per 100,000 live births. Recent scientific reports indicate a potentially higher prevalence of infanticide than previously assumed in scientific literature.

In this study we analyze four cases of infant deaths suspected as neonatal homicide. The first case was referred to as a fall-related trauma during a sudden delivery at home. The external examination revealed bruises, ecchymoses, "raccoon eyes," and a nosebleed. Autopsy findings included intracranial hematoma, subdural hemorrhage, and parietal fractures. The histological findings showed subarachnoid hemorrhage. The second case regards a newborn observed in the emergency room for breathing difficulties with no signs of trauma and a silent history. Surprisingly the autopsy revealed subdural and subarachnoid hemorrhages, retinal hemorrhages, and intraparenchymal cerebral and cerebellar contusions. The histological findings confirmed subarachnoid hemorrhage and retinal hemorrhage. The third case involves a two-month-old baby whose mother reported a vigorous hug to protect him from a dog. The external examination shows no signs of trauma, but the autopsy reveals subgaleal hemorrhage, temporal and parietal fractures, and subdural hemorrhage. The fourth case was presented to the emergency room due to cardiac arrest. The external examination revealed only minor signs of trauma. The autopsy showed cranial fractures, subdural, multiple costal fractures, and the fracture of the 11th thoracic vertebra. The microscopic findings included retinal hemorrhages.

In all cases, despite minimal external injuries, autopsy findings showed serious lesions. The multidisciplinary approach conducted by Postmortem Computed Tomography (PMCT), 3D reconstruction, and physical study of deformation of the cranic ovoid established that the first infant fell during delivery. In the other cases, there were unexpected and unexplainable intracranial hemorrhages, suggestive of homicide.

Infant Death; Multidisciplinary; Homicide

I96   Post-Traumatic Intracranial Aneurysms of Anterior Circulation: Always a Challenge in the Forensic Field

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Learning Overview: The goal of this presentation is to improve the forensic neuropathological approach so it is possible to highlight the traces of aneurysm or traumatic dissection after a long period of time from the rupture.

Impact Statement: This methodical and multidisciplinary approach will impact the forensic science community by clarifying, on the one hand, how to distinguish traumatic primary aneurysm of a cerebral posterior communicating artery, and, on the other hand, improve the understanding of possible secondary trauma-related damage, not related with medical malpractice.[1]

The neuropathological features seen in rare cases with Post-Traumatic intracranial Pseudo-Aneurysm (PTPA) of posterior circulation associated Subarachnoid Hemorrhage (SAH) are presented. A 40-year-old man, victim of an assault, died in the hospital after six days and neurosurgery treatment, with few external traumatic signs. TC showed multiple fractures on the face. The clinical cause of death was an Intracerebral Hemorrhage (ICH) in the frontal lobes and SAH. In these cases, Computed Tomography (CT) and Digital Subtraction Angiography (DSA) is suggested to identify a pseudoaneurysm, but negative angiography does not exclude the possibility of vascular injury.[2]

An autopsy was ordered by the prosecutor to differentiate between traumatic hemorrhage and hemorrhage due to a natural disease (rupture of aneurysm during a strong emotion). Neuropathological examination showed a massive subarachnoid hemorrhage over the surface of the brain (fronto-temporo-parietal lobe) and the presence of a metal coil within the left internal carotid. After seriated transverse cuts along the left cerebral posterior communicating artery, a dissection of the left internal carotid artery and left anterior cerebral artery, radiated from the posterior communicating artery to the other vascular structures was highlighted.[3] Brain samples from both cases were studied on differently stained sections. Microscopically the wall of the vessels showed characteristic alterations seen in traumatic pseudoaneurysm secondary to violent dynamics.[4-8] The underlying cause of ICH and SAH were anterior trauma of the head with stretching of the vessels and contusion of the front-temporo-parietal lobe by contrecoup. Secondly, the man reported ischemia of hypoperfused areas and cessation of brain activity. Therefore, homicide was the manner of death. In addition to neuroradiological investigation, serial cuts of the suspected area, in this case the aneurysm treated with coil, and evaluation of the vessel wall is suggested as a useful diagnostic procedure. Angiography can greatly improve the quality of the assessment. This work will present the phases of this assessment by presenting some innovations, which can be useful to rule out medical negligence or a natural event and confirm a homicide.

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Autopsy; Vascular; Neuropathology

I97 **Analyzing the Past to Help Shape Our Children's Futures: A Ten-Year Review of Pediatric Deaths in Arapahoe County, Colorado**

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Learning Overview: The goal of this presentation is to present a review and analysis of pediatric deaths in Arapahoe County, CO, for the past ten years, specifically focusing on methods of suicide between sexes and manner of death between races.

Impact Statement: A multi-year investigation of trends and differences in mortality across age, sex, race, and circumstances of death revealed statistically significant differences across different sexes and races. This presentation will impact the forensic science community by emphasizing the importance of targeted education for different populations in pediatrics to decrease mortality.

Since 2015, Colorado has surpassed the national average for pediatric (defined as individuals under 18 years of age) mortality per 100,000 annually.[1] Arapahoe County specifically, while the third-largest county in the state, is second-highest in suicides for the pediatric cohort.[2,3] From an extensive publication review, no data analysis has been published on multiple aspects of pediatric mortality in Arapahoe County. An annual report published by the Office of the Coroner of Arapahoe County contains strict data, without statistical analysis over multiple years. Similarly, census data and CDC data present strictly numbers without statistical analysis performed for differences between groups or outcomes within the past ten years. Given a rise in pediatric mortality, this research is essential to characterize who is at risk and most vulnerable.

The primary objective of this study was to analyze age, sex, race, manner of death, cause of death, and location of fatal incident in all deaths in cases of pediatric mortality in Arapahoe County between January 1, 2012, and January 1, 2022. Data analysis was performed to determine statistical significance by calculating expected values, incidence rate (per 100,000), degrees of freedom, critical values, and chi squared. Given the small sample size, the confidence intervals were calculated using difference of proportions and Fisher's exact test. When comparing method of death in sexes for suicide manner of death in the pediatric population, our analysis revealed the incidence of males who commit suicide by firearm and incidence of females who commit suicide by hanging is statistically higher than their opposite sex counterpart method of suicide. The data also revealed that the incidence of non-Hispanic pediatric Blacks who die from accidental asphyxiation is statistically higher than their same-sex, non-Hispanic Caucasian counterparts. These differences demonstrate the importance of education and awareness, which can be used to target specific populations and, ultimately, decrease mortality in the Arapahoe County pediatric population.

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Pediatric; Mortality; Statistics

I98 Scuba Diving, Extreme Sports, and Undetected Cardiac Pathologies. Is There a Need for Further Clinical Examinations to Make These Activities Safer for Health?

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Learning Overview: Scuba diving is a popular activity among athletes and tourists; however, immersions are not riskless for the human body, stressing the cardiorespiratory system due to the high pressures. After attending this presentation, attendees will be informed about a peculiar case of sudden death during a diving activity involving a non-competitive diver with unknown hypertrophic heart disease undiagnosed on standard non-competitive athlete screening tests.

Impact Statement: This presentation will impact the forensic science community by reflecting on the need to proceed, in non-competitive subjects, with deepened and more specific cardiac tests in scuba diving to identify complex and potentially fatal pathological conditions such as hypertrophic heart disease.

Scuba diving is a sport, but also a recreational activity, involving immersion in various underwater environments, both fresh and saltwater, such as seas, rivers, and lakes. This fascinating activity can be done independently or with the support of qualified personnel accompanied by proper equipment and specific techniques. Due to the high level of physical effort, specific physical requirements are necessary to join in this activity.[1-3]

During a normal session of diving, the body is exposed to high pressure, which causes the collapse of the pulmonary alveoli. It is necessary to inhale compressed air to prevent lung collapse, by insufflation of high alveolar gas pressures, which exposes the pulmonary blood to hyperbaric pressure.[4 5]

This work presents the case of a 53-year-old healthy woman practicing non-competitive scuba diving activities. The electrocardiography performed before the immersion did not show pathological abnormalities. After the first dive of the series, the woman presented a temporary cough. During the second dive, the instructor noticed a strange behavior and decided to bring the woman to the surface, controlling the ascent. Once on the boat, the woman began coughing violently, vomiting, and suddenly passed out. When medical assistance arrived, the patient was found to be in asystole with fixed mydriasis. After the resuscitation maneuvers, the heartbeat was restarted, and the woman was taken to the nearest hospital. A Computed Tomography (CT) angiography of the brain showed findings indicating massive and diffuse cerebral hypoxia but no signs of air embolism. The patient immediately underwent hyperbaric treatment without any benefits and was declared deceased four days later. On suspicion of a death correlated to malfunctioning of diving equipment, an autopsy was performed.

At the autopsy, the heart weighed 300grams, with left ventricle wall thickness measuring 11 millimeters anteriorly, 10 millimeters laterally, 9.5 millimeters posteriorly, and 15 millimeters of the interventricular septum. Cardiac chambers were significantly reduced in volume, and the oval foramen was patent, while the heart valves or coronary arteries were normal. Postmortem Magnetic Resonance Imaging (PMMRI) confirmed these findings. Histological examination of brain tissue showed hypoxic changes in neuronal elements, while in the myocardium, ubiquitous signs of hypertrophy and myocyte disarray with fibrotic foci were found. Subendocardial arterioles showed a mid-adventitial thickening, an element found in the endocardium too. These findings strongly suggested a diagnosis of hypertrophic heart disease. This cardiac pathology was the leading cause of the initial illness, explaining the rapid ascents and gas embolism that led to death.

In conclusion, it is essential to emphasize the need for more specific tests in current sports investigations, especially in extreme and physically activities such as scuba diving, even for non-agonist subjects, to diagnose cardiac pathologies often not detected by standard cardiac examinations such as electrocardiography.

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Diagnosis; Cardiac Death; Prevention

I99   Death by Frostbite: From Theory to Reality

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Learning Overview: After attending this presentation, attendees will have a better understanding of the macroscopic and histological features of death by frostbite.

Impact Statement: This presentation will impact the forensic science community by presenting a case in which site inspection, autopsy, and histological examination simultaneously revealed the rare forensic and anatomopathological characteristic findings of hypothermia.

Hypothermia is a systemic condition in which the core body temperature is below 35°C and the body loses its thermoregulatory capabilities and is not able to generate heat. A body temperature below 25°C is responsible for death caused by an arrest of the vital functions. Postmortem diagnosis of death by hypothermia is often a medicolegal dilemma: in most cases, a multidisciplinary approach is necessary to combine circumstantial findings, external examination, autopsy evidence (mostly non-specific), and microscopic and biochemical findings, in order to assess the diagnosis of fatal hypothermia.

The authors present a case of an elderly 85-year-old man suffering from Alzheimer's disease who was reported missing. After seven days of research, the lifeless body of the elderly missing man was found in a nearby abandoned country field. The body was found completely dressed in the upper part of the body, but his trousers were lowered on his legs down to the thighs. Shoes were not worn and were placed near the body. Forensic examinations were required in order to clarify time, cause, and modalities of death.

During the first cadaveric inspection, the body temperature was lower than the ambient temperature, the hypostases were reddish and localized in the posterior areas of the body, rigidity was diffuse to the main articular districts. External examination showed numerous abrasions on both forearms and thighs, some of which were roughly parallel, as well as purplish areas of discoloration on both elbows and, knees and gray brownish on both hands (frost erythema). No macroscopic alterations of heart, lungs, and brain were detected. At the opening of the stomach, the presence of widespread reddish hemorrhagic spots, mostly localized in the antral region, was evidenced (Wischnewski spot). The liver had a poorly recognizable architecture.

Microscopic observation of tissue sections stained with hematoxylin and eosin showed marked polivisceral congestion, micro and macrovesicular hepatic steatosis, as well as vacuolization of the basal cells of renal tubules (Armanni–Ebstein phenomenon). The gastric wall showed edema and vascular congestion involving mucosa and submucosal layers and focal erosions of the mucosal surface consisting of necrotic foci delimitated by a dense lympho-histiocytic infiltrate. Toxicological analysis performed in order to detect the presence of alcohol and/or drug abuse substances were negative. The cause of death deriving from our medicolegal examination was hypothermia in a subject with hypertensive heart disease, Alzheimer's disease, and epileptic syndrome.

This case describes most of the features and circumstances surrounding hypothermia-related deaths. Diagnosis of cold-related death is based on unspecific data and can only be made thanks to exhaustive, complete, and integrate medicolegal investigations.

In the case presented, site inspection, autopsy, and histological examination simultaneously revealed the rare forensic and anatomopathological characteristic findings of hypothermia, in accordance with the previously reported literature: paradoxical undressing, tanatochronological phenomena, frost erythema, polivisceral congestion, Wischnewski spot, and subnuclear vacuolization of renal tubular epithelial cells.[1-17]

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Frostbite Death; Paradoxical Undressing; Wischnewski Spots

I100   Too Hot to Handle: A Review of Carbon Monoxide Levels in Fire Fatalities

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Learning Overview: This presentation utilizes a retrospective review of cases to evaluate the Carbon Monoxide (CO) levels in fatalities relating to fires. The goal of this presentation is to seek to impart a better understanding of what happens to the CO levels as temperatures rise and fire damage occurs and how to interpret CO results in fire fatalities.

Impact Statement: This presentation will impact the forensic pathology community by presenting a better understanding of CO levels and what they mean in fire fatalities and determining cause of death. This will provide documented research evaluating CO levels that can be used in offices nationwide.

This study aims to understand how CO levels accumulate during the burning processes in fire fatalities and if at some point during the burning processes, CO is boiled out of the blood of more thoroughly burned descendants. This was an autopsy-based single-center retrospective cohort study of all fatalities involving thermal or inhalation injuries in which CO levels were collected at autopsy from January 2019 to June 2023 (N=92). Total body burn severity was classified on a scale from 0-6 with 1 point given for burn type (1-3) and 1 point given for total body area covered by burns (< 30%, 31-60% and greater than 61%) based on autopsy photos and autopsy reports.

Carbon monoxide levels were collected from blood samples taken from decedents at the time of autopsy. The average overall CO level from our 92 descendants was 24.5. The group with the highest averaged CO level of 43 were the descendants with a burn level of 1. The group with the lowest average CO level of 17.57 were the descendants with a burn level of 6. Based on our sample of thermal fatalities, there appears to be a bimodal distribution of CO blood levels with the lowest average CO level belonging to the decedent population with the greatest burden of total body burns and burn levels of 1 and 4 representing the peak average CO levels.

From this data observed based on the cases at our facility, it would appear that CO levels do dissipate after third-degree burns have covered around 60% of total body area (level 4 burn classification). As of now, there is no specific literature we were able to find discussing varying CO levels with regard to decedent burn burden, which makes interpretation of this lab result by medical examiners less predictive of cause of death. This is an important area of research to consider when evaluating blunt force injury versus fire injury, correctly classifying the cause of death, and helping families process the death of a loved one.

Carbon Monoxide; Fire Fatality; Inhalation Injury

I101   Smoke and Mirrors: A Bullet-Less Intraoral Gunshot Wound

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Learning Overview: This presentation will provide attendees with information regarding an unusual accidental intraoral gunshot wound involving a muzzleloader.

Impact Statement: This presentation will impact the forensic science community by emphasizing the level of trauma that ignited gun powder alone can cause and the importance of scene investigation and collaboration among disciplines to appropriately determine the manner of death.

A 30-year-old White male was hunting with two other individuals when a .50-caliber muzzleloader stopped firing due to wet gunpowder. They removed a projectile from the barrel of the firearm. One individual was sitting on a rack and was holding the weapon vertically, with the barrel pointing up, and was attempting to dry out the primer point containing black gunpowder by heating the area with a lighter. Reportedly, the decedent was holding the firearm in his left hand with the muzzle approximately at chest height and was attempting to blow air into the barrel to remove the powder out of the primer cap. The combined actions led to the powder being ignited and sending a blast down the barrel into the decedent's mouth. Emergency services were contacted, but no resuscitative measures were performed. At the scene, there were no observed defects or burns of the face.

Autopsy examination demonstrated bilateral periorbital contusions, lacerations and abrasions of the mouth, contusion of the gingiva, laceration of the tongue, chipped upper incisors, maxillary and mandibular fractures, fractures of the nasal turbinates, basilar skull fractures, diffuse subarachnoid hemorrhage, hemorrhage within the temporal muscles, and right temporal contusion. Copious soot was present on the hands and within the oral cavity. No projectiles were noted on radiographic examination. The right arm length was 30½" and the left arm length was 29¾". The firearm was an older model muzzleloader with a barrel length of 28". Abundant soot was present near the muzzle on the barrel of the firearm. At the initial evaluation, no projectiles remained within the firearm. Based on the information and examination of the firearm, the cause of death was listed as an intraoral gunshot wound and the manner of death was determined to be accident.

It is unusual to have a firearm death solely from the gunpowder ignition in the absence of a projectile emission. Throughout the research process for this case, no other studies have been found including a firearm-related gunpowder death nor a death regarding a bullet-less gunshot wound. The only type of gunpowder death-related studies that could be found included firework factory deaths. The lack of studies, or representation, for this type of accident is in part due to the outdated and less common use of a muzzleloader. The uncommon weapon, combined with the uncommon circumstances, led to an uncommon death.

Accident; Firearms; Death Investigation

I102 The Management of Deaths in Railway Stations: Investigation Methodologies and Safety Prospects for Public Health

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Learning Overview: After attending this presentation, attendees will understand that railway stations are places where different causes of death may occur.

Impact Statement: This presentation will impact the forensic science community by demonstrating, in light of the cases analyzed at the Institute of Forensic Medicine of Catanzaro, the importance of registering new safety standards for railway stations, especially to discourage frequenting railway stations for purposes other than those of the traveler.

Introduction: Railway stations are environments with a large number of travelers, people who pass through without taking the train, and people who use it as a home. The railway stations do not have strict security checks and for this reason access is open to all. Statistics show that numerous deaths occur every year in railway stations.

Case Series: In Case 1, the presence of a male body was found in front of the platform. Under the left arm a syringe with needle was found and on both elbows were signs of multiple acupunctures. Case 2 concerns a boy found dead on the rails following a railway investment. Fragments of brain material and fragments of the skull were also found 15 meters away and scattered along the entire route of the tracks, as proof of the extremely violent impact that occurred. His cell phone was handed over to the investigators in order to carry out investigations of the contents, also delimiting the point where the train impacted on the body. In Case 3, a middle-aged man passing by the tracks did not notice the acoustic signal emitted by the train and, therefore, was run over despite the operator having activated the braking mechanisms. In Case 4, a man voluntarily stood on the tracks while the train was passing. In Case 5, human remains were found during some renovation work at a railway station. In Case 6, a homeless person was found dead in the underpass of a railway station.

Materials and Methods: The work concerned an analysis of cases on bodies found in the railway stations and studied at the Institute of Legal Medicine of Catanzaro by performing inspections, autopsy, psychological autopsy, and histological and toxicological analyses.

Results: In Case 1, the autopsy demonstrated death from cardio-respiratory arrest from combined intoxication of narcotics (cocaine and heroin) and drugs (benzodiazepines), as confirmed by the results of the chemical-toxicological examination. In Case 2, the victim presented himself with his back to the direction of the train in an attempt to film the arriving train with an extreme selfie; when the boy noticed the train, he tried in vain to reach one of the escape routes from the side, and was impacted from the rear. In Case 3, the investigations confirmed that it was an accidental event as the video surveillance images showed that the man, after noticing the train, was trying to jump to avoid impact with the train. The autopsy showed fractured limbs with head trauma. In Case 5, the analysis with C14 demonstrated that they were ancient remains and, therefore, of non-judicial jurisdiction. In Case 6, the autopsy demonstrated a natural death from pulmonary thromboembolism in the absence of external injury.

Conclusions: We emphasize the importance of preparing our stations with new safety standards for greater prevention of deaths by accidental, suicidal, or homicidal methods. Prevention by surveillance of the station as a place where crimes of all kinds occur is important (drug dealing, violence, theft, suicides on the tracks). The proposal is to favor new legislation to draw up a surveillance and close monitoring protocol with the increase of systems for the safety of people who use the airport for transport purposes, investing in video surveillance systems. Contrary to airports, not all train stations prevent access to the platforms to those without a travel ticket and are not equipped with security checks at the entrance as they have very large halls and dangerous underpasses accessible to all. Furthermore, it would also be possible to install anti-suicide barriers, already existing in some subway stops.

Train; Accident; Forensic Analysis

I103   The Analysis of Gunshot Wounds in Gun Tests on Biological Matrices: An Experimental Study on Pig Heads

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Learning Overview: After attending this presentation, attendees will understand the importance of studying injuries caused by firearms to define shooting distance, caliber, and type of weapon.

Impact Statement: This presentation will impact the forensic science community by demonstrating the innovation of an experimental study carried out on biological matrices with the reproduction of lesions with different firearms and calibers at standard distances.

Introduction: Firearms are devices that exploit the strong thrust of the gases developed by the deflagration of explosive mixtures. The lesions produced on the basis of the distance, caliber, and type of weapon used have reproducible characteristics, which can be analyzed and used in order to partially reconstruct the ballistic dynamics. Therefore, the verification of injuries from firearms is a matter of forensic study as the possibility of attributing any damage to a specific weapon is fundamental in carrying out medical-legal examinations.

Materials and Methods: In the proposed study, the experimentation took place at an open-air shooting range in Calabria (Crotone) set up for carrying out ballistics practice in compliance with the rules and for the safety of the operators. Seventeen firearms of different calibers and barrel length were used, and 21 pig heads were used as targets that were drawn from three different distances (contact, 15cm, and 5m) in order to observe different characteristics of the lesions found on the biological matrices. The pig heads were supplied by industrially produced slaughterhouses. In this study, terminal ballistics were analyzed, in particular the entry bore, powder soot, and smoke soiling measurement as well as powder tattooing, presence of the pattern, and unburned elements.

Results: The analysis showed that in contact wounds, the entry hole had a diameter between a minimum of 0.5cm and a maximum of 2cm and only sometimes showed a starry shape, a smoke soiling with a maximum thickness of 4cm, absence of powder soot and unburnt elements, and only in some cases the presence of the muzzle imprint. Following shots fired at a distance of 15cm, the entry hole had a diameter between a minimum of 0.2cm and a maximum of 1.8cm and smoke soiling with a maximum thickness of 2.5cm; moreover, in some cases, the pattern and the powder soot were present with a maximum thickness of 0.4cm and in only one case were there unburnt elements. Finally, at a distance of 5m, the entrance hole had a diameter between a minimum of 0.3cm and a maximum of 3.5cm and was sometimes oval in shape; in some cases, the pattern and powder soot had a maximum thickness of 0.3cm; in no case (at a distance of 5m) were the smoke soiling and unburnt elements present.

Discussion: In the scientific literature, there are numerous studies regarding firearm injuries in order to evaluate the morphology of the injuries, residues present inside, and any ballistic evaluations. However, no scientific works with evaluation on biological matrices were found as in the experimental study we carried out, the results of which led to the finding of reproducible pathognomonic elements and attributable to specific characteristics of the weapon and/or to the variables due to the different dynamics of shoot. Therefore, from our analysis, it emerges that a dynamic and experimental study of injuries on biological matrices is fundamental for a correct medicolegal approach in judicial cases relating to firearm injuries. In conclusion, it represents a fundamental scientific method in order to reproduce, recognize, and evaluate the peculiar elements of such injuries through the use of the most common possible weapons as a fundamental aid in judicial cases with the application, on the firing tests, of evaluable and predetermined distances as well as the use of biological matrices for educational purposes for forensic personnel in training.

Handguns; Gun Test; Forensic Analysis

I104   Georgia: Home to the Carpet Capital of the World

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WITHDRAWN

I105   Penile Torsion as a Method of Identification

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Learning Overview: After attending this presentation, attendees will be aware of the potential for using documented congenital anomalies (such as penile torsion) to differentiate between identical twin infants.

Impact Statement: The presentation will impact the forensic science community by encouraging attendees to explore alternative methods of establishing identity in cases of unidentified infants.

Penile torsion is an abnormal twisting of the penis, which is congenital, but may not be identified until later in life. It is most often associated with other urogenital anomalies, especially hypospadias, but can occur in isolation.[1] With this condition, the penis appears rotated on its axis, almost always to the left. This report presents a case where a penile torsion was used for identification purposes.

The body of an infant boy was found abandoned at a house where he and his family (both parents and a twin sibling) had been living. The parents left the scene with the other infant after finding the decedent unresponsive and before the arrival of emergency personnel. Because the two infants were twins and no adult caregiver was available to provide an identification, the baby was initially regarded as unidentified.

Further investigation indicated that both adults and both babies were co-sleeping on a twin bed. When the parents awoke in the morning, they found one infant lying supine and unresponsive between his father and the wall. In phone calls, both parents identified the deceased infant as A and the living infant as B. Neither parent was willing to speak in person to law enforcement or medical examiner personnel due to outstanding warrants for drug charges.

Review of the birth records for twins A and B revealed that they were the product of a monochorionic/diamniotic pregnancy complicated by maternal substance abuse. The twins were born at 36 weeks gestation and were both hospitalized prior to discharge. During this hospitalization, Baby A was diagnosed with penile torsion (not present in his twin B); Baby B was diagnosed with a different congenital anomaly of the penis (not present in Baby A).

At autopsy, Baby A was noted to be a well-developed infant, with no injuries noted. Postmortem toxicologic analysis revealed the presence of methamphetamine in Baby A's blood. While there is no safe level of methamphetamine for infants and children, the level detected was low and probably did not cause Baby A's death. Because Baby A was reportedly co-sleeping with two adults on an unsafe sleep surface prior to being found unresponsive, asphyxia cannot be ruled out as a cause of death. The autopsy examination did not (and commonly does not in similar cases) demonstrate definitive signs of asphyxiation.

An informal consultation with a pediatric urologist confirmed that Baby A had mild penile torsion and did not have the penile condition identified in his twin brother B. This confirmation, in combination with telephone conversations with both parents, was deemed sufficient to definitively identify the infant as A.

Penile torsion is a common condition. The incidence of isolated penile torsion has been variably reported between 1.7% and 27%, with many mild cases likely going unrecognized.[1,2] It usually causes no symptoms, especially if the penis is rotated less than 90 degrees. We report the first case where penile torsion is used as a form of body identification during an autopsy. Our case highlights the possibility of alternative methods of establishing identity in cases where we have a clear previous recognized congenital defect or medical condition.

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Forensic Science; Postmortem Infant Identification; Penile Torsion

I106   The Role of the Forensic Laboratory in the Investigation of Postmortem Cardiac Markers for the Diagnosis of the Cause of Death: A Comparison of Forensic Utilities

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Learning Overview: After attending this presentation, attendees will understand the utility of measuring cardiac markers on cadaveric blood samples.

Impact Statement: This presentation will impact the forensic science community by demonstrating the advantages and limitations of laboratory investigations in defining the causes of death.

Introduction: Laboratory investigations on postmortem biological samples represent a new frontier of forensic research. Previous studies have already suggested the possible application of cardiac serum biomarkers, such as Troponin T and I and CK-MB, in the investigation of myocardial lesions in autopsy cases.[1] This study aims to investigate the contribution that markers of cardiac damage can have with respect to the cause of death variable. Recently, several authors have conducted postmortem biochemical analyzes of cTn I and cTn T in different body fluids, and cardiac troponins have proved to be of great value in the postmortem diagnosis of cardiac death.[2] Nevertheless, the application of these investigations for diagnostic purposes on the cadaver still has limitations related to the lack of experimental analyzes carried out. Furthermore, to date there are no standardized protocols for the use of cardiac markers in autopsy and forensics.

Materials and Methods: This study was conducted at the Institute of Legal Medicine-Magna Graecia University of Catanzaro on a total of 35 cadavers, divided into two large clusters with respect to the cause of death, namely Sudden Cardiac Deaths (SCD) and deaths of another nature. The SCD cluster was further divided into two subgroups based on the presence or absence of signs of myocardial infarction on histological investigations and the time of onset of ischemia (acute/subacute). The control group was divided into further subgroups: hanging, septic shock, pulmonary thromboembolism, car accident polytrauma, polytrauma from falling from heights, and gunshot wounds. Peripheral blood was subjected to immunofluorescence analysis after centrifugation for five minutes at 4,000rpm. The sample was then diluted with saline to a standard extent to allow for analysis. The markers examined were: Troponin I, CK-MB, myoglobin, pro-BNP, and D-dimer.

Results: The comparison between the values obtained documents a greater increase in the values of the markers of ischemic damage in the SCD cluster (mean values CK-MB=32.84ng/ml, TnI 20.38ng/ml, MYO=185.9ng/ml ml) compared to the control group (mean values CK-MB=29.78ng/ml, TnI 10.56ng/ml and MYO=112.7ng/ml). A minor variability between the two groups was observed for two other markers (SCD group: pro-BNP=5pg/ml and D-dimer=2,380ng/ml; control group: pro-BNP=20pg/ml and D-dimer=2,197.3ng/ml). A greater susceptibility has been highlighted for D-dimer in cases of polytrauma and pulmonary thromboembolism. The greatest advantage in the use of markers lies in the diagnosis of cases of myocardial ischemia, although an interesting application is also possible in cases involving large-scale traumas (with an increase above all in the case of traumatic lesions of the myocardium) or pulmonary thromboembolisms, for which comparison with autopsy data is essential. The extension of postmortem laboratory analyzes to myocardial damage indices could have great forensic utility in the future; especially, the study of a larger case series could reserve surprising data that are still little known. The disadvantage in the use of these data still consists in the lack of definition of standard pathological ranges on cadavers and in the possibility of false positives in case of iatrogenic lesions of the heart.

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Forensic Analysis; Biomarkers; Sudden Death

I107   Jay Dix Day Memorial Lecture Series

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Learning Overview: A proper medicolegal death investigation is a multidisciplinary process that often involves non-medical personnel as well as medical professionals. This annual lecture series provides non-forensic pathologist forensic scientists a basic review of selected topics in forensic pathology in order to increase familiarity and understanding and enhance inter-discipline communication.

This year's lecturers will discuss the medicolegal investigation of postmortem changes; deaths due to firearm injuries; deaths involving children; and deaths temporally associated with law enforcement apprehension and custody.

Impact Statement: This presentation will impact the forensic science community by presenting a comprehensive review of what causes and contributes to deaths related to the previously specified topics. Attendees will be able to systematically evaluate deaths in which the previously specified topics may have played a role that they encounter in their daily practices.

Following cessation of life, the human body undergoes a variety of progressive changes leading to its ultimate breakdown. Intrinsic (autolysis and putrefaction) and extrinsic (environmental conditions, animal/insect activity, funeral/burial procedures) factors affect the time course of this process and the appearance of the body/tissue. Proper evaluation of postmortem changes may be helpful in estimating the time since death and evaluating accuracy and reliability of other investigative information. Postmortem changes must be distinguished from antemortem disease/injury, may mask or obliterate pre-existing disease/injury, and may affect the performance, reliability, and interpretation of laboratory analyses. This lecture will review the postmortem changes, their causes and appearances, their proper recognition, their significance and the usefulness and limitations of utilizing this information in a medicolegal death investigation, ((Presenter—Joseph Prahlow, MD)

Firearm fatalities are a major cause of non-accidental morbidity and mortality in the United States. Multiple factors and mechanisms are involved in producing firearm injuries. Understanding and evaluating firearm injuries requires basic understanding of how these injuries are produced and how to distinguish them from other types of trauma. This lecture will provide a comprehensive review of these issues. (Presenter—Andrew Baker, MD)

Although humans are designed to live in air, a considerable amount of time is spent in and around watery environments. Deaths causally related to water can occur within or outside of water, and water may or may not have played a role in the deaths of decedents found in water. Investigation of deaths potentially related to water immersion requires specialized knowledge of drowning physiology as well as the effects of efforts to adapt to a watery environment (eg., SCUBA, pre-swim hyperventilation). (Presenter—James Caruso, MD)

Human life requires the uptake and utilization of oxygen along with the release of metabolic waste. Failure of these processes leads to asphyxia. Proper evaluation of asphyxia deaths requires knowledge of the entities that cause asphyxia, pathophysiologic mechanisms, asphyxia death scenarios, and factors that contribute to death. This lecture provides comprehensive discussion of the investigation of deaths that may involve asphyxia. (Presenter—Susan Ely, MD)

The death of an infant or child is a devastating event for the survivors and is accorded significant attention by society. Pediatric deaths may be caused by a wide variety of diseases and injuries. Accurate recognition of the cause, mechanism, and manner of death has important implications for the survivors, other interested investigative and health agencies, and society in general. Recognition of factors involved in pediatric deaths can help in enhancing the safety of other family members and serve as a basis for formulating death prevention strategies. This lecture will discuss the investigation and interpretation of findings in pediatric deaths that are of medicolegal interest. (Presenter—Katherine Maloney, MD)

Sharp force injuries are one of the major categories of mechanical injury. They result from the mechanical division of tissues by sharp or pointed objects. Sharp force injuries include stab wounds, cuts (incised wounds), and chop wounds, the latter being caused by relatively heavy-edged objects such as a machete or axe. Multiple mechanisms play a role in injury and deaths involving sharp force injuries. Understanding and evaluating injuries and deaths in which sharp force injuries may have played a role requires basic knowledge of injuries caused by sharp forces and how to distinguish them from other types of trauma; recognition of patterned injuries; and recognition of injury patterns (e.g., defensive wounds, "hesitation marks"). This lecture will provide a comprehensive review of these issues. (Presenter—James Gill, MD)

Death Investigation; Pediatric; Cause of Death

I108 **Necrophagous Insect Species Succession on Decomposed Pig Carcasses During North Dakota's Summer Months**

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Learning Overview: After attending this presentation, attendees will have a better understanding of the decomposition process dynamics during the summer months in North Dakota; this work represents the first postmortem entomofauna diversity report for this state.

Impact Statement: This presentation will impact the forensic science community by providing new information on the necrophagous insect species diversity and dynamics on decomposed pig carcasses in North Dakota, while the resulted data will strengthen the forensic entomology research in this state and will serve as reference data to be used during death investigations in the Great Plains region.

Forensic entomology is a worldwide-accepted method used during death investigations, primarily for the minimum Postmortem Interval (minPMI) estimation. After death, necrophagous insect species will colonize the body and will shift in diversity throughout the decomposition stages. Using the oldest development stage and insect colonization patterns, a forensic entomologist can estimate the minPMI. Both insect development and succession are influenced by numerous factors, among which the habitat and temperature play a crucial role. Certain species are cold tolerant, while others are active only during high temperatures, being habitat specific. Hence, it is crucially important to have a reference inventory with the necrophagous insect species diversity and colonization patterns from all habitats and geographic locations.

Since no forensic entomology inventory was previously performed in North Dakota, this research aimed to provide the first necrophagous entomofauna diversity report for this state. The experiment was performed in July 2022 and June 2023 on Mekinock Field Station (47°57'15.8"N 97°25'44.1"W) University of North Dakota research land. During each of the experimental months, three pig carcasses (50kg each) were purchased from a local pig farm, euthanized by captive blitz bolt at the farm, and placed in the field. The temperature was recorded hourly via temperature data loggers, while the relative humidity and precipitation data were obtained from the closest weather station to the research site (Grand Forks International Airport Station). Both adult and immature stages were collected daily and taxonomically identified and confirmed via DNA Barcoding using the mitochondrial gene Cytochrome Oxidase b subunit I (COI).

During both experimental periods, Diptera and Coleoptera species were identified, with ten new presence records for North Dakota. Blowflies (Diptera: Calliphoridae) were the predominant species collected and identified from the decomposed pig carcasses, while *Phormia regina* Meigen, 1826 was the primary colonizer.

The resulted data provided new and useful information on the primary forensic insect colonizers and a preliminary inventory for the state of North Dakota, namely, for Grand Forks County, encompassing information on carcass decomposition above the ground, colonization patterns of associated necrophagous insect species, including new records for this region and environmental factors involved in the decomposition process during the months of July 2022 and June 2023.

Forensic Entomology; Decomposition; North Dakota

I109 The Effects of Common Insecticides on Blow Fly (Diptera: Calliphoridae) Oviposition in the Field

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Learning Overview: After attending this presentation, attendees will be informed about the effects of commonly used insecticides on blow fly oviposition and decomposition in the field.

Impact Statement: This presentation will impact the forensic science community by providing new information on the effects of insecticides on forensically relevant species in the field.

Blow flies (Diptera: Calliphoridae) arrive to remains within minutes and insect colonization can occur soon after in the form of oviposition (egg laying). This is known as the Time Of Colonization (TOC), which forensic entomologists use to estimate a minimum Postmortem Interval (minPMI) or time between insect colonization and discovery of the remains. There are many factors, however, that can influence blow fly arrival and oviposition. The presence of chemicals (accidental or intentional), including insecticides, can mask the odors of decomposition, resulting in changes in insect detection and oviposition. Chemicals such as insecticides can additionally have an impact on the decomposition process. Bifenthrin (pyrethroid) and clothianidin (neonicotinoid) are two insecticides that are widely used in the United States in agriculture and farming.[1] In the insect's nervous system, bifenthrin targets the voltage-gated sodium channels in neurons while clothianidin targets the nicotinic acetylcholine receptor (nAChR).[2,3] Both modes of action result in insect paralysis and eventual death. The goal of this study was to determine if these two insecticides have an effect on blow fly arrival to remains, blow fly oviposition, and the decomposition rate of pig carcasses in the field. If the insecticides are capable of concealing the decomposition odors (or causing high mortality of insects), then all insecticide concentrations are expected to result in delayed first oviposition events. Additionally, the presence of insecticide will delay the decomposition process by repelling blow flies in the field.

To test this, field experiments were conducted at Purdue University during Summer 2023. A total of 24 stillborn pigs (mean weight = 733.5g) were obtained. Twelve pigs were used to conduct the bifenthrin experiments and 12 pigs were used to conduct the clothianidin experiments two weeks later. All treatments were tested in triplicate. To test each insecticide, pigs were assigned a number and insecticide concentration at random and placed in the field 15.24 meters apart. Then, each carcass was sprayed with one of the following concentrations of bifenthrin—73,519.08ppm (high), 735.19ppm (medium), and 73.51ppm (low)— or clothianidin—2,995.66ppm (high), 29.95ppm (medium), and 2.99ppm (low). Control carcasses were sprayed with water. Once all carcasses were treated, observations and time to the first oviposition event were recorded. When eggs were first observed, a small sample (approximately 100 eggs) was collected and reared to identify flies to species. Additionally, photographs were taken every hour until the first oviposition event and once a day after day 2 to document the progression of decomposition.

The first oviposition event during the bifenthrin trials varied among concentrations. The mean time to oviposition was as follows: control = 120min (±0 SE), low = 100min (±20 SE) medium = 60min (±0 SE), and high = 280min (±20 SE). The most common fly species obtained from the egg collections was *Phormia regina* Meigen, 1826. The decomposition rate also varied between carcasses. All carcasses in the control and low/medium concentrations of bifenthrin reached the skeletal stage by day 6, while the carcasses in the high concentration were still in the advanced decay stage by day 18. These results will be crucial during death investigations where pesticide use or presence is suspected, particularly in areas where agriculture and farming are prevalent. Understanding how commonly used insecticides affect forensically relevant species and the decomposition process will allow forensic entomologists to provide more accurate TOC/minPMI estimates.

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Forensic Entomology; Blow Flies; Pesticides

I110 It Boils Down to This: An Initial Evaluation, Using the Forensically Important Blow Fly *Cochliomyia macellaria*, on the Usefulness of Gene Expression Following the Common Method of Boiling Larvae

Joshua Smith*, Environmental Toxicology, Lubbock, TX

Learning Overview: After attending this presentation, attendees will have learned about approaches to making the molecular research being done in forensic entomology with gene expression more applicable to operational casework settings. Included in the presentation will be discussion about some of the challenges in connecting forensic entomology research to casework settings and ideas for addressing these challenges.

Impact Statement: This presentation will impact the forensic science community by highlighting differences in how samples are treated for research versus in operational casework environments. This presentation includes a specific method but helps highlight how experimental design for research should consider practical aspects of downstream applications. Results presented may aid in developing guidelines for forensic entomology gene expression research in the future.

There is a need to develop methodology that better connects the parameters used in research with the circumstances common in forensic entomology casework. A common application of forensic entomology is to use the age of an insect larval specimen removed from a body to provide an estimate of the minimum time since death.[1] Traditional methods of estimating age include comparing a physical feature, such as length, to a reference growth curve. There have been more recent attempts to estimate age through non-physical features, such as gene expression. Most of the work using gene expression in forensic entomology has focused on helping estimate insect age for developmental stages where size is inconsistent, such as the post-feeding larval or intrapuparial stages. There is an opportunity, however, to use gene expression to increase the precision of estimates in the feeding larval stages by focusing on genes that may influence development rates. It is common practice to boil larval samples prior to measurement, which makes the specimens extend for measuring larval length. Most references growth curves use this approach, but this is in contrast with gene expression work, which often places larval specimens live in storage solution and stores them at subzero temperatures. While preserving RNA well, these practices are known to cause larvae to shrink.[2] This difference in storage method based on traditional versus gene expression application has led to species reared under similar growth conditions being considerably different in size (compare cohort size ranges in 3 to 4). For gene expression work to be more compatible with established reference data sets based on boiled larvae, the feasibility of utilizing gene expression in boiled samples needs to be assessed.

Here, the success of generating the appropriate gene expression profile from a wide range of boiled larval samples was evaluated. Using the forensically important blow fly *Cochliomyia macellaria* (Fabricius, 1775)*,* larval samples from the same cohort were placed into two groups: boiled vs. non-boiled. Following the treatment of being boiled or not boiled, samples were stored in RNA*later*® at 4°C. RNA from larval samples was extracted at three different time points: 1–2 weeks, 4–5 weeks, or 8 weeks post storage in RNA*later*®. Resulting extracts were reverse transcribed, amplified with primers for the gene *transformer*, and visualized on gels to determine if gene expression was of high enough quality to determine sex. To ensure the method works across larval ages, two separate replicates were used: one replicate included larger feeding third instar larvae, while the other replicate included smaller second instar larvae. A total of 150 larvae were evaluated, 75 each from the boiled and non-boiled groups. A range of larvae sizes from 3.22 to 16.85mm were represented. For the larger specimens, rates of gene expression success were similar between the boiled and non-boiled group, at 95% and 92%, respectively. For the smaller specimens, the results were less consistent between the boiled and non-boiled group, at 64% and 100%. Overall, the results show that non-quantitative gene expression is viable for boiled samples, but further optimization is required to improve results for smaller larvae. It is likely RNA degradation is more problematic in the smaller larvae as there is less overall total RNA than larger conspecifics.

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Entomology; Method Validation; Forensic Science

I111   Baits, Bodies, and Bugs: An Analysis of Forensic Entomology in Arizona

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Learning Overview: After attending this presentation, attendees will be informed about the importance of forensic entomology through findings of both research and casework. Attendees will learn about forensic entomology in the Southwestern United States, a severely understudied area.

Impact Statement: This presentation will impact the forensic science community by being the first of its kind to compare forensically relevant blow flies across baited traps and animal and human remains in the state of Arizona. It will provide valuable information as a guideline for future research in this field.

Forensic entomology, the use of insects and arthropods to aid in legal investigations, is a subdiscipline of forensic science that has grown over recent years. This increase in use is due to the beneficial nature of these insects and the answers they can provide investigators. Insects can be used to answer various questions including body relocation, the presence of drugs/toxins, and help determine how long remains have been present by determining the insects age.[1-3] Even though this field is growing, it is still not nearly as common as other subdisciplines. We see this field more heavily utilized in areas where forensic entomologists are located, leaving large gaps of knowledge in the field, especially in areas that are environmentally unique.

The southwestern United States contains the Sonoran Desert, which extends south into Mexico. This desert system is unique, being extremely hot and arid, with biannual monsoons. Excluding one recent publication, forensic entomology literature has not been published in over 30 years in Arizona, with the last relevant published study in the 1980s. To help reduce this gap in knowledge, a year-long blow fly survey was conducted on Arizona State University's West Campus in Phoenix, AZ, to determine the relevant species in the area. In addition, we collected blow flies from ten carcasses (nine swine carcasses and one bovine carcass) to compare to the survey results and validated both methods with collections from human remains across the state. Our survey indicated that there were eight species present in the Phoenix area, with *Lucilia sericata* (Meigen, 1826)*, Calliphora latifrons* Hough, 1899*,* and *Lucilia mexicana* Macquart, 1843, as the predominant species. Collections from animal remains collected an additional species, *Cochliomyia macellaria* (Fabricius, 1775)*.* Last, all species collected from human remains were captured during our survey or from animal remains. These findings will provide baseline data for when species are expected to be present as well as specify which species should be of focus for future research in this area.

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Blow Flies; Crime Scene Investigation; Desert

I112   Feline Forensics: Revealing the Unique Decomposition and Forensically Relevant Blow Flies (Diptera: Calliphoridae) Associated With Cats

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Learning Overview: After attending this presentation, attendees will be informed regarding a general overview of forensic veterinary science, time of colonization, oviposition behavior of forensically relevant flies, and unique decomposition of cats (*Felis catus* Linnaeus, 1758).

Impact Statement: This research will impact the forensic science community because there is limited data about veterinary medicine within the forensic science fields. This research will aid established forensic entomologists and pathologists because there are important findings that seem to be unique to cats and possibly other animals with fur. Understanding these unique findings has considerable implications in future investigations involving neglect and abuse of cats. These implications include locating oviposition sites in atypical areas, understanding which blow fly species to expect colonizing cats, and developing a scoring system for decomposition of animals with fur. Applying the findings would assist forensic entomologists, pathologists, and veterinarians in their determination of neglect and abuse timeframes.

Forensic veterinary science is a growing discipline within the veterinary and forensic fields, including forensic entomology and pathology. Limited data exists on the time of colonization, oviposition behavior, and relevant blow fly species regarding animals with fur. Additionally, due to the sheer number of animal models used, determining the stages of decomposition for specific species is challenging. Typically, determining what stage of decomposition a body is in is subjective to the observer due to the qualitative methods of describing the morphological changes visually. By implementing a scoring system, the total body score, the observer is able to quantify decomposition and attempts to minimize observational bias. The purpose of this study was to examine insect colonization and decomposition of cats (*Felis catus* Linnaeus, 1758) with light and dark fur colors. The information from this study will aid in understanding the initial colonization period of forensically relevant flies and stages of decomposition, which can contribute to estimate the minimum postmortem interval.

Twelve domestic short-haired cats were placed in cages 15.2m apart in a grassy field Indiana. Weather data (temperature, precipitation, sun/cloud exposure, humidity), insect activity, time to oviposition, and decomposition changes were documented. Eggs from initial oviposition events were collected and reared to identify the primary colonizing species. Although time to first oviposition event were not different between the treatments, fur color does affect fly colonization, and cats with dark fur had more oviposition events than cats with light fur ($t = 2.639$, $df = 4$, $p = 0.029$). Three species of *Lucilia* Robineau-Desvoidy, 1830 (Diptera: Calliphoridae) colonized the cats on the initial day of placement. Total Body Scores (TBS) were used to quantify decomposition characteristics and changes. An exponential relationship between TBS and accumulated degree days was documented. Overall, there was not a significant difference in the rate of decomposition or total body scores between the treatments ($t = 1.126$, $df = 11$, $p = 0.14$). These findings will aid in locating initial oviposition sites, shed light on which blow fly species to expect colonizing cats, and time of colonization estimates. Additionally, understanding the stages of decomposition and the unique characteristics for animals with fur can assist investigators—forensic entomologists, pathologists, or veterinarians—determining timeframes for neglect or abuse.

Entomology; Decomposition; Veterinary Medicine

I113   How Can Forensic Entomology Contribute to Solving Rhinoceros Poaching in South Africa?

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Learning Overview: This presentation will utilize insect data collected and preserved at crime scenes involving rhinoceros remains to calculate the best estimate of the minimum Postmortem Interval (minPMI). The laws relating to this activity in the context of the judicial system in South Africa will be briefly discussed. After attending this presentation, attendees will better understand this type of evidence and its importance in determining the time since death of poached rhinoceroses.

Impact Statement: This presentation will impact the forensic science community by showing the consequences of poaching rhinoceros in South Africa as well as how forensic entomology is aiding in prosecuting its perpetrators. Forensic entomology has impacted many outcomes related to homicides and suspicious deaths among humans, and this discipline is now being utilized to help solve the deaths of threatened wildlife. This promotes forensic collaboration between police, wildlife experts, and the general public.

The Republic of South Africa (RSA) has been active in rhinoceros conservation for over 30 years, and this has resulted in a rebound in the number of rhinoceros populations.[1] Of the two species found in South Africa, white rhinoceros numbers have recovered most, but, despite the best efforts of the RSA, the black rhino remains in the top ten of the International Union for the Conservation of Nature red list of critically endangered species. This fact has not gone unnoticed in the RSA, with every effort now made to stop rhinoceros poaching (ranger teams, canine units, satellite tracking). However, between 2014 and 2021, 6,684 rhinoceros have been killed and dehorned in South Africa by poachers, generally a part of organized crime syndicates.[2]

This study examined the insect evidence collected from 49 illegally hunted rhinoceros carcasses between this period across five provinces of South Africa. We report on the role of Forensic Entomology (FE) in investigating 19 of these cases. When insects are present on remains, FE is the discipline utilized to estimate the time since death or Postmortem Interval (PMI). Once death occurs, there is a period designated the pre-colonization interval when insects are absent. This is followed by insect colonization (typically blow flies) and following discovery, collection, identification, and determination of the life stages of immatures, the forensic entomologist can calculate the minimum PMI (minPMI). From the 19 remains, a total of 74 samples of insect evidence were collected. The specimens consisted of 18 species from 12 families belonging to 3 insect orders. Although many Dipteran and Coleopteran species were found on and around each carcass, the predominant species were from the genus *Chrysomya* Robineau-Desvoidy.

This study discusses six insect species from the Dipteran and Coleopteran orders that were utilized to provide the best estimate of the minPMI. The species of fly larvae used in each case to estimate the minPMI were as follows: *Chrysomya marginalis* (Wiedemann) (13 cases), *Chrysomya chloropyga* (Wiedemann) (2 cases)*, Chrysomya albiceps* (Wiedemann) (1 case) and *Chrysomya megacephala* (Fabricius) (1 case). Two species of Coleoptera with 1 specimen per case were also involved in the minPMI estimations: *Dermestes maculatus* (DeGeer) and *Thanatophilus micans* (Fabricius). The success of forensic entomology in contributing to solve these crimes is evident in the feedback from the investigating parties and the results of court cases where insect evidence was presented. Finally, two FE expert witness statements from the 19 cases have been presented to the court. One perpetrator has received a cumulative sentence of 34 years imprisonment for poaching rhinoceros.

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Entomology; Postmortem Interval; Animal Remains

I114   Winter Decomposition in North Dakota and Vertebrate Scavengers

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Learning Overview: After attending this presentation, attendees will have a better understanding of outdoor decomposition during North Dakota winters, an environment dominated by negative temperatures and heavy snow, and on the importance of using experimental designs more relatable to real-case scenarios, namely not using cages to protect pig carcasses from vertebrate scavengers.

Impact Statement: This presentation will impact the forensic science community by providing a unique view of the decomposition process during North Dakota winters by emphasizing the importance of not using protective cages when investigating decomposition within extreme cold environments.

The decomposition process for above-the-ground (exposed) carcasses is usually divided into five stages: fresh, bloat, active decay, advanced decay, and skeletal remains. The chronology of these stages will be influenced by numerous factors, especially by the environmental temperature. Lower temperatures will slow down the decomposition process, while vertebrates will be able to scavenge the remains. Scarce information is available on decomposition from cold climates, and even fewer studies were performed on pig carcasses without using protective cages.

The current research aimed to provide a unique view on decomposition during North Dakota winters, using pig carcasses as human analogs not protected by cages from vertebrate scavengers. The carcasses were placed in the field December 2022, on Mekinock Field Station (47°57'11.5"N 97°25'42.3"W) University of North Dakota research land and monitored until the snow melted in May 2023. The monitoring took place daily via trail cameras with motion sensors, and vertebrate scavengers' activity was investigated throughout the entire decomposition process. The reason for not using cages and using field cameras came from the previous winter experiment (2021–2022), when three pig carcasses (55kg each) were placed in the field, protected by cages, and scavenger activity was recorded March throughout the end of April. As such, the need to understand the vertebrate scavenger behavior from this area arose. The temperature was recorded hourly for the entire experimental period via temperature data loggers, while the snow (precipitation) records were obtained from the closest weather station, situated 10km from the research site.

The temperature fluctuated between -32°C and 30.3°C along the five experimental months, with a snow depth of up to 130cm. The carcasses were covered by snow; however, the main vertebrate scavengers, represented by coyote (*Canis latrans* Say, 1823) and foxes (*Vulpes* sp. Garsault, 1764), dug up corridors to reach and consume the carcasses, mostly after sunset. The presence of these two canids was not surprising as these species are often encountered in the Northern United States.

Decomposition studies are of tremendous help to understand the factors that induce variation in the chronology of the decomposition stages, invertebrate and vertebrate diversity, and colonization patterns to be of use to the forensic science field by providing an overview on decomposition and an inventory of the primary decomposers for death investigations. Future endeavors will include monitoring decomposition with and without protective cages to better understand both invertebrate and vertebrate behaviors from this geographical location.

Decomposition; Scavengers; Winter

I115   Assessing the Impacts of Prescription Medications and Metabolites on Larval Blow Fly Development

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Learning Overview: The goal of this presentation is to create a discussion about: (1) bridging laboratory results with field conditions encountered by forensic entomologists, and (2) how metabolites of parent drugs may influence larval development.

Impact Statement: This presentation will impact the forensic science community by presenting entomological postmortem estimations using developmental data assuming that no drugs or toxins are present in a decedent.[1] This project is the first to compare controlled laboratory experimental results to those of human donor's fluids and total field decomposition.

Blow flies are primary decomposers of vertebrate tissue. Thus, toxicants present in human remains may impact carrion insect physiology and behavior, potentially resulting in inaccurate minimum Postmortem Interval (minPMI) estimates.[2] Previous entomotoxicology studies demonstrate that certain drug classes (e.g., barbiturates, analgesics) can decrease body size, development rate, and survivorship of blow fly larvae.[3-7] Though numerous entomotoxicology studies have been conducted under controlled laboratory conditions, none contain validations of data with human remains in the field. We aimed to investigate the effects of commonly prescribed drugs on forensically important blow fly phenotypes. Specifically, we examined the impacts of morphine, lorazepam, and the secondary metabolites of each drug on the: (1) developmental duration (larval, pupal, and total), (2) body size (larval, pupal), and (3) survivorship of blow flies. We also examined the impacts of human decomposition fluid containing these drugs on the fly phenotypes of interest.

All experiments were performed with a laboratory colony of the black blow fly, *Phormia regina* (Meigen). Each experiment followed fly development from first instar larva through adult emergence. Each replicate within a treatment was comprised of primary drugs of interest (Morphine [M]; Lorazepam [L]), metabolites (Morphine-3-Glucoronide [M3G]; lorazepam-Glucoronide [LG]), or drug/metabolite combinations (L:M, M:M3G, L:LG, M3G:LG) blended into ~100g of organic, antibiotic-free lean pork. Additional treatments using field-collected human decomposition fluid were made in the same manner. Decomposition fluid samples were originally collected from four human donors decomposing at the Anthropology Research Facility (ARF) at the University of Tennessee. Confirmation of morphine and lorazepam in the fluids was confirmed by High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS). Control replicates consisting only of pork were also implemented in the study design. Each replicate was inoculated with N = 100 first instar larvae and maintained at 25°C and 70% relative humidity with a 12:12 light:dark cycle. Four replicates were run per treatment. Length and width were taken for each larva, development stage was assessed via microscopic examination of the posterior spiracles, and mass of pupae and adults was taken. Survivorship was determined for each developmental stage. Data was analyzed using a two-way Analysis Of Variance (ANOVA) with a post-hoc Tukey Honest Significant Difference (HSD) test.

Drug treatment significantly impacted larval duration ($P <0.001$) and total duration ($P <0.001$), but not pupal duration ($P = 0.408$). Treatments M, L, and M:M3G took significantly longer to reach pupation compared to all other treatments (all $P <0.050$). However, no significant difference was observed for L:M, M3G, or controls for pupal mass, larval duration, or total duration. While drug treatment was not significantly related to pupal emergence time, the glucuronide active group appears to also produce variability in this stage, the effects of which are still under investigation. Additionally, preliminary data indicates that the control larvae, raised on pure lean pork, are all significantly different in size compared to each drug or decomposition treatment ($p <0.05$) and *P. regina* pupae associated with glucuronides were smaller with lower survivorship. Pupal and adult weights were closely correlated, as expected biologically ($P <0.001$). *P. regina* larvae associated with individuals who tested positive for morphine ($P <0.001$) and lorazepam ($P <0.003$) were shorter, consistent with preliminary experimental results. Larvae on lorazepam treatments experienced the lowest survivorship, while larvae exposed to morphine was similar to the control group. Overall, these preliminary results indicate morphine, lorazepam, and their metabolites impact *P. regina* larval development. Continued research aims to determine if developmental durations associated with specific drugs and metabolites in this study significantly impact minPMI estimations in the field.

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Entomology; Toxicity; Decomposition

I116 A Decade Into Developing a Microbiome Tool for Predicting the Postmortem Interval: Where Are We Now and Where Are We Going?

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Learning Overview: After attending this presentation, attendees will have acquired a current understanding of the state of science underlying predictions of the Postmortem Interval (PMI) using microbiome data.

Impact Statement: This presentation will impact the forensic science community by providing an overview of advances and remaining knowledge gaps for developing a tool to predict the PMI based on microbiome data.

Time of death is important for death investigations, particularly criminal investigations for which alibis must be verified. However, it can be difficult to estimate the PMI after the first several days of death, especially in the absence of other physical evidence such as last known communications or insect activity. Over the past decade, the proposal investigators have demonstrated the power of microbiome-based estimates of PMI with mouse and swine model systems (2011-DN-BX-K533), and translated estimates to humans by utilizing multiple United States anthropological forensic research facilities (2011-DN-BX-K533, 2015-DN-BX-K016, 2016-DN-BX-0194).[1-5] Recent research by the investigators generated a machine learning model utilizing 16S rRNA gene amplicon data from skin and soil samples associated with 36 human cadavers collected daily for 21 days from three forensic facilities, which predicts PMI within approximately +/- 3 days over the first 21 days postmortem.[5] Thus, this new tool could provide useful accuracy for crime scene investigations.[6]

However, several knowledge gaps remain. First, we need to expand the 36-body PMI microbiome database by collecting similar sample types from additional human cadavers from facilities that are in climate types not yet represented in the PMI database. Second, we need to collect and utilize test samples that are completely independent from samples used to train our predictive model. To address these gaps, newly National Institute of Justice (NIJ) -funded (15PNIJ-22-GG-04402-MUMU) research is underway to bring a cold Köppen-Geiger classified climate type into the database so that three of the major United States climate types are represented in the postmortem microbiome database. The new and existing data will together train a new machine learning model for estimating PMI that will be more generalizable across climates represented in the United States. Second, an independent test set of samples from cadavers that are not part of the 54-body training set will be used to validate the predictive model. Test set samples will be collected from cadavers from locations represented in the training set and also from new locations not represented in the training set. From these data, generalizability and accuracy of the model will be assessed for predicting PMI across climates, individual locations, and decomposition time frames in the United States. Additionally, we will discuss predictive modeling algorithms utilized, and potential future directions in this area[6]. Finally, we will highlight potential paths to technology adoption by the forensic community.[7]

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Microbiology; Postmortem Interval; Forensic DNA

I117 Understanding Seasonal Differences in Postmortem Microbiome Development for Forensic Practice

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Learning Overview: After attending this presentation, attendees will better understand how postmortem microbiome progression is impacted by variable environmental conditions (i.e., temperature, relative humidity, and rainfall) brought on by seasonal differences. "Microbiome" refers to the community of microorganisms that exist on or in any given environment, with the focus here being given to bacteria. Attendees will also be introduced to how the postmortem microbiome of carrion is assessed and analyzed using 16S amplicon sequencing.

Impact Statement: This presentation will demonstrate to the forensic science community the impact seasonal differences in rainfall, temperature, and humidity can have not only on the duration of decomposition, but also how the postmortem microbiome of carrion develops over time. Attendees will also be introduced to the growing research on postmortem microbiomes and their potential utility in death investigation—particularly in the determination of a Postmortem Interval (PMI). These impact goals will be accomplished by analyzing differences in the alpha- and beta-diversity of bacterial communities associated with replicate carrion (i.e., samples from mouths, rectal cavities, and on skin) in the Great Lakes region during summer and autumn. The abundance and composition of bacteria over time will describe bacterial succession as decomposition occurs in relation to dynamic abiotic factors.

It is well understood that abiotic factors play a large role in microbial development, as the influence exerted by things like temperature, relative humidity, and resource availability directly determines the growth of certain bacterial species. Bacteria have unique maximum, minimum, and optimal growth temperature ranges that dictate their ability to not only survive on carrion, but proliferate as well.[3,6] Relative Humidity (RH) is an important consideration for similar reasons, but most culturable bacteria demonstrate a shared RH requirement of ≥60%.[3,5] In combination, abiotic factors work to govern the sustainability of bacterial life in environments like those found on, in, and surrounding decomposing remains. The development of the bacteria in these environments over time is called bacterial community succession.

The research being presented aims to show the forensic science community that precipitation and temperature change with declining average daylight hours, resulting in cascading effects that directly affect both the composition and development of microbiomes on carrion and likely affect how carrion bacterial communities go through succession. Measurable and significant differences in the relative abundance of certain species of bacteria can help to better estimate PMI as well as provide insight into what, alongside macroinvertebrate decomposers, is driving the decomposition of vertebrate remains.[1,4,7,8] It is also important to ensure that the reference data used in PMI determination is as representative as possible and evaluating whether or not microbiome community composition seasonally varies significantly will greatly improve how postmortem microbiome profiles can be interpreted as evidence.

Data for this experiment were collected using six stillborn pigs as models during the summer and autumn of 2022, with body site sampling taking place twice a day until complete skeletonization of the remains was observed. In the summer, this process took 7 days, whereas in the fall this took 32 days. Temperature measurements from data loggers ranged from 8°C to 39°C in the summer and –2.3°C to 35.4°C in the fall. Samples were taken using sterile cotton swabs by wiping the swab head along transects on the carcass skin and within its mouth and rectal cavities for 30 seconds each. The DNA from the swabs was extracted using the DNeasy® Blood & Tissue kit that then underwent 16S amplicon sequencing according to the 16S Illumina® amplicon protocol. Polymerase Chain Reaction (PCR) performed prior to sequencing took advantage of primers 515F-806R to target the V4 region of SSU rRNA. Sequencing data were then analyzed using the microbiome informatics platform QIIME2®. An Analysis of Variance (ANOVA) comparing sampling seasons showed that there was a statistically significant difference in the level of phylogenetic diversity (Faith PD) observed between summer and fall. Fall demonstrated a significantly lower level of phylogenetic diversity overall when compared to the summer prior ($P < 0.001$). Shannon diversity, a measure of richness and diversity within a community, differed as well between the seasons ($P < 0.001$). A Permutational Multivariate Analysis Of Variance (PERMANOVA) significance test for group-level differences between seasons demonstrated that it may also have a significant effect on overall microbiome composition ($P = 0.01$). A PERMANOVA testing season and sampling days suggested that there is almost certainly a significant difference in microbiome composition over time ($P = 0.001$).

Existing scientific endeavors aim to model the postmortem microbiome and develop a microbial clock of death, one that can be compared to snapshots of the postmortem microbiome found on human remains to derive an approximate estimate of PMI.[1,2,4] Evaluating how microbial succession changes over time and among or between seasons will strengthen the reliability of a microbial clock and is important in further pushing for more corroborative evidence to be used in the ultimate determination of PMI.

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Microbiology; Forensic Analysis; Next Generation Sequencing

I118   Microbiome Across the Pond: Decomposition in North Dakota Extreme Winter Temperatures Versus United Kingdom Summer Temperatures and Their Effect on the Microbiome for PMI Estimation

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Learning Overview: After attending this presentation, attendees will understand the differences that extreme winter versus mild summer temperatures have on the decomposition of pig carcasses and on their microbial communities and how this may affect the estimation of Postmortem Interval (PMI) in different scenarios.

Impact Statement: This presentation will impact the forensic science community by highlighting how the postmortem microbial clock applies to highly different environmental contexts and will serve as a reference for the evaluation of the accuracy of the microbial clock in such circumstances.

The study of the microbial populations colonizing the body and their succession over time has been investigated in forensics to estimate the time elapsed since death (PMI). Accurate "microbial clocks" based on the succession of specific bacterial populations and originally developed on mice or rats in controlled (laboratory) conditions or buried in the soil gave high levels of accuracy for PMI estimation.[1,2] However, it is well known that the environmental conditions such as humidity, temperature, and precipitations play a key role for insect activity and microbial successions, affecting the decomposition rate overall. It is still not clear how this affects the capability of the microbial clock to correctly estimate PMI.

In this study, the decomposition of three pig carcasses in an extreme winter environment in North Dakota was compared with the decomposition of six pig carcasses in a mild summer environment in the United Kingdom. Both morphological (e.g., Total Body Scores [TBS]) and microbiome (internal and external nose swabs) data were collected at selected time points as long as possible (e.g., until when the noses were completely colonized by maggots, making the swabbing impossible). The North Dakota experiment started in November 2021 and lasted for 23 weeks, with sample collection in triplicate from each pig every week (total samples n=414), whereas the United Kingdom experiment started in June 2023 and lasted for 11 days, with sample collection in duplicate from each pig at 28 selected time points (time 0, then every 3 hours for 24 hours, every 6 hours for following 48 hours, every 12 hours for subsequent 72 hours, and every 24 hours for the last 120 hours, total samples n=672). Then 16s rRNA gene sequencing was performed on an Illumina® MiSeq® platform. Data were pre-processed and analyzed using QIIME2® with Silva v.138 and DADA2 on R studio; random forest modeling for PMI estimation was also conducted on R studio.

Distinct decomposition rates and TBS were observed between the two experiments. The most abundant phyla identified during the decomposition process were Proteobacteria, Firmicutes, Actinobacteriota and Bacteroidota; alpha diversity indices decreased with increasing PMIs and were significantly lower in interior swabs compared with exterior ones. In terms of PMI prediction, internal nose swabs appeared to be more accurate than exterior ones. Comprehensive results of the PMI estimation modeling in both environments will be presented at the conference, along with the corresponding prediction accuracy and errors and with recommendations on the best sampling strategies for obtaining reliable microbial data for PMI estimation in different contexts.

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16s rRNA Sequencing; Metabarcoding; PMI

I119   Developing a Multi-Omics Bone Biomolecular Signature by Integrating Metabolomics, Lipidomics, and Proteomics to Estimate Postmortem Interval

Andrea Bonicelli*, University of Central Lancashire, Preston, England; Noemi Procopio, University of Central Lancashire, Preston, England

Learning Overview: After attending this presentation, attendees will better understand the potential of Liquid Chromatography/Mass Spectrometry (LC/MS) -based multi-omics analysis for long Postmortem Interval (PMI) estimation based on bone tissues, including the accuracy rate and the associated prediction errors.

Impact Statement: This presentation will impact the forensic science community by proposing the first comprehensive multi-omics tool for the estimation of PMI, setting up strong foundations for future improvements of the approach.

In recent decades, omics techniques have become increasingly prevalent in forensic science, particularly for estimating PMI. When a person dies and their metabolic activities cease, bone biomolecules undergo time-dependent modifications influenced by the environmental conditions. For early PMIs, metabolomics on biofluids has demonstrated significant potential for PMI estimation, while structurally and thermally more stable proteins and lipids have shown promising results for estimating longer time intervals.[1,2] By combining these three classes of biomolecules, it is possible to cover a wide range of PMIs and achieve a level of accuracy and precision currently lacking in traditional methods.[3] This study represents the first and the largest systematic investigation, to our knowledge, of a wide postmortem time interval of human bone biomolecules for PMI estimation, utilizing a multi-omics approach.

The researchers analyzed non-treated bone fragments from the anterior portion of the tibia of 73 individuals from two human taphonomy facilities: the Forensic Anthropology Center at Texas State University and the Southeast Texas Applied Forensic Science Facility at Sam Houston State University. The PMI of the samples ranged from zero to seven years. To extract proteins, metabolites, and lipids from the bone powder, specific methods developed for this purpose were employed. Peptides were injected into a reverse phase C18 column and analyzed using an Exploris™ 480 Quadrupole-Orbitrap™ Mass Spectrometer. Metabolites and lipids were injected into Hydrophilic Interaction Chromatography (HILIC) and C18 columns, respectively, and analyzed using a high-resolution quadrupole Time-Of-Flight (q-TOF) mass spectrometer. The proteomic, metabolomic, and lipidomic datasets were then processed via Progenesis QI for Proteomics and Progenesis QI, respectively, for Tandem Mass Spectrometry (MS/MS) identification and relative quantification. Preliminary results from a subsample of 30 individuals demonstrated an estimation error of less than one year when using a random forest algorithm that combined metabolites and proteins. Certain metabolites (e.g., hypoxanthine, creatinine, uric acid) and proteins (e.g., vimentin, histone proteins, hemoglobin) consistently exhibited correlations (either positive or negative) with PMI, indicating that they could serve as valid markers for PMI estimation. By replacing the PMI range measured in accumulated degree days and by adding the additional samples, the researchers aim to confirm the identified markers and potentially discover new ones, leading to the development of robust machine learning models for PMI estimation based on bone biomolecules.

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Postmortem Interval; Multi-Omics; Bone

I120   A Metabolomics Investigation of Postmortem Human Pericardial Fluid

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Learning Overview: After attending this presentation, attendees will learn about the potential application of Nuclear Magnetic Resonance (NMR) metabolomics in a particular biofluid, namely pericardial fluid, easy to collect during autopsies. Such an approach allows one to investigate Postmortem Intervals (PMIs) in which traditional approaches cannot be applied or can be alternative/additional to other matrices (e.g., vitreous humor).

Impact Statement: This presentation will impact the forensic community by displaying results based on a metabolomic investigation conducted on postmortem human pericardial fluid. Although proof-of-concept, this experiment suggests that metabolome composition seems to be correlated with PMI, whereas extraction protocols, age, sex, and cause of death do not affect it.

Introduction: Due to its peculiar anatomy and physiology, the pericardial fluid is a biological matrix of particular interest in the forensic field. Despite this, the available literature has mainly focused on postmortem biochemistry and forensic toxicology, while to the best of the authors' knowledge, postmortem metabolomics has never been applied. Similarly, estimation of the time since death or PMI based on pericardial fluids has still rarely been attempted.

Objectives: We applied a metabolomic approach based on ^{1}H nuclear magnetic resonance spectroscopy to ascertain the feasibility of monitoring postmortem metabolite changes on human pericardial fluids with the aim of building a multivariate regression model for PMI estimation.[1]

Methods: Pericardial fluid samples were collected in 24 consecutive judicial autopsies in a time frame ranging from 16 to 170h after death. The only exclusion criterion was the quantitative and/or qualitative alteration of the sample. Two different extraction protocols were applied for low molecular weight metabolites selection, namely ultrafiltration and liquid-liquid extraction. Our metabolomic approach was based on the use of ^{1}H nuclear magnetic resonance and multivariate statistical data analysis.

Results: The pericardial fluid samples treated with the two experimental protocols did not show significant differences in the distribution of the metabolites detected. A PMI estimation model based on 18 pericardial fluid samples was validated with an independent set of 6 samples, giving a prediction error of 33–34h depending on the experimental protocol used. By narrowing the window to PMIs below 100h, the prediction power of the model was significantly improved with an error of 13–15h depending on the extraction protocol. Choline, glycine, ethanolamine, and hypoxanthine were the most relevant metabolites in the prediction model.

Conclusion: The present study, although preliminary, shows that pericardial fluid samples collected from a real forensic scenario represent a biofluid of interest for postmortem metabolomics with particular regard to the estimation of the time since death. Most relevant metabolites in this model were already found to be correlated to PMI in different biological matrices in both human and animal models.

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Postmortem Interval; Metabolite; Omics

I121   Infant Postmortem Microbiome Biomarkers: An Initial Survey

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Learning Overview: After attending this presentation, attendees will better understand the postmortem microbiome for infant deaths. The use of postmortem microbial communities in adults has demonstrated its ability to discern discrete body sites and its potential use of biological biomarkers during death investigation (e.g., postmortem interval range estimate). But limited data exists on the variability of postmortem microbial communities for humans in the infant age range. This presentation will show the postmortem microbiome associations of infants from samples collected during routine death investigations.

Impact Statement: This presentation will impact the forensic community and practitioners by providing a foundational dataset of postmortem microbial signatures after infant death. As evidenced by the constant flux of the antemortem microbiome from person-to-person and across disease status, more robust data across different age ranges and demographics are needed of postmortem microbiota to provide a more cohesive understanding of these communities for forensic investigators, especially in cases of suspected sudden unexpected infant death.

Postmortem microbial communities were collected from a cross-sectional survey of 50 infant death cases. All samples were gathered by the Wayne County Examiner's Office in Detroit, MI, during routine death investigation. For the purposes of this study, manner of death was categorized as: natural, accidental, non-accidental, or control. Natural death included cases of circumstances of death attributed to natural, pre-determined causes, such as a pre-existing genetic condition. Accidental death involved chance circumstances, such as accidental drownings or airway obstruction unrelated to co-sleeping. In contrast, non-accidental deaths resulted from intentional harm caused by another, such as blunt force trauma. Finally, control cases were those where circumstances of death were due to asphyxia, airway obstruction, or over-lay associated with co-sleeping. Postmortem microbial DNA was isolated and quantified following a modified manufacturer protocol for commercially available kits, a fluorometer, and a microchannel-based automated electrophoresis system. Targeted amplicon (16S rRNA V4 gene region) and shallow-shotgun whole genome high-throughput sequencing was conducted on individual sample libraries. Resulting postmortem microbial community data were analyzed using open source bioinformatic pipelines and statistical software.

Bacterial community composition differed among postmortem infant body sites (i.e., rectum-cardiac blood, rectum-ears, rectum-mouth, rectum-nose, rectum-trabecular space, and cardiac blood-nose), with highly significant (KW, fdr corrected, $p < 0.01$) differences between bacterial families. Rectal samples shared the most similar compositions across cases, with higher abundances of the families *Oscillospiraceae*, *Bacteroidaceae*, and *Bifidobacteriaceae.* Beta-diversity of the postmortem microbiomes was analyzed using weighted UniFrac distances of bacterial communities from whole genome sequencing and results showed a nearly statistically significant ($p = 0.132$) effect of manner of death. The predictive power and performance of three separate machine learning models to predict infant manner of death suggest potential biomarkers for manner of death and body site exist across the 50 cases sampled.

Through life and after death, the microbiome is becoming a powerful tool to provide insight into the human health condition; with no exception made to aid in estimating the postmortem interval and creating emergent models to discern manner of infant death. The results from this dataset are important for future research as they provide foundational information regarding postmortem microbial variability during infant death investigation. It is critical that these initial datasets are validated with larger sample sizes and across increased geographic regions (e.g., state, national, global). These results will further support the creation of standardized, best practice recommendations and workflows for the analysis of microbiomes in routine death investigation.

Microbiology; Infant Death; Next Generation Sequencing

I122   A Longitudinal Study for PMI Estimation Via GC/MS Metabolomics Applied to Porcine Muscle Tissue

Andrea Bonicelli*, University of Central Lancashire, Preston, England; Halima Al-Balushi, University of Central Lancashire, Suhar, Shamal al Batinah, Oman; Zoha Khan-perveen, University of Central Lancashire, Preston, England; Alberto Chighine, Forensic Unit, Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Sardegna, Italy; Irene van Zwieten, University of Central Lancashire, Preston, England; Peter Cross, University of Central Lancashire, Preston, England; Noemi Procopio, University of Central Lancashire, Preston, England

Learning Overview: After attending this presentation, attendees will be aware of the potential that Gas Chromatography/Mass Spectrometry (GC/MS) metabolomics has for Postmortem Interval (PMI) estimation on muscle tissues, including the accuracy rate and the associated prediction errors, in a temperate United Kingdom climate.

Impact Statement: This presentation will impact the forensic community by proposing a cheap and affordable way to perform PMI estimation on soft tissues via GC/MS that may be applied in the future for improved forensic outcomes.

The estimation of the PMI is the subject of extensive debate in the field of forensics. While current methods for estimating short-term PMIs rely on biochemical and morphological examinations of the deceased body, there is a growing interest in biomolecular techniques like metabolomics. This increased interest is a result of the advancements in analytical platforms and data analysis methods. Furthermore, it is well known that in order to produce accurate estimation models, it is essential to perform longitudinal studies that can take into account environmental conditions such as humidity, temperature, and precipitations as well as the biological variability between individuals.[1] These environmental factors play a key role for insect activity and microbial successions, overall affecting the decomposition rate.

The present study involved the placement of three male pigs (*Sus scrofa*) aged six months in June 2023, which were left decomposing for a duration of 11 days. Sample collection was conducted for each pig at 28 specific time points. The sampling schedule included time 0, followed by collections every 3 hours during the first 24 hours, every 6 hours for the subsequent 48 hours, every 12 hours for the following 72 hours, and every 24 hours for the last 120 hours. Pigs were positioned on the right side, and muscle sampling was performed on left biceps femoris. In order to limit exogenous variables, skin was sutured between each sampling. In total, 90 samples (one sample per pig at all timepoints for three pigs) were considered for the current metabolomics study. The muscle tissue was processed in a Precellys® beads homogenizer and metabolites were extracted using a methanol-water solution. Samples were analyzed in an Agilent® 5977C GC/MSD, data pre-processed in MS-Dial and analyzed in R (v. 4.2.2).[2] Partial least square regression was used to produce a PMI prediction model with an error of approximately 12 hours over a 216-hour PMI. Several metabolites (e.g., hypoxanthine, hypotaurine, and ethanolamine) were found correlating with PMI and confirm results from previous studies in both animal and human models.[3,4]

Overall, this proof-of-concept real-life experiment suggests that the use of GC/MS-based metabolomics could be an additional and affordable platform to estimate PMI from muscle material for time windows in which traditional methods cannot be applied (e.g., over 48-72 hours).

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Postmortem Interval; Muscle; Metabolomics

I123   The Mass Grave Project: The Impact of Freezing and Burial on Personal Identification Through Human DNA Analysis

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Learning Overview: After attending this presentation, attendees will have acquired insights into the various possibilities of sampling of biological material from deceased individuals for human identification, particularly in complex situations such as mass graves or mass disasters.

Impact Statement: This presentation will impact the forensic science community by providing recommendations on the type of tissue and biological samples that can be collected non-invasively (e.g., avoiding bones and teeth) from cadavers in different stages of decomposition to achieve successful human genetic identification. In particular, the different conditions of storage or burial (fresh before freezing, fresh after freezing and thawing, and actively decaying after exhumation from single or mass graves), and actions aimed at reducing potential cross-contamination *in situ* will be considered.

The Mass Grave Project (MGP) is a multidisciplinary study being conducted within the Forensic Anthropology Center at Texas State University (FACTS) aimed at comprehensively studying the complete process of human decomposition within mass graves on macroscopic, microscopic, and biomolecular levels.[1,2] In particular, it focuses on the examination of an experimental mass grave made up by six donors, and of three additional single graves, to develop investigative techniques including grave (remote) detection, excavation, evidence analysis, and documentation.

At present, the extraction of DNA from decomposed human remains is carried out using labor-intensive and invasive techniques, specifically targeting hard tissues such as bones and teeth. This substudy of the MGP aims to gain insight into the impact of freezing bodies on human DNA to assess the appropriateness and reliability of utilizing frozen human donations for genetic research purposes and at evaluating the potential of utilizing DNA profiling from decomposed soft tissues to facilitate identification in mass burial scenarios. Therefore, the first aim of this substudy was to evaluate the completeness of the genetic information obtained from swabs taken from the skin, oral cavity, and rectum upon the arrival of the donors at FACTS after their freezing for a period ranging from 11 to 455 days and thawing prior to their burial for 18 months. The second aim was to investigate the post-burial DNA profiling of different soft tissue sampling sites (skin, oral, periocular, and rectal swabs, nails, cartilage, and soft tissue such as muscles and internal organs), their performance, and their correlation with the degree of decomposition, the characteristics of the burial (single or multiple), and the position of the donor in the mass grave.

Results showed that the quality of DNA profiles decreased from fresh to thawed and finally to decomposed. Interestingly, the DNA results were not influenced by the degree of macroscopic tissue decomposition or the body's position within the mass grave. In the mass grave, signs of DNA cross contamination were observed, along with higher levels of human DNA preservation compared to the individual graves. These results contribute to our understanding of how freezing and burial affect genetic identification in forensic investigations as well as to the development of improved strategies to identify individuals in mass graves. Based on the study findings, several recommendations are proposed for forensic practice and future research in challenging contexts such as mass graves and mass disasters.

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2. Mickleburgh H.L., Wescott D.J. (2021). The Mass Grave Project: An experimental taphonomic study aimed at advancing methods of detection and documentation of mass graves and human identification. *European Meeting on Forensic Archaeology, Geneva*.

Mass Graves; Mass Disaster; Identification

I124   The Recovery of Corpses From Aquatic Environments: Procedural Standards to Maximize Evidence Recovery

Denise Gemmellaro*, Kean University, Dept. of Biology, Union, NJ; Gloria Raise, New Jersey Institute of Technology, Maplewood, NJ; Andrea Zaferes*, Forensic Aquatic Consulting & Training, LLC, Shokan, NY

Learning Overview: After attending this presentation, attendees will be aware that practitioners need procedures, equipment, and training to recover corpses from aquatic environments, that it is common for basic death investigation standards to be broken when corpses are located and transported from the aquatic environment to land, and what solutions are available to optimize evidence preservation.

Impact Statement: This presentation will impact the forensic science community by providing questions to ask the personnel in their areas of jurisdiction who are tasked with recovering submerged corpses to assess their current practices and protocols in regard to preserving evidence by documenting and bagging corpses prior to moving them and searching the immediate areas under and surrounding the corpse for additional evidence.

The recovery and documentation of corpses from aquatic environments can be a complex and challenging process. Personnel normally tasked with body recovery on land may not be able to access the body if they are not forensic divers or swift water/ice technicians. Currents, low or zero visibility, frigid temperatures, entanglements, debris, confined space, contaminants, and deep water all increase safety risks, loss of evidence, and possibilities of damage to corpses. As these risks increase, so does the risk of evidence loss and the need for proper handling of corpses.

When a body is recovered from still or moving water environments, it is imperative to ensure the integrity of the corpse itself as well as any type of evidence associated with it. Commonly, once a corpse is identified in water, efforts are made to transport it to a boat or shore. Once it reaches those locations, it is removed from the water and placed in a body bag. This procedure may result in evidence loss and contamination. Loss may include clothing or objects on the body, trace evidence, necrophagous and necrophilous arthropods, and even tissue or body parts, depending on the stage of decomposition. For these reasons, it has been advocated that bodies in water are documented and bagged prior to transport to the surface. Unfortunately, there are no specific universally accepted guidelines or standards to instruct investigators and responders in how to recover corpses in water that will maximize evidence preservation.

We present a research project to illustrate differences between bodies recovered from aquatic environments when they are placed in a body bag while they are still in the water and bodies that are bagged after removal from the water. Eight clothed swine carcasses and adult water-rescue mannequins with objects in pockets and trace evidence (fluorescent fabrics and paint residue for an easier visualization) placed on the clothing and skin were placed in a standing fresh water environment in New Jersey and allowed to decompose until floating decay was reached; four carcasses and four mannequins were recovered during the fresh stage (24h after placement), while the remaining ones were recovered during the floating/bloat stage. Two groups of public safety divers were tasked with recovering the carcasses and the mannequins (two carcasses and two mannequins per group per stage). One group of divers was provided with underwater documentation and bagging protocols, equipment, and training. The other group was just told to bring the carcasses to the shore without using body bags and with no instructions. The carcasses and mannequins were then sampled to determine the degree of evidence preservation and loss. Documentation of the "corpse" prior to transport was also evaluated. The objective of this preliminary research was to produce a report that may lead to more formal standards for the recovery of corpses from water.

Decomposition; Aquatic Environment; Body Bags

I125   **Developing an Introductory Forensic Biology Graduate Course: Preparing Future Forensic Biologists**

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WITHDRAWN

I126   Standards Development Activities in Medicolegal Death Investigation and Forensic Pathology

J. Keith Pinckard, Travis County Medical Examiner's Office, Austin, TX*

Learning Overview: After attending this presentation, attendees will have a better understanding of the documentary standards development process and the current state of affairs of documentary standards within the fields of forensic pathology and death investigation.

Impact Statement: This presentation will impact the forensic science community by creating greater awareness of how standards are developed and the players involved in standards development in the medicolegal death investigation realm, as well as their implementation.

The Organization of Scientific Area Committees for Forensic Science (OSAC) was created to strengthen the nation's use of forensic science "by facilitating the development of technically sound standards and guidelines and encouraging their use throughout the forensic science community." OSAC, however, is not a Standards Development Organization (SDO) and thus cannot publish documentary standards on its own. OSAC has many discipline-specific subcommittees that conceive of and draft standards that would be useful to the forensic science community. These draft standards are then submitted to appropriate SDOs that put them through defined, consensus-based processes to vet and publish them. Once a standard has been published by an SDO, OSAC then evaluates it for suitability to be placed on the OSAC Registry, which is a centralized repository for high-quality, technically sound standards.

Standards development in the field of forensic pathology is unique in that it is the practice of medicine and thus, consensus-based, documentary standards are not appropriate. Professional practice standards developed by a professional practice organization (such as the National Association of Medical Examiners) would be the appropriate venue for standards development for the medical practice of forensic pathology. The history of how OSAC has navigated this issue will be discussed in terms of how the scope of standards to be conceived, drafted, and developed has been delineated by the OSAC Medicolegal Death Investigation (MDI) Subcommittee.

During this presentation, the state of standards development within medicolegal death investigation and forensic pathology will be discussed to include the current status of each of the individual standards within the standards development process. These include draft standards being developed by the OSAC MDI Subcommittee, draft standards that have been sent to an SDO for further development, and SDO-published standards being considered for placement on the OSAC Registry. In addition, priorities for future standards development will be discussed.

Standards; Forensic Science; Medicolegal Death Investigation

I127 Systematized International Capacity Development for Forensic Pathology in Low-Resource Jurisdictions: A Proposed Model

Alfredo Eugene Walker*, University of Ottawa, Ottawa, ON, Canada

Learning Overview: After attending this presentation, attendees will be able to: (1) describe the Medicolegal Death Investigation International Community of Practice (MLDI ICOP) and its purpose and functions; (2) discuss a blueprint for international capacity-building in forensic pathology using the MLDI ICOP as a launch pad; and (3) describe the development of postgraduate training in anatomical pathology in the Caribbean and how an opportunity arose out of adversity and resulted in the Jamaican model of training in forensic pathology.

Impact Statement: This presentation will impact the forensic science community by sensitizing international practitioners as to the major issue of lack of adequate forensic pathologists that exists internationally but predominantly affects low-income countries and negatively impacts the criminal justice system in these jurisdictions, while simultaneously providing a model for a workable solution that can be implemented with appropriate stakeholder engagement.

Most international jurisdictions (inclusive of the United States and Canada) do not have enough Forensic Pathologists (FPs) for their medicolegal death investigation workload. The United States has <50% of the required forensic pathologists and the National Association of Medical Examiners (NAME) has embarked on medical student education and recruitment drive to remedy this. The salary gap between FPs and Anatomical Pathologists (APs) in the United States is mostly responsible for chronic inability to recruit and retain FPs.

Some jurisdictions (esp. low-resource countries) do not have postgraduate training programs in either anatomical pathology and/or forensic pathology and so those so inclined have to seek training opportunities overseas in better-resourced regions (e.g., United Kingdom, Australia, United States, Canada, etc.). However, numerous issues with IMGs being able to pursue postgrad medical training overseas exist. Navigating the respective medical qualifying examinations in the United States , Canada and the United Kingdom are prohibitive in this regard for a combination of financial and other reasons.

This presentation will cover a proposed two-pronged approach that will aim to alleviate this shortfall in both the short and long terms by utilizing the training, examining and certifying bodies that are currently in existence internationally and bringing their collective experience in this area under one internationally accredited training, examining, and certifying body that can provided a recognized postgraduate qualification that can be used in low-resourced jurisdictions but would be equal in international standard.

The Short Term Objectives will consist of:

- Targeted training of autopsy pathologists in forensic pathology.

- Development and delivery of accredited online didactic lecture series in forensic pathology - International Forensic Pathology Fellowship Programme (IFPFP).

- Facilitation of Clinical Observerships and Elective Attachments (United States, Canada, United Kingdom, Australia, South Africa)

- Candidates prepared to challenge the United Kingdom exams in FP.

- ØFRCPath (RCPath)

- Long Term

- Creation of an accredited, internationally recognized training, examination, and certification body in forensic pathology, with input academics drawn from the existing international examining bodies (RCPath UK, ABPath, RCPSC, RCPA, etc.).

Capacity Development; Forensic Pathology; Training

I128   2023 State Legislative Encroachments Against Forensic Pathology Professionalism and Independence: Harbingers of Future Threats and Opportunity for Strategic Planning and Action

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Learning Overview: After attending this presentation, attendees will understand the importance of monitoring and evaluating state legislative proposals through a prioritized, anticipatory process. Vigilance in monitoring is warranted due to the recent uptick in legislative proposals and passed bills that dictate processes and outcomes in Medicolegal Death Investigations (MDIs). The information obtained by attendees will enable proactive and informed responses to proposed legislation that encroaches on the independence of forensic pathology and MDIs.

Impact Statement: This presentation will impact the forensic science community by exposing threats to the independence and neutrality of MDIs and the medical practice of forensic pathology and by encouraging forward-leaning responses to ward off such threats.

MDIs are performed by coroner or medical examiner offices to explain the occurrence of unexpected, suspicious, and violent deaths and to diagnose threats and offer preventative guidance for avoidance of premature death in the living. Forensic pathologists are essential to MDI as they employ specialized medical training to reach medical diagnosis relating to cause and manner of death. Forensic pathologists use external examination, medical records, pathology, histology, toxicology, and a surgical procedure known as the autopsy to determine the presence or absence of diseases, poisons, or external factors that caused death. The Centers for Disease Control and Prevention Vital Statistics System, which provides foundational, baseline data for innumerable federal, state, and local entities, relies universally on accurate and objective autopsy, toxicology, and related findings as to cause and manner of death.

In 2023, the independence of forensic pathology in the context of MDI was directly attacked by the introduction of various state legislative proposals and bills passed into law. These legislative proposals and laws erode the ability of forensic pathologists to maintain impartiality and objectivity, mandate particular findings on death certificates, and allow non-physicians to perform autopsies. The cause for alarm is heightened by the apparent lack of scientific and medical underpinnings in the bills and laws.

In Texas, HB 6 and companion SB 645 mandated that death certificates include the term "Fentanyl Poisoning" if a toxicology examination reveals fentanyl or fentanyl-related substances in previously identified lethal amounts and autopsy results are consistent with opioid overdose as the cause of death. Illinois SB 1086 mandated the inclusion of "Fentanyl Poisoning" on death certificates under similar circumstances. These bills undermine the independence of forensic pathologists to consider all the facts and circumstances in a case and additionally assumes there is a universally agreed-upon lethal concentration of fentanyl, which there is not. On June 14, 2023, Texas HB 6 was signed into law.

Indiana's HB 1286, another bill relating to the fentanyl crisis, mandated testing body fluids for any amount of xylazine, including trace amounts, if the coroner reasonably believes that the cause of death was due to overdose. Legislative proposals such as HB 1286 would increase the workload and costs for state health departments and toxicology laboratories, as well as compromising forensic pathologist independence. HB 1286 was signed into law on April 20, 2023.

In Oregon, SB 953 was engrossed by the Senate on April 17, 2023, after a March introduction, with a heading that reads, in pertinent part: "Provides that a physician assistant or nurse practitioner may be appointed as county medical examiner or assistant county medical examiner and may perform autopsies in deaths requiring investigation. Directs Chief Medical Examiner to provide training and supervision to physician assistants and nurse practitioners in performance of their duties." While the language of the bill was softened slightly in the amendment process, the bill was recommended for passage in committee and went to fiscal review and public comment before dying at adjournment of legislature.

Vigilant monitoring of state-specific legislation is essential to remaining in front of changes so that temporally effective, scientifically informed responses can be formulated. Attendees will be provided with governmental and open-source electronic resources that can be utilized to track bills and legislative movement in their respective states.

Conclusion: Laws and regulations that intrude into the independence of the medical diagnosis of death threatens the independence of medicine writ large. Heightened vigilance is called for among all those working in forensic science, and particularly in MDI systems, to monitor, challenge, and resist evolving legislative activity that threatens to encroach and erode the independence of forensic pathology diagnosis and conclusions.

Legislation; Fentanyl Toxicity; Xylazine

I129   The 2022 NFLIS Medical Examiner and Coroner (MEC) Survey Findings

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Learning Overview: After attending this presentation, attendees will understand the findings from the Drug Enforcement Administration's (DEA's) National Forensic Laboratory Information System (NFLIS) 2022 MEC office survey.

Impact Statement: This presentation will impact the forensic science community by providing updated information on toxicology-related death investigation practices of MECs across the United States who participated in the survey.

The National Forensic Laboratory Information System (NFLIS) is a program of the DEA, Diversion Control Division. NFLIS has systematically collected drug identification results from case submission to federal, state, and local forensic laboratories that analyze controlled and non-controlled substances secured in law enforcement operations for over 25 years and more recently expanded to include data from both toxicology laboratories and MEC offices. As part of the expansion, profiles of all MEC offices were developed to include information about those offices and these profiles are further refined and updated through completion of periodic surveys. This presentation will provide information from the findings of the 2022 NFLIS-MEC survey of MECs conducted by the DEA to aid in these updates. This is the second time a survey was administered on behalf of NFLIS to all the MECs in the United States. This 2022 survey covered MEC office data from the 2021 calendar year and will help inform DEA as they expand the NFLIS-MEC component while providing important information about the MEC community regarding office practices, resources, and demographics.

The presentation will present information from the 1,606 MECs across the nation who responded to the survey (45% of whom served small jurisdictions, 42% served medium jurisdictions, and 13% served large jurisdictions), including providing information on who these respondents were. It will present information about the MEC community, including who has an internal toxicology laboratory and who uses off-site laboratories, what the responsibilities are of varying types of offices, such as the over 95% who determine cause and manner of death, 88% who perform scene investigations, with only 74% who order toxicology testing, along with information on computerized case management systems, or lack thereof, among MECs and how this compares regionally and according to population size.

This presentation will also provide information related to investigations of drug-related deaths to include information about variations in practices such as use of toxicology screening tools, testing practices of the 62% of responding MECs who indicated the testing they request varies based on case circumstances, the frequency of both specific toxicology testing and quantitation, and death certification practices and timing. MECs reported almost 1.5 million cases for the reference year; this caseload information will be reviewed, both for overall caseloads and for overdose caseloads, with manners of death for these over 100,000 overdose cases examined, both when compared by region and population.

Coroner; Medical Examiner; NFLIS

I130   Preserving the Silent Witnesses: Ensuring Long-Term Maintenance of Insect Evidence in Forensic Investigations

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Learning Overview: After attending this presentation, attendees will have an understanding of the parallels between entomological collections in museums and collections of forensic insect evidence placed in storage. Additionally, the relevance of museum expertise in maintaining and safeguarding insect samples to facilitate the reanalysis of entomological evidence will be discussed.

Impact Statement: This presentation will impact the forensic science community by raising awareness about the crucial aspect of long-term maintenance of insect evidence in forensic investigations. By exploring past challenges and successful strategies, we hope to equip forensic professionals with the knowledge to preserve and utilize insect samples effectively in future cases, contributing to accurate and just resolutions.

Insects play a pivotal role in forensic investigations, offering crucial insights into crime scene dynamics, time-since-death estimation, and other vital parameters related to criminal cases. Numerous papers and book chapters have detailed methods for collecting and preserving insects during investigations, both at the crime scene and during autopsy procedures. After collection, it is recommended that insects be preserved in ethanol 70/80% (immature specimens) or dry (adult specimens). Preservation is a mechanism to facilitate the morphological analyses of insects for the identification of the species and the estimation of larval age. However, they can also be preserved in ethanol 100% or in the freezer at -20°C, allowing for molecular and toxicological analyses, respectively. Following the collection and correct preservation of specimens, forensic entomology best practice suggests performing the analyses soon after or within a few weeks to avoid alterations in their morphology (especially larval length) that would affect the outcome of the analyses.

However, a significant gap persists in the awareness of untrained personnel on how to curate preserved insect evidence, held as police evidence, over the long term, which is critical as these invaluable specimens may serve as crucial evidence during cold case reviews. This is of particular importance as Forensic Entomologists (FE) are often asked to analyze old cases where, although insect material was collected, it was not considered in the initial investigation. Quite often an FE may also be asked to reanalyze cases in the light of new forensic entomology research, analyze current cases in which bodies are found associated with poorly preserved insect specimens (highly decomposed or dry due to the time elapsed since death or condition of the storage environment), and provide expertise in paleo-entomology and archeoentomology cases.

This presentation will address two main topics. The first topic will be to discuss the techniques used in entomology museum curation designed to preserve insect specimens over the long term. The second topic will discuss the current tools to facilitate the reanalysis of poorly preserved and poorly maintained insect evidence.

The presentation will provide insights into cases where inadequate maintenance of insect samples has proved challenging and offer practical tips on extracting information from poorly maintained insect specimens over extended periods.

Entomology; Preservation; Crime Scene Investigation

I131   The Toxic Workplace in Forensics

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Learning Overview: The goal of this presentation is to provide a personality-based and organizational framework for understanding the roots, propagation, and effects of workplace toxicity as it applies to the forensic community. These effects impair workplace productivity, decrease the retention of quality personnel, and cripple personal and organizational morale. It is essential that individuals not only recognize the features of toxicity in the workplace but understand the required interventions to mitigate the detrimental effects of these negative traits and possibly resolve them completely. The ultimate goal is to apply effective interventions that stop the cycle of toxic leaders breeding toxic cultures, and toxic cultures developing more toxic leaders.

Impact Statement: This presentation will impact the forensic community by defining the relevant terminology, identifying toxic characteristics, demonstrating the impact of the toxicity in the forensic community, and provide a framework to apply best practices on a personal and organizational level. The purpose of this presentation is not to "call out" individual offenders or "toxic" offices, but to educate the audience as to the traits and characteristics of a toxic work environment and provide tools to maintain personal, professional, and organizational resiliency.

The "toxic workplace" is a term used to describe an unhealthy, stressful work environment that creates and propagates hostile working and interpersonal conditions. This "counterproductive leadership," as the Army defines it, is a widespread problem across many industries and sectors that manifests in many different forms. These hostile personalities and workplace conditions manifest themselves in different ways, resulting in employees suffering from a range of physical and mental health problems, poor workplace productivity, and high employee turnover. Toxic leadership is arguably the largest contributor to the lack of workplace satisfaction and employee turnover. This phenomenon greatly impacts the forensic community by contributing to the already critical personnel shortages throughout the field. Toxic leaders and institutions directly hurt recruitment of talented individuals and the retention of qualified personnel within the field of forensics.

There are several effective models that are useful to understanding toxicity from a personality-based approach and how these personality traits can effectively destroy an organization. Understanding the "Big 5" personality traits of extroversion, agreeableness, openness, conscientiousness, and neuroticism are critical to understanding interpersonal relationships, in addition to how the extremes of any of these traits can contribute to a toxic environment. The "Dark Triad" of personality (narcissism, psychopathy, and Machiavellianism) are defined and discussed in a forensic context. The terminology of the toxic workplace will be defined and discussed such as bullying, gaslighting, narcissism, and harassment. The six main characteristics of toxic leadership will also be discussed: autocratic, narcissistic, manipulative, intimidating, overly competitive, and discriminatory. The role of toxic leaders will be discussed in the context of the "toxic triangle," which also includes susceptible followers and a conducing environment. Survival in toxic environments is incredibly difficult as your choices are to conform with this bad behavior, collude with it to get promoted, or leave the environment completely, which is often ruinous to one's career.

Properly understanding the characteristics of workplace toxicity is critical to developing interventions designed to mitigate the negative effects of workplace toxicity on both a personal and organizational level. Personal well-being overflows into professional well-being so building resiliency in one's personal life is one of the best practices to deal with a toxic workplace. Organizational resilience is a concept that builds organizational trust and makes the organization less likely to crumble and reach mission failure due to toxic forces. Resilient organizations are prepared, adaptable, collaborative, trustworthy, and responsible. Ideally, these characteristics are spread through an organization in both a top-down and bottom-up distribution. Applying these concepts in the forensic workplace setting will improve workplace satisfaction, productivity, and resiliency. Personal and professional well-being and job satisfaction are critical to personnel retention and recruitment in the forensic community.

Behavioral Science; Bullying; Personality Disorder

I132   But What Does It Mean? A Rationale for a Standardized Means of Death Classification System

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Learning Overview: After attending this presentation, attendees will understand the definition of "means of death" and the need for a standardized classification system around "means of death" among the medical examiner and coroner community.

Impact Statement: This presentation will impact the forensic science community by highlighting the challenges with current systems in informing stakeholders and the general public on population-based mortality statistics in the country.

One of the primary roles of a medical examiner and/or coroner is to help the public understand how and why a person has died. Aggregate data is valuable for a community and public health officials when evaluating the health of a county, state, and nation. The medicolegal community has standardized definitions for cause of death and manner of death as well as a standardized population-based tool (the death certificate) to report that information on a national level. In terms of interoperability across county and state lines, that is often where the story stops. However, the level of information and detail collected by individual offices (the primary data) can better inform the general public and help establish mortality trends to inform public health initiatives. This study will review the practices of selected offices across the country with readily accessible annual reports available online and highlight the challenges with interpretation of the data presented within.

An internet search was conducted to identify medical examiner/coroner offices with annual reports available for review on-line. Offices were selected from around the United States.[1-8] The annual reports were analyzed to record how information was aggregated to inform the public of incidences, proportions, and trends of different types of death. Within major death classifications including causes of death and manners of death, offices varied in the way information was aggregated and presented within each group, ranging from the use of "causes," "mechanisms," "circumstances," "means," "submanner," "case code," and "method.". One office did not designate a heading for the categories presented. It was clear, however, that the offices were intending to highlight the same information but did not have a standardized system of doing so. For example, within the "accidental deaths," all offices indicated several deaths due to "drugs," but each had a different way of presenting this information. Whereas if a standardized "means of death" classification system was available, individuals interested in aggregate data across county/state lines would be able to quickly analyze reports from various offices and make informed interpretations.

The definition of the word "means" is an action or system by which a result is brought about or to achieve an end. "Means of death" represents a broad classification system than encompasses deaths from all manners and a variety of causes. For example, a death with a means of "drug" could be queried to identify all drug-related deaths regardless of manner; the same with "gun," "fall," etc. The ability to quickly analyze drug-related deaths from all manners gives a comprehensive view of the true burden of the drug epidemic rather than just those stratified by cause (which is fraught with inconsistencies) and/or manner (which is dependent on a multitude of factors). There is a push for data standardization, modernization, and interoperability at the national level. If a system for categorizing deaths in comprehensive "buckets" could be utilized by all offices, then information could be queried, validated, and reported in an easy-to-interpret manner to help inform public health policy and the overall health of our nation. Future work will propose a "means of death" classification schema and a call for collaboration to refine and adopt the "means of death" system.

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Means of Death; Data Standardization; Interoperability

I133   Plastic Bag Suffocation: A Case Series and an Effective Protocol Proposal

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Learning Overview: After attending this presentation, attendees will better understand the management of plastic bag suffocation. During the past 13 years, we have dealt with an exceptionally large number of plastic bag suffocations, especially suicides, which led us to design a protocol for these kinds of situations, since they are quite rare in forensic practice. Our aim is to present the major epidemiological, microscopical, and psychiatric findings of our experience and ultimately propose a protocol to be applied when plastic bags are used as a mean for asphyxia.

Impact Statement: This presentation will impact the forensic science community by providing an effective protocol based on our experience: an accurate crime scene investigation, followed by gross examination of the affected systems and organs, together with histological and immunohistochemical analyses of the damaged tissues are mandatory.

Plastic bag suffocation is an uncommon cause of death that is rarely encountered in forensic practice and can be suicidal, homicidal, or accidental, where the latter is mainly observed in the pediatric population.[1,2] Hence, it may be a challenge for the forensic pathologist to recognize and collect pieces of evidence that can suggest the nature of the event. A thorough comparison between the ante- and postmortem data by investigating the compatibility of the forensic findings with the hypothesized dynamics is crucial to determine whether the act was self-inflicted or not. Additionally, the identification of signs or patterns related to the psychiatric dimension of suicide can guide the medical examiner in the analysis of the event.

During the past 13 years (2010-2023), 13 cases in which the cause of death was plastic bag suffocation were registered. In each case, crime scene investigation, together with the study of both the circumstantial evidence and the medical charts when available, followed by a full complete autopsy and a routine microscopic histological examination were performed. Eleven out of the 13 cases were ultimately recognized as suicides, while one, which was initially marked as a suicide, was indeed confirmed to be a homicide. Only one case was filed as accidental.

At the autoptic examination, upper and lower airways were removed *en bloc* together with the heart and major blood vessels to highlight the characteristic signs of asphyxia (i.e., petechial hemorrhages on the pleural and pericardial surfaces, pulmonary emphysema, and widespread visceral congestion).

Microscopically, all the collected lung specimens showed acute congestive hyperemia and focal hemorrhage adjacent to overextended pulmonary alveoli, which is known as hemorrhagic-dysoric syndrome.[3] Moreover, immunohistochemistry was performed to analyze the pattern of expression of asphyxia-related markers in pulmonary tissue (i.e., surfactant protein A (SP-A), hypoxia induced factor 1-alpha (HIF-1α), Oxygen-regulated protein 150 (ORP 150), CD68 and cyclooxygenase-2 (COX-2)).[4-6] This was especially useful in the homicide investigation, as multiple phases of aggression were recognized where the plastic bag suffocation was the ultimate cause of death as immunohistochemical signs of vital breathing were recognized.

From a psychiatric point of view, only five of them had a clinical history of psychiatric disorder, while two were terminally ill patients in treatment with anti-anxiety medication. Half of them had signs of previous suicide attempts, which were carried out shortly before the final act. Interestingly, 3 out of 13 cases occurred in an extremely restricted period (1 month), within the same geographical area. All three cases were covered by local newspapers and the sites of the events were only a few miles from one another. This is known as the Werther effect, (i.e., media-induced imitation effects of suicidal behavior where publicized suicide acts as a trigger for the next suicide by a susceptible or suggestible person, resulting in an actual suicide cluster).[7]

Overall, the study population showed various epidemiological backgrounds and temporal distribution of the plastic bag suffocation phenomenon, where the number of cases was exceptionally high when compared to the existing literature, considering the rarity of occurrence. The use of a standardized protocol to deal with this uncommon occurrence is crucial to achieve accurate reconstruction of the event.

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Asphyxiation; Histological; Plastics

I134   A Case of Ductus Venosus Agenesis (DVA) in a Stillborn Baby: The Postmortem Computed Tomography (CT) Scan and Autopsy Findings

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Learning Overview: After attending this presentation, attendees will understand the importance of a prenatal diagnosis of DVA and of the possible conditions associated.

Impact Statement: This presentation will impact the forensic science community by showing the case of a stillborn baby with a rare malformation of the fetal venous circulation and presenting a particular autopsy section that must be realized in order to best highlight and describe this anomaly. Deepening these aspects from a medicolegal point of view could also be extremely useful in cases of professional liability.

The DVA is a rare malformation of the fetal circulation with a variable prognosis that depends partly on the presence of associated conditions (such as cardiac or extracardiac abnormalities). The ductus venosus connects the portal and embryonic venous circulation into the inferior vena cava and has a crucial role in fetal circulation, allowing oxygenated blood from the placenta to bypass the liver. When it is absent, the umbilical vein connection to the venous system may be extrahepatic or intrahepatic, via the portal venous system.[1] A systematic evaluation of the ductus venosus in the late first trimester routine ultrasonography has become part of daily clinical practice that has led to the increased number of DVA cases published in the literature.[2] However, this is still a rare condition with a reported low prevalence between 1 in 2,532 and 1 in 556 fetuses.[3,4]

In the case presented, a 33-year-old woman, at 40 (+5) weeks of gestation, entered the emergency room with: rupture of the membranes, drainage of dark material, and pathological fetal beats on the cardiotocographic tracing. An emergency cesarean section was performed, but unfortunately the baby was born dead.

The pregnancy had not been followed up consistently. An ultrasound in the 25th week had shown marked hydrops fetalis and polyhydramnios. All the tests for possible infectious diseases were negative. The parents refused amniocentesis and other investigations. An autopsy was requested to investigate the cause of death.

During the external examination, the following was observed: considerable facial congestion with widespread petechiae, a low auricular implant, and a batracian abdomen. The actual autoptic examination revealed hemorrhagic petechiae at the subcutaneous and muscular tissues of the head, thorax, and abdomen, some asymmetries of the anatomical structures (for example, at the level of the sternocleidomastoid muscles and the diameter of the jugular veins), lymph nodes in the context of the chest presenting an increased volume, effusion of reddish liquid in the pleural cavities, collapsed lungs, and petechiae on the surface of the heart and the lungs. A fundamental element that emerged was finally represented by the finding of agenesis of the venous duct of Arantius. The umbilical circulation was separated from the portal circulation.

The following histopathological analysis of the placenta showed, at the level of the chorionic villi, edema and numerous erythroblast and fetal nucleated red blood cells in the capillaries. These specific findings are often associated with anemia and hydrops fetalis of non-immune (cardiological, genetic, hematological, infectious) or immune causes (Rh incompatibility or other minor types of incompatibility).[5] Serial pregnancy checks would have allowed an adequate neonatal counseling, even more important in such cases.

The hope of this case report is to stimulate physicians and pathologists to deepen the studies on this rare condition. In fact, it would be desirable that in these particular cases the anatomical dissection was performed by pathologists who are experts in fetal pathologies.

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Ductus Venosus Agenesia; Fetal Venous Malformations; Autopsy on a Stillborn Baby

I135   Minimally Invasive Postmortem Autopsies Using CT-Guided Biopsies in Suspected Cases of Pneumonia: A Feasibility Study

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Learning Overview: After attending this presentation, attendees will understand how bacterial cultures obtained using Postmortem Computed Tomography (PMCT) -guided biopsies can be utilized in cases of suspected bacterial pneumonia as a surrogate to full autopsy. A detailed protocol for percutaneous PMCT-guided biopsies will be described, as well as sensitivity/specificity analyses using data from an ongoing validation study, allowing for comparative consideration of this novel, cost- and time-efficient approach to identify Cause Of Death (COD).

Impact Statement: This presentation will impact the forensic science community by sharing novel data, including positive predictive value and efficiency/cost-savings projections that support PMCT biopsies as an accurate and efficient alternative to traditional autopsy in cases of suspected bacterial pneumonia. This less-invasive method would benefit populations averse to autopsy based on religious and cultural grounds.

The improvement in efficiency that this novel protocol can provide is significant given a current need for upward of 1,000 forensic pathologists to work full-time and, according to the National Academy of Sciences Report, only 400-500 forensic pathologists are currently practicing in the United States.[1-4] Of the studies that have successfully implemented PMCT-guided biopsy for determining cause of death, few have investigated its' application to suspected bacterial pneumonia.[5-7] This is an ongoing, blinded validation study comparing PMCT-guided biopsies, incorporating both bacterial culture and histologic review, to traditional full autopsies with histologic lung sections and bacterial cultures taken at the time of autopsy.

To date, eight cases have been entered into the study out of a proposed 30. Cases identified as suspicious for pneumonia by initial review of case history and PMCT are considered for the study; if autopsy and postmortem cultures are included in the initial, blinded workup by the triaging pathologist, the case is included in the study. Within a 20-minute window prior to a blinded, traditional full-autopsy, 20-gauge CT-guided needle core biopsies are procured using sterile technique from each lung with a portion of samples sent to bacterial culture and the remainder submitted for histologic sections. Needle biopsies target areas of highest suspicion of pneumonia; a commiserate sample from the contralateral lung is taken to act as an internal control. A COD is derived using the case history and PMCT-guided biopsy sample histology and culture findings, then compared to the official COD, entered by the blinded pathologist performing a traditional full autopsy with correspondent tissue histology and culture analysis. The sensitivity and specificity of the novel PMCT-guided biopsy protocol are then calculated, using the results of traditional full autopsy as the gold-standard reference. Out of eight cases, eight have had concordant positive and/or negative results—both in terms of histologic findings and bacterial cultures—between traditional full autopsy and PCMT-guided biopsy techniques. The sterile technique of PMCT-guided biopsies avails culture results with fewer artifactual-contaminating organisms, averaging 1–2 organisms isolated, compared to the 2–3 isolated by traditional postmortem culture.

To date, our study clearly supports PMCT-guided biopsies as a sensitive, specific, efficient, cost-effective, and histologically-accurate means of diagnosis in suspected cases of COD due to bacterial pneumonia. This novel approach to medicolegal death investigation can be utilized to combat the forensic pathologist shortage and to address religious and cultural objections to conventional autopsy.

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Postmortem CT; Biopsy; Bacterial Pneumonia

I136   An Analysis of Autopsy-Radiology Discordance in Cases of Fatal Blunt Force Injury

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Learning Overview: After attending this presentation, attendees will learn about the interpretation of Postmortem Computed Tomography (PMCT) in cases of fatal blunt trauma.

Impact Statement: PMCT is increasingly used by forensic pathologists in the death investigation setting to augment traditional methods of determining the cause of death. Blunt force injuries are frequently well-depicted on PMCT, which may enable the forensic pathologist to forgo a full autopsy in non-suspicious cases. This presentation will impact the forensic science community by informing attendees that understanding the relative strengths and weaknesses of autopsy and PMCT in blunt force injury cases is critical for realizing the potential of PMCT to maintain, or even increase, accuracy while reducing the pathologist's workload.

Background: A previous double-blinded study compared the findings at autopsy, reported by a forensic pathologist, to the findings from whole body PMCT, reported by a radiologist, for 167 cases of fatal blunt force trauma in adults and 67 cases of pediatric trauma.[1] That study concluded that autopsy and PMCT are both imperfect at detecting injuries, with similar overall sensitivities. Here, we report a detailed analysis of only the discordant findings from the trauma cohorts of the previous study (i.e., findings observed at autopsy but not PMCT, and findings observed at PMCT but not autopsy).

Methods: The whole body PMCT study for each case was reviewed by a second radiologist, who rated each discordant finding as: (1) CT true miss (injury found at Autopsy is not visible in CT); (2) CT false miss (injury found at Autopsy is visible in CT but not recorded); (3) CT true find (injury missed at Autopsy is visible in CT); (4) CT false find (injury not found at Autopsy is not visible in CT); (5) Inconclusive (injury is ambiguous); or (6) Terminology Issue (same injury was found at both Autopsy and Radiology but described differently).

Results: A total of 2,830 discordant findings were reviewed. In 2,001 instances of autopsy-radiology discordance (71%), the second radiologist agreed with the first radiologist's interpretation, comprising 696 CT True Misses (i.e., findings reported at autopsy that were not detectable at CT by either radiologist) and 1,305 CT True Finds (findings missed at autopsy but detected at CT by both radiologists). In 521 instances of autopsy-radiology discordance (18%), the second radiologist disagreed with the previous radiologist's interpretation, comprising 402 CT False Misses (findings reported at autopsy, missed by the previous radiologist, but seen by the second radiologist) and 119 CT False Finds (findings reported by the first radiologist, but not reported at autopsy and not seen by the second radiologist). The second radiologist judged 225 previous instances of discordance (8%) to be inconclusive (i.e., the finding was not sufficiently clear to assign it to another category), and 83 of the previous discordant findings (3%) were judged to be a disagreement in the terminology used, rather than true discordance. The most common types of injuries that resulted in CT True Misses (findings seen at autopsy but not detected at PMCT by either radiologist) were external injuries (abrasions, contusions, and lacerations) and lacerations of organs (brain, heart, lung, and other internal organs). The most common type of injury that resulted in CT True Finds (findings missed at autopsy but seen at PMCT by both radiologists) were fractures. More than 25% of all discordant findings analyzed were fractures missed at autopsy but found at PMCT by both radiologists, while fractures deemed as CT False Finds represented <1% of discordant findings. The most common inconclusive findings involved internal organ injuries (hematomas and lacerations), followed by fractures. Fractures were also the most common injury classified as a "terminology issue."

Conclusions: Overall, the agreement between the first and second radiologist was 71%. Of 2,830 discordant findings, 46% were findings missed at autopsy, but detected by both radiologists at PMCT, while 25% were findings observed at autopsy and not detected by either radiologist at PMCT. Inter-rater agreement cannot be similarly assessed for autopsy. This analysis confirms that in fatal blunt force trauma cases, PMCT detects many fractures that would be otherwise missed if only an autopsy were performed, while radiologists rarely "overcall" fractures. External injuries were the most common type of injury confirmed to be undetectable at PMCT, highlighting the importance of the external exam in cases that will not receive an autopsy.

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Autopsy; Postmortem CT; Accuracy

J1   A Pathologically Impulsive Reaction and Instantaneous Perpetration of a Double Crime With a Firearm

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WITHDRAWN

J2   A Polygraph Examination of a Rape and Murder Suspect: A Case Study

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Learning Overview: This presentation will utilize data obtained through forensic polygraph examination at a crime lab to exhibit the importance of polygraph examination in a forensic setting. After attending this presentation, attendees will be in a position to understand the importance of polygraph examination when no other forensic evidence is available.

Impact Statement: This presentation will impact the forensic science community by sharing the results of a polygraph examination. Forensic program managers and top management will learn that if there is no other forensic evidence available, polygraph examination is still a useful investigative tool to uncover the truth and give an alternative perspective for the investigation of criminal cases.

In February 2018, a minor 6-year-old girl was missing from her home after 5:00 p.m. The family and relatives searched but did not find the girl. They initiated a complaint to police regarding the missing minor girl. After a couple of days, her dead body was found in a dirty pond. There was no other forensic evidence available except one suspect, a relative who denied any involvement. The objective of the polygraph examination was to determine the veracity of the suspect's statement regarding his involvement.

After a briefing from the case investigation officer, the Forensic Assessment Interview Technique (FAINT) and Integrated Zone Comparison Technique (IZCT) were used during the polygraph examination in the present study.[1,2] After collecting three polygraph charts using an LX 5000 instrument with software version 11.8.6, data was analyzed by using the computerized Academy for Scientific Investigation Training (ASIT) Algorithm for polygraph charts analysis (IZCT) and Weighted Scoring Systems for FAINT, respectively.[3,4]

During the initial interview, proper consent was obtained from the suspect. During the examination, the suspect denied the allegation that he raped and murdered the young girl. The examination results were analyzed as deceptive. Subsequently, he was interrogated, which led to his confession of rape, murder, and dumping her dead body in nearby dirty pond. He also admitted that after the rape and murder, he washed the dead body of the victim. The case went to trial in the Anti-Terrorism Court of Multan and the polygraph results were used as a corroborative piece of evidence. He was convicted and in addition to receiving the death penalty, he also received lifetime imprisonment and Rs. 2.5 million in fines.

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Rape and Murder; IZCT; FAINT

J3　　　Internet Gaming Disorder: An Invisible Addiction With Real Damage

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Learning Overview: This presentation seeks to illustrate a study on the correlation between emotional regulation and Internet Gaming Disorder (IGD) and how it impairs the subject's ability to self-control and the related forensic consequences.

Impact Statement: This presentation will impact the forensic science community by presenting a possible approach in framing immutability in subjects suffering from gaming addiction, comparing impaired emotional regulation with the ones occurring in other forms of addiction.

Introduction: Addiction in not just about substance use, but also regards other addictive problematic behaviors, such as gaming, smart phone abuse, and gambling. IGD consists of a pattern of behaviors characterized by the need to play, over-use, abstinence, and addiction, with negative effects on individuals' health and social interactions.[1] Clinical research shows that despite the existence of different kinds of addiction, they share similar etiopathogenetic mechanisms of emotional dysregulation.[2,3] In this study, we explore the possible correlation between emotional regulation styles and addiction in a non-clinical sample.

Methods: The target sample for this study were individuals scheduled into the University of Naples Federico II mailing lists (students, doctoral candidates, resident physicians, researchers, associate professors, full professors, and administrative staff). Two validated questionnaires were used: MULTICAGE CAD-4 and the Emotion Regulation Scale - ERI.[4,5] MULTICAGE CAD-4 is a screening tool for various kinds of addictions; the Emotion Regulation Scale explores three styles of emotional regulation: integrative, suppressive, and dysregulated. Both questionnaires were completed online by a total of 1,655 subjects.

Results: The statistical analysis showed that emotional dysregulation is related to a high risk of developing addictive behaviors. In IGD and in other addictions related to internet and smart phone usage, a suppressive regulation style of negative emotions also implies a high risk of developing addictive behaviors.

Conclusion: We found the presence of a correlation between emotional dysregulation and IGD. This dysregulation may lead to irrational and impulsive behaviors. Within the forensic area, results highlight the need to understand if subjects affected by IGD can exert choice about antisocial behaviors related to addiction or if they are forced to make constrained choices to satisfy an allegedly overpowering desire, like in substance addictions. Consequently, there is the possibility that, like in substance addictions, their capability to be juridically liable for their own actions is reduced or excluded.

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Addiction; Psychological Behavior; Insanity

J4   **Sexual Sadism Toward a Minor and Diogenes Syndrome With the Hiding of a Cadaver**

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WITHDRAWN

J5   The Criminal and Forensic Psychology of a Case of Filicide by Decapitation of a Minor

Bernat-Noël N. Tiffon Nonis*, Consultoria En Psicologia Legal Y Forense, Dr. Bernat-N. Tiffon, Barcelona, Catalonia, Spain

WITHDRAWN

J6   **The Insurpassable Fear as Justification in a Crime Perpetrated in the Municipal Police Corps**

Bernat-Noël N. Tiffon Nonis*, Consultoria En Psicologia Legal Y Forense, Dr. Bernat-N. Tiffon, Barcelona, Catalonia, Spain

WITHDRAWN

J7   **The Psychological Profile of the Missing Person as a Method Speeds Up the Team's Work in Identifying the Reasons for the Disappearance**

Laura Volpini*, PhD, Sapienza University of Rome Italy and Scientific Committee for missing persons in Italy, Roma, Lazio, Italy; Luciano Garofano*, Italian Academy of Forensic Science, Parma, Emilia-Romagna, Italy; Maria Gaia Pensieri, PhD, UPM and Scientific Committee for missing persons in Italy, Rome, Lazio, Italy

WITHDRAWN

J8   The Treatment Decision-Making Capacity (TDMC) in Children and Adolescents Undergoing Orthopedic Surgery: A Comparison With Their Parents

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Learning Overview: Data on the medical decision-making capacity in children and adolescents are scarce. This lack is not in line with the need underlined by various national and international legislations to involve minors in the medical decision-making process, in the absence of legal validity. In the case of minors, the consent must be legally expressed by the person exercising parental responsibility. After attending this presentation, attendees will realize that the initial evidence from samples of minors indicated there is a possibility for adequate decision-making regarding care, but with limitations.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the results suggest there are grounds for the progressive involvement and autonomy of children in medical choices.

Objective: This study was conducted to assess TDMC in a sample of minors undergoing orthopedic surgery and compare it with their parents' TDMC regarding the same treatment. We also aimed to evaluate possible associations between the TDMC of minors and their cognitive functioning.

Methods: We recruited n=50 consecutive young patients (aged 14–18 years) undergoing orthopedic surgery from a single university children's hospital. The patients were matched and compared with their parents, whose legal consent for surgery was clinically obtained by the treating staff. The MacArthur Competence Assessment Tool for Treatment (MacCAT-T) was used to measure minor and parent TDMC, focusing on the characteristics of the diagnosis and proposed surgery. The Kaufman Brief Intelligence Test, Second Edition, (KBIT-2) was administered to minors to measure their verbal and non-verbal intelligence.

Results: The MacCAT-T scores proved that most of the young patients showed good capacity to *understand* diagnosis and treatment information as well as a good ability of adequately recognizing their diagnosis/symptoms and possible surgery implications. More than 80% of the young patients scored in the higher range of MacCAT-T *reasoning*, thus providing evidence of good capacity to critically reason and to draw adequate conclusions about possible surgery treatment effects in everyday life. Eighty percent of the young patients were able to express a clear and non-ambivalent treatment choice. We found no significant differences between young patients and parents in MacCAT-T understanding and appreciating mean scores, while the young patients scored significantly slightly lower in reasoning and expressing a choice. The analysis of MacCAT-T subscales items showed a better capacity of the adults in *appreciating treatment*, *comparative thinking,* and *logical consistency*. Spearman's correlation coefficient disclosed a positive association between Total IQ and TDMC (Rho = 0.291, p <0.01).

Conclusion: The sample of minors undergoing orthopedic surgery showed good TDMC, substantially in line with those of their parents. These results in a real clinical setting broaden the initial evidence suggesting the existence of foundations for guaranteeing progressive involvement and autonomy of minors in medical choices.

Mental Assessment; Forensic Psychiatry; Psychological Behavior

J9   Adverse Childhood Experiences and Antisocial Behavior Among Incarcerated Individuals: Unveiling the Link and Implications for Mental Health Support in Correctional Facilities

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Learning Overview: The goal of this presentation is to examine the correlation between Adverse Childhood Experiences (ACEs) and the emergence of antisocial behavior within the incarcerated population, emphasizing the significance of mental health assistance in correctional institutions.

Impact Statement: The impact of this research on the forensic science community is profound, as it reveals a favorable correlation between adverse childhood experiences and antisocial behavior in incarcerated individuals. It enhances assessment and profiling within the criminal justice system while underscoring the urgency of comprehensive mental health assessments.

Prior studies have consistently demonstrated a robust association between ACEs and psychotic disorders, suggesting that early-life trauma has a substantial influence on an individual's personality and mental health. ACEs comprise a range of abusive, neglectful, and dysfunctional household circumstances. These experiences have a detrimental impact on the individual's ability to regulate emotions and develop cognitively in a healthy manner. Consequently, those who have experienced ACEs face an increased likelihood of developing psychiatric illnesses such as depression, anxiety, Post-Traumatic Stress Disorder (PTSD), and substance misuse disorders during adulthood. Moreover, it is important to note that the occurrence of such traumatic experiences has the potential to significantly modify the structure of the brain and the functioning of stress-response systems. This, in turn, may heighten the susceptibility to various mental health difficulties that may persist throughout the course of an individual's lifetime.[1] Moreover, this interplay between ACEs and adult psychopathology significantly contributes to the formation of criminal behavior.[2,3] However, minimal research exists on how mental illness and early traumatic childhood experiences influence antisocial behavior.

This study aimed to investigate the complex interplay between ACEs and the development of antisocial behavior among incarcerated individuals, particularly those charged or accused of sexual assault, homicide, and physical assault within Tihar Jail, New Delhi. A comprehensive array of assessment instruments, including the Modified Adverse Childhood Experience Questionnaire, the Life Event Scale, the Patient Health Questionnaire-9, the Civilian Version of the Posttraumatic Stress Disorder Checklist, the Generalized Anxiety Disorder-7, and the Empathy and Aggression Scale, were utilized to achieve the aim and objective of the present study. The research findings unveiled a positive association between adverse childhood experiences and antisocial behavior, underscoring the paramount importance of early trauma in the etiology of criminal conduct among incarcerated individuals. Moreover, the study provided insights into a significant occurrence of mental health illnesses and contemplation of self-harm within the inmate population, emphasizing the urgent need for the implementation of mental health support within correctional facilities.

A future way forward in the present study will be delineated, and the detailed results will be presented in the textual, graphical, and tabular forms in the presentation.

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Forensic Psychiatry; Correctional Settings; Traumatic Life Events

J10   Brainwashing in Cults: Sexual Abuse and Delayed Treatment Until It Is Too Late

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Learning Overview: After attending this presentation, attendees will better understand the complex and multi-faceted structures of psychological cults and the associated risk of sexual abuse and violence.[1]

Impact Statement: This presentation will impact the forensic science community by showing that several sociological characteristics are relevant to the victims to engage in sexual relations. These include: the group members' deep-seated belief in the leader's charisma, who is revered and on whom the victims are dependent; isolation; emotional dependence on him; and the leader's mental and physical control over them.[2]

A 29-year-old woman began to live in a Meditation center, located in an isolated "cocoon-like" environment tightly controlled through mental and psychological methods, where she personally provided services and money to the group. This system was based on "holistic theory" that people would improve inner evolution and interpersonal relationships by admiring the leader at the top of the social hierarchy. During theoretical-practical seminars, "the Master" instilled energy in the cultists through his own hands to promote their well-being. He imposed "tantric practices," group sexual activities aimed at the pursuit of spiritual healing. Precise indications were provided about the diet, including periods of fasting to purify oneself as well as homeopathic methods suggested for the treatment of headaches.

The adherence to the cult implied the acceptance of rules and beliefs that pervaded all aspects of the woman's life and, through suggestions/manipulation, undermined her integrity and decision-making autonomy, making her completely dependent on the leader's determination. In this context, she was conditioned by the Master, who minimized the danger of a bleeding nevus that he excised with a kitchen knife in the meditation center. The Master led the woman to think that she wanted the pain for herself and caused the bleeding. She therefore showed a reduced ability to criticize her own acts as well as to foresee their consequences. She was induced to undergo pseudo-treatments without any scientific validation and died of undiagnosed metastatic melanoma at the age of 40 after a few months.

The delay in treatment due to the recommendations made by the Master negatively affected the chances of survival of the patient. He was sentenced to three years imprisonment for manslaughter. Emphasizing sociological characteristics of victims, the court maintained that the defendant exploited his lofty spiritual and charismatic status for his personal sexual gain and, accordingly, that the sexual relations practiced were an abuse of the defendant's power.

A multidisciplinary approach is key in understanding the crucial questions pertaining to sexual abuse of consenting adults in informal social settings, such as spiritual relations and cults.[3]

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Cults; Brainwashing; Sexual Abuse

J11   When Forensic Patients Want to Cross the Border: The Canadian Experience

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Learning Overview: After attending this presentation, attendees will be aware of the International Transfer Act in Canada. Attendees will know about the challenges that mental programs face when the receiving jurisdiction does not have the same level of support and control.

Impact Statement: This presentation will impact the forensic science community by making attendees aware of the gap in legislation between jurisdiction and the need to be mindful of the resources available by others to take care of forensic patients.

Canada is a country rich in diversity, notably because of the cultural mosaic of its population. Canada not only welcomes foreigners to live on its soil, but also permits them to become Canadian citizens, with similar rights, liberties, and mandates as individuals born in Canada. Diversity is present everywhere, including patients of the Forensic Mental Health system. Four types of "foreign" patients can be identified: (1) a patient who does not have any legal status in Canada but has established their life in Canada; (2) a patient who does not have any legal status in Canada, and who has not established any form of long-term living in Canada; (3) a patient who is a Canadian citizen but is primarily a citizen of another country; and (4) a patient who is primarily a Canadian citizen but also has citizenship from another country.

The challenges are different depending on which category the patient falls into. Indeed, the patient can either be willing to stay but is at risk of deportation, or the patient wants to go back to his/her home country but is detained under the authority of the Review Board of the Province or Territory.

There are Canadian laws, but there are also international treaties or legal agreements between Canada and other countries, which define the options available in terms of deportation and extradition, ensuring that a sentence will be exercised by the judicial system of the welcoming country.[1] The Canadian Review Board (Forensic) system does not have any equivalent in other countries; therefore, it may be difficult for another country to ensure the same level of monitoring and protection of the public as defined in the Review Board dispositions of each patient. This gap, which does not permit an easy transfer of patients from Canada to another country, certainly causes socio-economically impacting situations for these foreign patients.

The purpose of this presentation is to make an analogy with the population involved in the correctional system and to determine how the custodial environment of the forensic system could apply to this similar situation.

Reference:
[1.] International Transfer of Offenders Act S.C. 2004, c. 21.

Mental Health; Safety; Transfer

J12   Fitness to Stand Trial Under Canadian Criminal Law: A New Leaf After Bharwani

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Learning Overview: The goal of this presentation is to present an assessment for fitness to stand trial in Canada, the recent evolution of the law, and the expected impact on the forensic programs.

Impact Statement: This presentation will impact the forensic science community by helping attendees appreciate the fact that our disciplines evolve and their practitioners have to adapt quickly to the judicial and legislative bodies.

A key tenet of the criminal law in Canada, and indeed in perhaps all Common Law jurisdictions, is the right of an accused person to meaningfully participate in their own defense. It is a matter of procedural fairness that accords with the principles of fundamental justice. If an accused person, at any stage of the defense proceedings before a verdict is rendered, suffers from a mental disorder rendering them unable to conduct a defense or to instruct counsel, they may be deemed unfit to stand trial. Under section 2 of the Criminal Code of Canada, being unfit to stand trial means that an accused is unable on account of mental disorder to: (1) understand the nature or object of the proceedings, (2) understand the possible consequences of the proceedings, or (3) communicate with counsel. Fitness to stand trial is assessed by a qualified mental health professional, typically a psychiatrist with forensic training. To nuance the federal legislation, we rely on the caselaw.

Up until recently, the seminal decision in the Canadian criminal law was *R. v. Taylor*.[1] In *R. v. Taylor*, the Court of Appeal for Ontario concluded that the "analytic capacity" test that required that the accused be able to act in their own best interests was too high a threshold. Instead, a "limited cognitive capacity" test is applied, which requires that the accused understand the judicial process and be able to instruct counsel was the correct test. Later decisions in *R. v. Morrissey* and *R. v. Jobb* confirmed the "limited cognitive capacity" test as the correct threshold.[2,3] The implications of a finding of unfit to stand trial on the accused person are significant, particularly in relation to their liberty interests and personal autonomy. Once assessed as unfit, the accused may be subject to court-mandated treatment orders and keep fit orders, which are essentially imposed involuntary treatment and detention.

In March of 2023, the Court of Appeal for Ontario released its 103-page decision in *R. v. Bharwani*, where it comprehensively revisited its earlier decision in *R. v. Taylor*.[1,4] While the 2023 decision does not overturn the 1992 decision, it provides clarification to the fitness to stand trial test, which in turn, will inform psychiatric assessments and expert testimony.

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1.   *R. v. Taylor* (1992), 17 C.R. (4th) 371.
2.   *R. v. Morrissey* (2007), 54 C.R. (6th) 313
3.   *R. v. Jobb* (2008), 239 C.C.C. (3d) 29
4.   *R. v. Bharwani*, 2023 ONCA 203.

Mental Health; Mental Assessment; Treatment

J13   Competence to Stand Trial Training in Forensic Psychiatry Fellowships

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Learning Overview: This presentation explores the readiness of forensic psychiatry fellows to perform Competency to Stand Trial (CST) evaluations by surveying the attitudes of board-certified forensic psychiatrists who have performed at least 50 CTS presentations post-fellowship training. After attending this presentation, attendees will be able to appreciate which formal assessment tools are taught during training and with what frequency, the range and average number of CST evaluations completed by trainees, the skill with which these were performed, and how often fellows testify in these types of hearings. Attendees will also be able to appreciate the most common errors committed by trainees in CST evaluations and aspects of the evaluation that trainees struggle with the most.

Impact Statement: This presentation will impact the forensic science community by shedding light on the readiness of forensic psychiatry trainees to competently perform CST evaluations and whether they are being adequately prepared by training programs in this regard. Moreover, by identifying potential areas for improvement, fellowship programs can adjust appropriately to better prepare trainees to perform this cornerstone of forensic psychiatry practice.

CST evaluations are the most commonly requested evaluations that forensic mental health experts are asked to opine on. In many jurisdictions, these are performed by non-forensically trained professionals due to the lack of access to trained forensic professionals. Even among graduates of fellowship programs, there are many who can make fundamental errors in these evaluations. This fact emphasizes the importance of all trainees being adequately taught and supervised on how to do these correctly, so as to minimize errors in evaluations or opinions. Additionally, due to the scarcity of board-certified forensic examiners, such individuals may be required to help train others in areas lacking access to experts, but where the benefits of getting evaluations done in a timely manner outweigh the risks of general psychiatrists being responsible for the bulk of these evaluations.

In order to determine the current state of forensic psychiatric training in regard to CST evaluations, it is important to gather the opinions of faculty regarding barriers to effective training, the average number of supervised evaluations performed, opinion on the degree of skill of graduates, and recommendations for what an ideal structure of training would look like (such as a minimum number of evaluations per fellowship year, the types of CST done, and the methods of report writing for the court).

Our survey seeks the opinions of experts in performing CST evaluations. The qualification for the survey is any board-certified forensic psychiatrist who has performed 50 or more CST evaluations post-training. The initial pool is then split off into those who have experience in fellowship training (the focus of the research in this presentation) and all experts (for general opinions on improving skill). The former group is then surveyed to determine the general range of the number of evaluations, types, methods, and challenges faced in training and recommendations for an ideal "gold-standard" program in regard to producing fellows with CST expertise.

In this session, we will present the ongoing research on this topic via the preliminary results of the survey, including, but not limited to, number of respondents, demographic information, years of experience, opinions on the various aspects of forensic psychiatry fellowship training, and opinions on the best ways in which to improve expertise for those who perform these evaluations. The presenters include a former deputy director with over six years in training forensic fellows and an incoming forensic psychiatry fellow.

Competence to Stand Trial; Forensic Psychiatry; Fellowship Training

J14   Exploring the Intersection of Mental Health and the Criminal Justice System in the United Kingdom

Claire Power*, William Harvey Research Institute, London, England; Fiona Wilcox, Westminster Coroner's Court, London, England; Nikolas Lemos, Cameron Forensic Medical Sciences, Barts and The London School of Medicine and Dentistry, San Francisco, CA; Richard Walters, Queen Mary University of London, London, England

Learning Overview: After attending this presentation, attendees will better understand the mental health assessments and legislation of the United Kingdom. Their significant roles before, during, and after trials will be discussed as well as how they can impact the outcome of sentencing.

Impact Statement: This presentation will impact the forensic science community by highlighting that there are no internationally agreed terms for sentencing regarding mental health. Influence of public opinion and bias will also be emphasized.

Since the Trial of Lunatics Act 1883, the mental health of the accused has had bearing on the outcome of criminal prosecutions in the United Kingdom. The history of relevant legislation will be reviewed to contextualize the differing protocols across the four nations of the United Kingdom. The new national guidelines for deaths relating to mental health will also be highlighted.

The involvement of forensic psychiatry has a profound impact on criminal proceedings, both for the prosecution and defense, affecting the likelihood of arrest, being charged, conviction, and sentencing. As the understanding of mental health is constantly adapting and, therefore, can result in old cases being revisited, the vital role of forensic psychiatry in the appeal process is explored as well as prisoner's rights.

Around the globe, there are differing medical and legal definitions of insanity, creating varying views on the impact mental health should have on criminal proceedings. Comparison is made between countries such as the United States and Japan. Comparative analysis is made of the mental health care infrastructure and support systems available in different countries and their impact on criminal proceedings. Illustrative cases of those charged and convicted in one jurisdiction then moved to another will be presented, emphasizing that what constitutes justice can greatly vary.

Recommendations for future research and policy initiatives to further improve the treatment and consideration of mental health in the criminal justice system will also be investigated, including the potential role of technology and artificial intelligence in improving mental health assessments and informing decisions in criminal proceedings

Finally, there will be a focus on social impact, questioning the role that public opinion has on the justice system. While disposal of criminal cases via hospital orders are often seen as more lenient than traditional criminal justice routes, they often involve greater restrictions, which may far out last the penal sentences they displace.

Mental Health; Criminal Sentencing; United Kingdom

J15   Trauma Responses and Coping Mechanisms in the Field of Forensics

Brianna Simental*, National University, San Diego, CA; Ismail M. Sebetan*, Forensic Science, National University, San Diego, CA; Paul Stein*, National University, Ramona, CA

Learning Overview: After attending this presentation, attendees will have a better understanding of the impact of trauma and stress on forensic professionals. Exposure to graphic and violent scenes leads to acute stress reactions, burnout, and various psychological difficulties. These professionals manage stress levels associated with their work by relevant coping styles.

Impact Statement: This presentation will impact the forensic community by exploring the correlations between work nature, trauma, stress, and mental health support. This understanding can inform policies, procedures, and training programs to mitigate these risks and create a safer and more supportive work environment.

Introduction: Professionals, forensic scientists, and crime scene investigators play a crucial role investigating and analyzing violent crime scenes and related evidence. They are exposed to graphic and traumatic content, which can lead to psychological effects, including trauma, compassion fatigue, and burnout. They face unique challenges in managing chronic trauma, and understanding these challenges is essential for them and their family's well-being.

Methodology: A survey questionnaire was used to gather insights from current and former forensic professionals, with the intent of understanding their experiences and the impact of trauma. The survey included demographic information and questions exploring trauma and coping strategies, including adapted questions from the Post-Traumatic Stress Disorder (PTSD) Checklist and the Brief-Cope questionnaire. The data was analyzed using SPSS and non-parametric statistical tests to determine significance and correlation (Chi Square and Spearman test).

Results: The data analysis showed a significant number, 64% of participants, stated that they had experienced distress or trauma during investigations. Most common sources were witnessing deaths of children and interacting with family members. The levels of stress and trauma experience were examined on a linear scale from 1–10. The results showed forensic professionals had an average of 5.6. There was a significant inverse correlation (Spearman test) between Field Experience and Trauma Exposure, $r = -0.455$ (p value <0.05). There was a correlation between occupational stress and personal life with being on "call" and divorce, Spearman test $r = 0.452$ (p value <0.05).

A significant percentage (68%) of participants exhibited symptoms of PTSD (Chi Square, p value < 0.05); 36% of these participants demonstrated a high severity stress level that correlated with PTSD ($r = 0.474$, p value <0.05). Interestingly, the survey revealed that 44% of participants used mental health resources provided by their agency and slightly more (52%) received outside treatment or counseling. There was also a significant inverse correlation ($r = 0.450$) between the length of time in the field and the mental health resources provided. Participants that had been in the field for a shorter period were more aware of their agency's resources than those with more time in the field. The Brief COPE questionnaire survey findings indicated that humor was the most frequently chosen coping strategy, which falls under the emotion-focused category. Relying excessively on emotion-focused coping mechanisms may increase the risk of stress-related illnesses.

Conclusions: The results showed that these professionals face frequent exposure to trauma, leading to conditions like compassion fatigue and PTSD. Understanding and addressing these challenges are crucial for providing available support mechanisms and resources. To achieve this, a culture should be established, including comprehensive training, open communication, peer-support networks, and prioritizing self-care. Managing these issues will create a forensic science community that can support the well-being of its professionals and enable them to continue to do their job efficiently and effectively.

Forensic Professionals; Post-Traumatic Stress Disorder; Coping Mechanisms

J16   Health and Wellness: A Study Assessing Stress Using Physiological and Self-Reported Data Among Medicolegal Death Investigators

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Learning Overview: This presentation will provide attendees with a better understanding of the aspects of medicolegal death investigation work that cause the most stress for Medicolegal Death Investigator (MDI) professionals and will reveal promising strategies for mitigating their impacts.

Impact Statement: This presentation will impact the forensic science community by providing needed information about the daily stressors experienced by MDIs and will highlight a promising free online tool to address MDI work-related stress.

MDIs conduct death investigations and certify the cause and manner of unnatural and unexplained deaths, providing crucial services and information to the public health and criminal legal communities. They also support communities and families during the most difficult of circumstances. As such, they experience high levels of stress. Yet, relatively little is known about which aspects of work cause the most stress, their impacts, and the kinds of resources needed to address it. RTI International partnered with the American Board of Medicolegal Death Investigators (ABMDI) and the International Association of Coroners and Medical Examiners (IACME) to conduct a research study to address this gap.

Our study involved 55 MDIs providing daily self-report measures and biometric data on work activities, sleep, and stress indicators over a six-week period, allowing the team to learn more about the types of activities that trigger stress. The team also tested the effectiveness of MDI Align, a mindfulness and wellness app developed through this project that is tailored to the needs of MDIs. Through the daily surveys in MDI Align, participants were asked to report issues and events experienced on each workday. Of the 661 days reported as having a work shift, participants identified stressors every day. The most frequently reported daily issue was fatigue, which was indicated in 56% of the recorded surveys. This was followed by working alone (32.5%) and excessive administrative duties (32.4%). Over the study duration, participants reported that their sleep over the past 24 hours was of poor quality about one quarter of the time. Participation in the study led to a significant reduction in self-reported sleep problems and depression and a significant increase in self-reported coping self-efficacy. Participants also demonstrated a significant decrease in physiological stress, as measured by heart rate variability, compared to the baseline measurement.

Medicolegal Death Investigator; Stress; Health and Wellness

J17   **Upholding the Constitutional Standard of Care: Correctional Mental Health Monitoring and Investigational Expert Work Across the Spectrum of Detention Centers**

Kahlil Johnson*, Kahlil Johnson Psychiatry LLC, Fulton, MD; Corina Freitas, Freitas and Associates LLC, Alexandria, VA

Learning Overview: After attending this presentation, attendees will better understand the significance of evidence-based practices in corrections, detentions, and other camps, the collaborative nature of Correctional Mental Health Monitoring and Investigational Expert work, and will have a better grasp of institutional issues.

Impact Statement: This presentation will impact the forensic science community by highlighting the crucial role of Correctional Mental Health Monitoring and Investigational Expert work in upholding the minimum constitutional standard of care for mentally ill inmates across the spectrum of detention centers. By focusing on early identification, evidence-based practices, and multidisciplinary collaboration, these experts aim to foster an environment that prioritizes the mental health and well-being of incarcerated individuals, ultimately contributing to a more just and humane justice system.

Correctional institutions grapple with the immense challenge of effectively managing incarcerated individuals with mental health disorders. This presentation will shed light on the pivotal role of Mental Health Monitoring and Investigational Expert work in upholding the United States Constitutional standard of care within the diverse landscape of detention centers. By focusing on evidence-based practices and multidisciplinary collaboration with medical, legal, and correctional experts, they strive to ensure the well-being and dignity of mentally ill inmates is upheld. By identifying mental health institutional issues during investigations with the United States Department of Justice, and other concerned bodies, the parties can hopefully come to agreement on what will improve individual and system-of-care outcomes as well as work to transform institutions to safer and more secure correctional environments.

Furthermore, this presentation will highlight the significance of evidence-based practices in guiding the work of these experts. By drawing on national standards of care in corrections as well as research-driven methodologies, suicide risk assessment, trauma-informed care, and psychopharmacology, they can guide the creation or improvement of comprehensive policies and procedures that align with best practices in correctional mental health care. This ensures that inmates receive the standard of care they are entitled to, even within the confines of a correctional setting.

The presentation emphasizes the collaborative nature of Correctional Mental Health Monitoring and Investigational Expert work. By fostering partnerships with mental health professionals, medical professionals, corrections officers, administrators, and external agencies, these experts create a seamless network of care, facilitating the smooth transition of mentally ill individuals between the correctional system and the community.

Correctional Settings; Forensic Psychiatry; Ethics

J18 Barriers to the Great Outdoors: Balancing Psychiatric Patients' Rights and Safety

Robert Polo*, University of Southern California, Los Angeles, CA

Learning Overview: After attending this presentation, attendees will better understand: (1) the mental health impact of access to the outdoors, (2) policies regarding access to the outdoors for psychiatric inpatients, (3) and ongoing barriers to providing fresh air.

Impact Statement: This presentation will impact the forensic science community by strengthening attendees' understanding of inpatient psychiatric patients' right to spend time outdoors and enhance their ability to critically examine barriers that limit access to the outdoors.

Current research shows that time outdoors positively influences mental health and well-being.[1,2] It has further been suggested that unhindered access to an outdoor space may reduce aggressive behavior in psychiatric inpatients.[3,4]

Despite the mental health benefits of time outdoors, barriers exist that can significantly reduce, or even eliminate, fresh air time during a psychiatric inpatient hospitalization. For example, a hospital in a densely populated urban setting may have limited outdoor space, forcing several units in a multistory building to share a small patio. Furthermore, architectural barriers may be compounded by other obstacles such as staffing shortages and safety considerations.

Internationally and nationally, governing bodies have established access to the outdoors as a right for psychiatric inpatients. Barriers to implementation of this right are elucidated by a case in Massachusetts. The state's "fresh air" bill faced over a decade of criticism from hospital administrators. Their arguments against enacting the bill included, for example, patient safety and staffing issues.[5] In spite of this, Massachusetts established "reasonable daily access to the outdoors" as a psychiatric inpatient's right in 2015.[6] This legislative victory for patients' rights was dampened by follow-up regulations enacted by the Massachusetts Department of Mental Health, which allowed for an exception if a hospital could not safely provide outdoor time for patients. Indeed, several hospitals sought waivers to the requirements of the "fresh air" bill, citing limited space.[7,8] Massachusetts provides an example of legislative, regulatory, and architectural barriers that hinder psychiatric inpatients' right to access the outdoors.

As of 2016, 17 states had similar legislation, highlighting inpatients' right to time outdoors as an actively evolving issue.[9] The "fresh air" law faced challenges that are not unique to Massachusetts. Psychiatric hospitals throughout the nation were built at different times, in different environments, and with different philosophies. In some cases, buildings erected for unrelated purposes were adapted to house psychiatric patients. Consequently, many hospitals were not designed with the patients' right to access the outdoors in mind. These architectural issues require funding that may not be available.

The ratification of patients' right to outdoor access does not change the structure of a building, but it does place pressure on clinicians to balance this right with patient safety. Some outdoor restrictions are justified, but practitioners should always carefully consider if they violate individual liberties or state policy. In addition to discussing challenges, this presentation will propose design elements that may increase psychiatric inpatients' access to the outdoors.

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Human Rights; Treatment; Mental Health

J19   **Has Care-ing Gone Too Far? An Overview of California's Controversial "CARE Court" for Outpatient Civil Commitment**

Collin Shumate*, USC Institute of Psychiatry and Law, Los Angeles, CA

Learning Overview: After attending this presentation, attendees will: (1) become familiar with the purpose and design of the Community Assistance, Recovery, and Empowerment (CARE) Act; (2) understand the context of the CARE Act within other civil commitment procedures; and (3) appreciate the social policy implications of outpatient civil commitment within their jurisdictions.

Impact Statement: This presentation will impact the forensic science community by: (1) increasing knowledge of different outpatient civil commitment procedures, and (2) teaching a model for performing CARE Court Evaluations according to the ethical principles of mental health professionals.

The crisis of homelessness continues to intensify in the United States. California has been called the "epicenter" of the homelessness crisis. California accounted for 30% of the country's total homeless population, although the state is home to less than 12% of Americans. The disparity is even more dramatic with respect to unsheltered individuals; half of all unsheltered people in the United States reside in California.[1]

While not all homeless individuals have a mental illness, the Treatment Advocacy Center estimates that one-third of the homeless population is experiencing a Serious Mental Illness (SMI). That ratio is even higher among those who have been chronically homeless.[2]

In response to the twin humanitarian crises of homelessness and SMI, in September 2022, California enacted "CARE Court." This newly established civil court is intended to "serve as an upstream intervention for the most severely impaired Californians to prevent avoidable psychiatric hospitalizations, incarceration, and … Mental Health Conservatorship."[3] Candidates for treatment through CARE Court are patients with schizophrenia spectrum disorders who are "not clinically stabilized in on-going voluntary treatment" and meet one of two additional criteria—either being (1) "unlikely to survive safely in the community without supervision," or (2) in need of services and supports to prevent involuntary psychiatric hospitalization.[4] An individual's tailored "CARE plan" can include "short-term stabilization medications, wellness and recovery supports, and connection to social services such as housing." Unlike prior legislation allowing counties to "opt-in" to outpatient civil commitment laws, all counties in California are required to have a "CARE Court" by December 2024.[5]

Californians across the political spectrum have embraced the CARE Court.[6] Criticisms about CARE Court have been raised by mental health and legal advocates. The CARE Act was passionately supported by some mental health organizations, but strongly opposed by other prominent advocacy groups such as Disability Rights California.[7]

This presentation will review the history of mandated outpatient psychiatric care, including social policy implications and legal controversies. The novel CARE Act will be compared with prior statutes mandating Assisted Outpatient Treatment (AOT). Last, this presentation will propose a model for performing evaluations of individuals referred to "CARE Court."

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Mental Health; Homelessness; Civil Commitment

J20   Bridging the Divide: A Comparative Analysis of Military and Civil Forensic Psychiatry

John F. Chaves*, University of Pennsylvania, Philadelphia, PA

Learning Overview: The goal of this presentation is to highlight the essential distinctions between Military and Civil Forensic Psychiatry, emphasizing the importance of tailored approaches to effectively meet the unique challenges in each domain. By fostering greater collaboration and understanding between these fields, professionals can strengthen the intersection of mental health and the legal system, ensuring the delivery of quality care and justice to both military personnel and civilians alike.

Impact Statement: This presentation will impact the forensic science community by improving community knowledge about the intricacies of military forensic psychiatry.

Forensic psychiatry, as an interdisciplinary field, plays a pivotal role in the legal system, offering expert insights into the intersection of mental health and the law. This presentation delves into the nuanced differences between Military and Civil Forensic Psychiatry, highlighting the unique challenges, ethical considerations, and distinctive practices that each domain encompasses. By understanding these contrasts, stakeholders can foster greater collaboration and develop tailored approaches to meet the diverse needs of their respective populations.

This presentation underscores that while both military and civil forensic psychiatry share a common goal of assessing and addressing mental health issues within a legal context, the populations they serve and the settings in which they operate significantly differ. Military forensic psychiatry deals with the mental health of service members, veterans, and individuals within the military justice system, navigating issues like Post-Traumatic Stress Disorder (PTSD) and combat-related mental health conditions. In contrast, civil forensic psychiatry involves civilians entangled in legal matters, such as criminal cases, civil litigation, and child custody disputes.

Furthermore, this presentation examines the distinct ethical dilemmas that practitioners encounter in each domain. Military forensic psychiatrists must balance their role as clinicians with obligations to military command and national security, potentially influencing their objectivity and independence. Conversely, civil forensic psychiatrists face challenges related to impartiality, confidentiality, and the duty to the court, striving to provide unbiased expert opinions while adhering to the legal standards.

This presentation also explores variations in assessment practices and methodologies between military and civil settings. Military forensic psychiatrists often conduct evaluations in highly structured environments, utilizing specialized assessment tools to determine fitness for duty and combat-related mental health claims. In contrast, civil forensic psychiatrists operate in diverse contexts, employing a wide array of psychological testing and interviewing techniques to assist courts in understanding issues like criminal responsibility and competency to stand trial.

Military; Forensic Psychiatry; Justice

J21   **Not Guilty by Reason of Insanity (NGRI) for Florida Teen Who Murdered Two Strangers and Engaged in Cannibalism: Implications for Forensic Mental Health Professionals**

Emily D. Gottfried*, Medical University of South Carolina, Charleston, SC; Christopher Fields*, Psychiatry, Medical University of South Carolina, Charleston, SC; Edward Thomas Lewis III*, Medical University of South Carolina, Charleston, SC; Diana Mullis*, Medical University of South Carolina, Charleston, SC

Learning Overview: After attending this presentation, attendees will understand factors that contributed to Austin Harrouff's rampage and resulted in the death of two strangers and accusations of cannibalism. Attendees will understand why initial reports concerned professionals that this was part of a wave of designer drug intoxication.

Impact Statement: This presentation will impact the forensic science community by exploring the forensic assessment of criminal defendants raising an insanity defense, early intervention with potentially violent individuals, and the stigma of mental illness delaying treatment and mental health professionals' ability to gauge dangerousness in the context of significant paranoid ideation. Reconstructing a timeline of Mr. Harrouff's psychiatric symptoms will help mental health professionals conceptualize how to best intervene with their own potentially violent patients.

The presenters will explore the multiple expert opinions from forensic psychiatric evaluation reports that were unsealed by the judge and published. These evaluation reports discussed Mr. Harrouff's legal insanity and will be used to contrast the expert opinions with lay opinions regarding what constitutes severe mental illness vs. antisocial behavior. The forensic science community will gain a better understanding of important factors that influence decisions regarding NGRI. The authors will also explore how NGRI cases with serious consequences affect public opinion, and in turn, potentially state law.

Although the killings of two strangers by Austin Harrouff in 2016 were a terrible tragedy, the details of this forensic case provide a wealth of educational material for forensic mental health clinicians, whether they are providing expert opinions or clinical psychological/psychiatric care.

Not Guilty by Reason of Insanity; Cannibalism; Bipolar Disorder

J22   Catfish: How the Internet Can Lead to a Double Tragedy

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Learning Overview: After attending this presentation, attendees will better understand how the internet and online applications adversely affect people psychologically, socially, and physically.[1] In some cases, controlling and aggressive behaviors can occur in the context of virtual interactions as cyber-dating abuse.

Impact Statement: This presentation will impact the forensic science community by showing the negative mental health effects, including distress, shame, and panic to anxiety, self-harm, and suicide, that can be experienced by people who have been catfished.

A 24-year-old man, described as handsome, sporty, and active, committed suicide by hanging. He had never exhibited any signs of mental instability. His parents searched for clues in their son's social media messages and texts and found over 8,000 messages on instant messaging platforms between him and a 22-year-old woman. For almost one year, the pair exchanged messages, always by text, without ever engaging in any phone or video calls or dates in person, although these were requested several times by the man. Dialogs mainly focused on the affective-sexual sphere with declarations of love, sexual fantasies, and planning of a long-lasting relationship, including marriage and building a family. In the last months, the woman's messages became aggressive through direct attacks such as threats, arguments, and insults against the man and his family. He became completely emotionally dependent on her to the point of being manipulated and continued to play along, begging for her attention and consideration. This psychological violence continued until he realized he was being catfished. The girl with whom he had been texting was entirely fictional, and the picture "she" sent him belonged to a differently named model. After discovering the scam, the man continued to ask for explanations in vain, receiving vague and aggressive answers for two months until he finally committed suicide.

After his death, his parents discovered that the false feminine profile was created by a 64-year-old homosexual man from a nearby city. They accused him of subjecting their son to a form of psychological torture and reported it to the prosecutor. Investigators found several fake female profiles in which he chatted with other men, inducing them to a virtual emotional relationship through jealousy attacks and threats.

The catfisher's psycho-pathological profile was analyzed. His behavior had traits of antisocial personality disorder and narcissistic disorder with malevolent and destructive connotations, as well as a constant tendency to affective manipulation malignant psychological manipulation implemented with pseudo-victimization.

The man was convicted of "impersonating a person" for personal gain according to Article 494 of the Italian penal code and only received a fine. Prosecutors opened an investigation into a possible charge of "death as a consequence of another crime," but charges were dismissed. Meanwhile, a popular Italian TV show produced a report about the story, tracking down the catfisher, who was easily identified in his small town. The man received death threats and insults and committed suicide a few days later.

This sad story shows that the internet and social networks provide tools that facilitate the creation of interpersonal relationships and communication between the members of a couple. However, negative effects have also been documented because people are more accessible and susceptible to being controlled and more at risk of interpersonal intrusion and harassment.[2] This is particularly true for people who use social media as an alternative space for online dating, allowing them to bypass feelings of fear due to the heightened level of trust in these platforms.[3]

In these cases, the criminological evaluation can permit an in-depth behavioral analysis of the relationship between the victim and the oppressor, where the laws and tools needed to protect people from catfishing could not be robust enough.

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Catfish; Suicide; Cyber-Dating Abuse

J23 Evaluating Handwriting Variance in the Detection of Deception: An Exploratory Study of Qualitative and Quantitative Approaches

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Learning Overview: After attending this presentation, attendees will have learned about the potential of detecting deception from handwritten statements through two different analytical approaches.

Impact Statement: Research into the detection of deception in handwriting impacts forensic science by providing a possible non-invasive tool to examine handwritten statements.

We demonstrate the results from a participant group of college students who reviewed truthful and deceptive handwriting statements. We compare their answers to the quantitative results from temporal recordings of the same handwritten statements. Our results include analysis and discussion of the strengths and weaknesses of both approaches with recommendations for further research.

In polygraph testing, the underlying stress associated with the act of deception causes a psychophysiological response in the body. Correspondingly, handwriting, with its sensitive connection to the nervous system, can exhibit variance from its normal pattern when a writer is experiencing stress. Handwriting research has shown that under stress, writers show changes in pressure, reaction time, and movement time as well as increases in horizonal expansion, size, and variability.[1-3] Prior research into detection deception using movement-based handwriting recordings and quantitative measures has shown slower writing times, increased pressure, and increased width during deceptive tasks.[4-6]

In this exploratory study, 12 college students participated in two scenarios that included writing a statement of an event that occurred to them recently, followed by being instructed to write about the same event a second time, but this time to "lie" about the event. Of the 12 subjects, 5 of the subjects performed a third scenario where they were asked to write about another event, but to report about it truthfully. All statements were written on paper with an inking pen that was placed on top of an electronic tablet. The statements were simultaneously recorded using NeuroScript® MovAlyzeR® software set at a sampling rate of 200 Hz. After completing all tasks, the experimenter asked the subject if they thought there were differences when comparing paragraph Scenarios 1 and 2. Nearly all subjects reported that they felt they were slower when writing the second, deceptive paragraph (Scenario 2).

A quantitative analysis of the recorded paragraphs was conducted where it was found that deceptive statements showed an increase in duration or signing time and an increase in length/stroke and width. Due to the limited number of participants, the increased measures during the deceptive task did not reflect statistical significance, but they showed concordance to prior research.

In a qualitative analysis of the handwritten statements, a second group of college students were provided with copies of the handwritten statements and asked to make determinations about which statement was deceptive. The statements were presented in random order to the college students. After forming a conclusion, the participants were asked to provide the reasons for their conclusions. Some participants relied on the language or the linguistics they read in the narrative, rather than handwriting observations, and responded that some of the statements seemed unrealistic or unbelievable.

A comparison of the quantitative and qualitative approaches is explored, including recommendations for further research.

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Handwriting; Psychological Behavior; Forensic Analysis

J24   Medicolegal Investigations in Cases of Death Due to Escape From Health Care Facilities in Psychiatric Subjects: The Management of the Prevention of Fatal Events

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Learning Overview: After attending this presentation, attendees will understand the importance of suitable surveillance systems for the safety of non-self-sufficient patients by describing cases of deaths due to escape from health facilities

Impact Statement: This presentation will impact the forensic science community by demonstrating the possibility of implementing such systems with respect for the health and dignity of the patient in order to avoid or reduce fatal events.

Introduction: Health care residences are facilities that offer care and support to more fragile patients such as the elderly, patients in need of physical rehabilitation or individuals with psychiatric disorders, and personalize treatment according to the specific needs of each individual. The care of patients suffering from mental health problems is a global public health issue which, unfortunately, is still often underestimated. However, the supervision of psychiatric patients in nursing homes is essential to protect patient safety. Psychiatric patients can be vulnerable to self-harm or other dangerous behaviors, so careful surveillance can help prevent accidents and ensure prompt intervention in case of emergencies. Adequate surveillance can also help define correct adherence to pharmacological treatment, monitor the subject's clinical progress over time, evaluate any progress so as to define the treatment plan in a personalized way or identify signs of deterioration. Among the concrete risks to be faced in these environments, there is also the possibility of patient elopement. In this work, we describe two cases in which psychiatric patients died following their escape from the facility where they resided.

Case Reports: In Case 1, an elderly woman was found dead near a little bridge in an open environment. The investigation determined that the woman was actually a patient suffering from dementia who had spontaneously left the nursing home during the night, passing unnoticed. In Case 2, an elderly man suffering from schizophrenia was found deceased and naked in a river. Investigation revealed the man had escaped from a nursing home located about 1km from the scene.

Results and Discussion: In Case 1, the autopsy determined that the woman had a vertebral fracture with signs of hypothermia due to the low winter temperatures at the time of the escape. The woman had managed to walk about 3km during the night; however, following a fall from the little bridge, she became immobilized, suffering from severe hypothermia until she died. In Case 2, the autopsy determined that the subject presented with multiple fractures attributable to a fall that occurred near the river, signs of hypothermia, and no evidence of drowning. The man had managed to escape by taking advantage of an emergency at the residence with the arrival of an ambulance and the opening of the gates. The man had managed to walk about 1km and subsequently fell into the river. Both cases concern psychiatric subjects; in both cases, there had been previous escape attempts with the completion of the escape during the hardest moments for surveillance (i.e., at night or during an emergency).

The reported cases emphasize the importance of implementing surveillance systems for these patients in order to protect their safety. It necessarily requires an accurate initial assessment to establish the degree of assistance needed by the patient; suitable visual surveillance, especially on the access and exit routes from the structure and, above all, at night or during an emergency. Furthermore, we propose improvement of surveillance systems to avoid fatal events with control checklists by attributing a score to the risk of escaping, especially on the basis of previous similar episodes. We also emphasize the possibility of using electronic bracelets capable of emitting signals in case of escapes outside the structure at unscheduled times. Vigilance should always be provided with the aim of promoting the health and recovery of the patient while respecting his/her rights and dignity.

Forensic Analysis; Forensic Psychiatry; Autopsy

J25   On Pathological Religiosity: Technical Reflection Points From an Expert Case Study

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Learning Overview: Through the analysis of four cases, this presentation aims to illustrate how the combination of dysfunctional religious beliefs and a culture of belonging, together with a premorbid personality, can influence an individual's conduct and assume relevance in the medicolegal field. Religions as a meaning system can affect the coping process, and the doctrines and religious beliefs within them can either exacerbate or reinforce psychopathological tendencies in emotionally vulnerable individuals.[1,2] After attending this presentation, attendees will be more aware of the importance of careful analysis of religious and cultural dimensions, along with historical and existential aspects, in criminological assessments.

Impact Statement: This presentation will impact the forensic science community by showing the anthropological and psychological relevance of the effects of the intersection of disturbed religiosity and psychopathological antecedents on behavior. Paying attention to this element can be useful in the prevention of deviant and criminal behavior, as well as in the treatment, prognostic, and curative approaches to these dynamics.

Four expert cases were examined. The first case involves a couple with inadequately treated psychiatric disorders and a pathological religious dimension. This combination of factors led to serious dysfunctional dynamics in the relationship, resulting in an unwanted pregnancy and a reparative marriage. The daughter was diagnosed with severe autism spectrum disorder after a few years. During the separation, the technical relevance concerns the identification of the most suitable legal entity to take custody of the ill child, currently hospitalized in a health care facility.

The second case concerns a man, an entrepreneur and a Mason, who, approaching a church community, completely changes his lifestyle and gets married. However, he later develops a desire to become a priest. After the birth of his second daughter, he has a psychotic episode and undergoes involuntary mental health commitment. Subsequently, he separates from his wife, undertakes the declaration of matrimonial nullity, and begins training for the permanent diaconate. The technical relevance in this case is twofold, as it is necessary to assess both the subject's psychological condition with reference to the marriage nullity and his psycho-physical fitness for religious life. An affirmative judgment on the first point motivates a negative one on the second.

The third case involves a couple belonging to the Mormon Church. The beginning of the relationship coincides with his adherence to that religion, of which she has been a member since childhood. Sharing this cult with all its rigidities, methodologies, and psychological pressures helps keep the couple's relationship firm, concealing differences and incompatibilities. The estrangement and subsequent abandonment of the Mormon Church causes strong relational conflicts leading to the separation of the couple. This crisis also affects the children, who become the object of triangulation.

The fourth case deals with possible sexual abuse by a grandfather belonging to the Christian Congregation of Jehovah's Witnesses against his minor granddaughter. The complex plot of this case of hypothetical intrafamilial abuse unfolds in a religious setting with a vindictive perspective and raises doubts about the granddaughter's ability to provide credible testimony due to her suggestibility.

The cases presented highlight the possibility that the religious dimension not only can turn in a phenomenon associated with individual or collective mental suffering, but the individual can also become inherently ill when deprived of authentic anthropological content.[3] Because negative aspects of religion such as religious struggle, negative beliefs, miscommunication, and misunderstandings can be damaging to mental health, it's essential that those involved in spiritual education pay special attention in dysfunctional and problematic religious attitudes.[4-8] Indeed, early recognition and appropriate management of such dynamics is crucial to fostering a healthy environment and spirituality.

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Behavioral Science; Forensic Science; Psychological Behavior

J26 Self-Stigma in Alcohol Use Disorder in the Context of Fitness-to-Drive Medical Assessments

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Learning Overview: After attending this presentation, attendees will understand how focused interventions in alcohol users may reduce self-stigma, encourage people to believe they can achieve their life goals, and circumvent further negative consequences that result from self-stigmatization.

Impact Statement: The presentation will impact the forensic science community by underscoring how targeted interventions in alcohol users aimed to reduce self-stigma may play a role in improving the prognosis of rehabilitation interventions.

According to the World Health Organization, Europe has the highest alcohol consumption level and the highest proportion of drinkers in the population. Although responsible for premature death and several diseases, alcohol use remains widely diffused, trivialized, and anchored in daily life. On the other hand, alcohol use disorder is less accepted as a disease by the lay public, and pathological users are highly stigmatized. Moreover, the process of stigmatization does not stop outside those stigmatized. Indeed, self-stigma denotes a cognitive and emotional process within the stigmatized subject, taking place when a person internalizes prevalent negative views about "someone like him/her" and applies these views to himself/herself, thus endorsing stereotypes and viewing them as relevant and anticipating social rejection.

The aim of this study was to assess self-stigma in a population of alcohol consumers who underwent fitness-to-drive medical assessment following traffic offenses that occurred after consuming alcohol for whom a diagnosis of alcohol use disorder was recorded. A questionnaire was administrated before and after a psychosocial intervention focused on building motivation to change, with the aim of assessing whether focused interventions may reduce self-stigma, encourage people to believe they can achieve their life goals, and circumvent further negative consequences that result from self-stigmatizations. A total of 102 individuals (78 males, 24 females) were recruited in the study. Twenty-two of them (16 males, 6 females) received a diagnosis of alcohol use disorder and did not reobtain their driving licenses. Based on existing procedure, persons with alcohol use disorder diagnosis were required to undergo a six-month psychoeducational treatment focused on building motivation to change. In 16 out of 22 cases, the responses provided in the second questionnaire clearly revealed poor agreement compared to first completion pertaining to all domains related to self-stigma. Globally considered, these results suggest that psychoeducational treatments focused, among others, on motivation to change, may contribute to improve one's self-esteem and a sense of self-value and self-worth, thus reducing self-stigma and stigma pressure and enhance recovery and empowerment. [1-9]

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Alcohol; Traffic Accident; Psychological Behavior

J27   Serial Killers: Does Gender Make a Difference?

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Learning Overview: After attending this presentation, attendees will be able to recognize and compare differences in male and female serial killers. The significant findings about the neglect of female serial killers in research and media coverage will be presented and discussed. This presentation emphasizes the importance of developing a new typology to profile female serial killers to enhance our understanding of these offenders and improve prevention, detection, apprehension, and conviction efforts.

Impact Statement: This presentation will impact the forensic science community by showing that a better classification system is needed to profile female serial killers. This will help improve the understanding about the development, the prevention, and their treatment. Historical research on serial killers has predominantly focused on men, neglecting the toll and human tragedy and motives of female serial killers.

Introduction: This study highlights the lesser-known female serial killers and explores why they are often overlooked compared to their male counterparts and the neglect of female serial killers in research. The current typologies and approaches to understanding serial killers are male-centered and fail to adequately represent female serial killers. Despite the lack of attention, statistics show that women are involved in violent crimes, including serial killings, and their numbers are increasing. The study suggests the need for a classification system that accurately profiles female serial killers.

Methodology: Data was derived from the *Radford University/Florida Gulf Coast University Serial Killer Database*, which contains information on over 4,000 male and female serial killers. The data was then analyzed for statistical differences using a *t*-test and chi-square test and the p-values obtained. The demographic backgrounds, motives, and methods of female and male serial killers were analyzed to determine the differences between the genders. Classification systems and individual case studies were examined. A proposed classification system called "Reed's Typology of Female Serial Killers" was used.

Results: The data analysis confirmed that a female serial killer is more diverse than her male counterpart. She starts her kills at a later age and continues killing throughout her life. Gender comparisons were also made examining demographic backgrounds with categorical variables such as physical, sexual, and psychological abuse as a child, head injuries, and age at first kill. The proposed classification system for both male and female serial killers did not significantly differ. Reed's typologies consisted of Emotionally Reactive Killer, Break with Reality Killer, For-Profit Killer (Monetary), Thrill and Cause Type, Caregiver, and Team Killer.

Conclusions: This study will add more information about female serial killers, their backgrounds, diagnoses, and trauma history to enable a comprehensive comparison with male serial killers and create a more accurate profile. It acknowledges that current understanding of female serial killers is limited to those who have been caught, and many still remain undetected. Female serial killers are often overlooked as suspects due to biases against their inherent violence, making prosecution and conviction challenging. The study employed both qualitative and quantitative analysis, utilizing an existing database and individual case studies, to examine differences between male and female serial killers and compare them according to a proposed classification system.

Forensic Psychology; Reed's Typology; Serial Killer

J28　The Importance of Surveillance and Storage of Drugs in Prison

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Learning Overview: After attending this presentation, attendees will understand the importance of supervising access to potentially lethal drugs in prison.

Impact Statement: This presentation will impact the forensic science community by demonstrating how it is necessary to adopt safety systems on lethal drugs, not only with respect to the prisoners but also to prison guards.

Introduction: Drugs can have numerous side effects, even lethality, if not correctly administered according to dosage. In fact, many drugs can be used for suicidal purposes: opioids, for example, can cause respiratory depression, severe sedation, and ataxia. Barbiturates taken intravenously cause respiratory depression and sedation. Every year in the United States, there are many cases of suicide by drug intoxication. In 2010, nearly 7,000 women died from overdosing on painkillers, such as paracetamol and ketoprofen, and more than 50,000 people died from an overdose of opioids such as morphine, oxycodone, and methadone. The most frequent intoxications are from opioids, benzodiazepines, and painkillers. Drug-induced suicides are common among drug addicts, psychiatric patients, and prisoners. In prisons, the problem of drug surveillance is amplified by the risk of theft or inappropriate use for self-harm or hetero-damaging purposes, even for accidental events such as Look Alike-Sound Alike (LASA) drugs. It is also necessary to consider the risk, known in the literature, of the security guards' burnout caused by work in stressful environments such as prisons.

Case Report: We report the case of the death of a prison guard, which occurred suddenly during the work shift. Autopsy and toxicological investigations showed that the subject had taken toxic doses of clonazepam before his death. Investigations showed that the guard had free access to drug stocks during his work shift, and he had been able to take toxic quantities due to the absence of safety systems.

Materials and Methods: An autopsy was carried out with toxicological and histopathological examinations. Testimonial data from the victim's colleagues were collected, and an investigation on the death scene was performed

Results and Discussion: Surveillance for life-threatening drugs is a global public health issue. The World Health Organization (WHO) estimates that hundreds of thousands of people die every year due to lethal adverse reactions to drugs or their improper use. These events may be due to errors in prescribing, administering, or monitoring drugs as well as unexpected drug interactions and adverse reactions. This problem is certainly more serious in prison, where it is necessary to maintain high levels of attention to the management of these substances. To date, few works in the scientific literature highlight the possible violations in the use of drugs in the prison environment. The case we have reported emphasizes how such violations can occur not only by prisoners, but also by prison guards, who should guarantee the custody and the correct administration of drugs. A recent scientific study has emphasized the high stress and suicide risk in prison guards, highlighting how the severity of this issue is still underestimated.

In our case, investigation showed that the prison guard, given the possibility of controlling access to drugs, was able to easily access the substance and take it. This work underlines the need for more control with the aim of avoiding free access to drugs by a single guard. To deal with this problem, we propose surveillance strategies such as an obligation to double check with at least two people for access and administration of highly dangerous drugs. From this point of view, access to drugs by prison staff should be accounted for through specific registers and confidential and computerized access codes. However, we emphasize the importance of keeping strategies in order to avoid errors in the administration such as "Tall Man" Lettering (ex., "LeTerRinG") and suitable separate storage spaces.

Prison Death; Surveillance; Drugs

J29   **The Influence of Traumatic Life Events and Subsequent Psychopathology on Recidivism: A Critical Review**

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Learning Overview: The goal of this presentation is to explain the correlation between Adverse Childhood Experiences (ACEs), mental illness, and their impact on recidivism rates among incarcerated prisoners.

Impact Statement: The presentation will have a huge impact on the forensic community as it reveals a strong connection between adverse childhood experiences, mental illness, and recidivism. The information has the potential to facilitate the development of tailored intervention methods aimed at mitigating the likelihood of recidivism within the criminal justice system.

According to Walmsley, there are more than 11.5 million people who are incarcerated worldwide.[1] According to the statistics provided by the National Crime Records Bureau (NCRB) in 2021, it has been observed that a proportion above 4% of individuals who have been released from incarceration in India are subsequently reconvicted for engaging in criminal activities.[2] Childhood experiences, specificallyACEs such as maltreatment, exert a substantial influence on subsequent criminal behavior.[3] Simultaneously, there is persistent concern regarding the global rates of recidivism and the prevalence of psychiatric problems among individuals who have been convicted.[4] The correlation between psychopathy and exposure to adverse childhood experiences exacerbates the probability of engaging in criminal behavior again. In order to address the recurring pattern of criminal conduct, it is imperative to get a comprehensive understanding of the complex interplay between childhood trauma, psychopathic characteristics, and mental well-being. This understanding is essential for the development of intervention strategies that are effective in achieving beneficial outcomes.

To address this gap, a systematic review was conducted using Boolean search methodologies in databases like Science Direct®, PubMed®, Web of Sciences®, and Google® Scholar. The search identified relevant articles published between 1990 and 2022, focusing on the impact of adverse childhood experiences and mental illness on recidivism rates. Twelve studies were examined, involving a total sample size of 3,532 individuals, with an average age of 46.75 years. The majority of the studies demonstrated a significant association between traumatic childhood experiences, mental illness, and the likelihood of recidivism.

This systematic review emphasizes the importance of addressing these risk variables and their influence on recidivism. It highlights the need for targeted rehabilitation and intervention programs that consider the impact of adverse childhood experiences and mental illness in the criminal justice system. Further research in this area is necessary to develop evidence-based interventions that can effectively address these underlying risk factors and contribute to reducing recidivism rates. The findings of this systematic review have profound implications for the forensic science community, prompting a comprehensive approach in understanding and addressing the complexities of criminal behavior and advocating for evidence-based interventions to improve outcomes for incarcerated individuals.

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Mental Health; Traumatic Life Events; Recidivism

J30   **The Role of Graphology in Forensic Investigations in Cases of Imputability Assessment Through the Analysis of the Writings of Prisoners**

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Learning Overview: After attending this presentation, attendees will know how expert graphology provides a contribution to the assessment of imputability.

Impact Statement: This presentation will impact the forensic science community by demonstrating the usefulness of graphology as a tool to support the work of the forensic physician and psychiatrist.

Introduction: The evaluation of the culprit's imputability considers both a clinical/objective aspect (infirmity), and legal ("ability to understand and to want" at the time of the crime) aspect. The ability to understand is the attitude of the subject to know external reality and understand his own actions. The ability to want is the attitude of the subject to self-determination and to control his impulses.

Generally, the coroner and the forensic psychiatrist must ascertain imputability to evaluate any pathologies excluding or diminishing the capacity of the offender. Currently, judges also rely on graphological advice to support the specialists mentioned above. Several studies have shown that the help of the graphologist is already effective for the evaluation of the inability to understand and to want determined by graphic, paragraphic, and extragraphic disturbances (anomalies of the graphic layout, potentially attributable to pathologies).

Materials and Methods: We analyzed six cases dealing with the assessment of imputability for which the judge has requested a forensic medical expert report/psychiatric forensic relating to the assessment of the social danger and the ability to understand and to will of the defendants at the time of the commission of the crimes.

Results: In the first case, the analysis of the manuscripts revealed several disturbances (partial chaoticity of the text; omissions; anticipations; transpositions; stereotypes; agrammatismi). Above-average quantum degree of graphic personality, expressed in sign terms by the "Intozzata II," which conditions normal reasoning skills for impressionability. The right-handed axial tendency denotes the search for affective support. The "Calibro Alto" is indicative of egoic overcompensation. The "Aggrovigliamenti" ruffle ideas and the "Lettere Addossate" generate apprehension that reduces the will. The writing is "Sciatta" for tendency to carelessness and "Stretta tra Parole" for superficiality of assessment. From the text, we see many graphic signs of incapacity to understand and to want. In two cases emerge graphic indicators conditioning the will of the writers that could reduce the ability to want. The graphisms present "Lettere Addossate," conduction on the staff "Titubante" and "Descendente," "Aste Concave" to the left that make willpower, pressure spasms, tangling, and "Inclinazione disomogenea/pendente."

In the other manuscripts, although they may lack some graphological indexes that support the state of awareness, we believe that there are no particular criticisms about imputability.

Discussion: We have seen that the first graphological parameter useful to understand the affective-volitional and intellectual process is graphic pressure, that is, the basic energy. Quantitatively, it is required that the graphic traits have a physiological differentiation between ascending/descending paths (Intozzata I modo). Qualitatively, continuity is considered in case of detached writing and regularity understood as non-interruption-interruptions in pressure, changes, burrs indicate suffering (Intozzata II modo). Continuity and regularity can be examined through the dynamic relationship between pressure intensity and amplitudes (Largo di lettera/Curva) of the graphic lines, indicators of the type of will and the way to free it. By integrating this relationship with movement (Ritmo), we understand how the ability to want interacts with the ability to understand.

We list further graphological signs that may negatively affect intellect and will: "Stretto di lettera; Alta; Spavalda: Aggrovigliata; Titubante; Intozzata II modo; Ricci della mitomania; Curva in grado eccessivo; Lettere addossate; Sciatta; Aste Concave a Destra; Aste Concave a Sinistra; Discendente."

Conclusions: Finally, the graphologist does not make a diagnosis but supports the doctor with the analysis of the signs and evidence of a disorder. The graphological evaluation of the writing composition is not standardized, and it moves from the analysis of the innumerable combinations to the more common patterns seen in more people; the conclusions are determined by the individual nuances and unique personal attributes.

Forensic Science; Forensic Psychiatry; Graphology

J31   Does Violent Pornography Beget Real-World Violence?

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Learning Overview: The objectives of this presentation are for attendees to become familiar with: (1) approaches in estimating sexual misconduct risk; (2) the current understanding of pornography's relation to criminal behaviors; and (3) connections specifically between serial violent crime and the consumption of violent pornography.

Impact Statement: This presentation will impact the forensic science community by improving attendees' competence regarding individuals who consume violent pornography and by offering ways to incorporate such knowledge into performing their legal or clinical duties.

Pornography and other depictions of sexuality have been treated differently across cultures and have long been matters of contention. In the modern western world, the controversy significantly escalated in the 1960s and 1970s when obscenity laws were challenged in various countries. Denmark became the first nation to legalize "hardcore" pornography, and researchers examining subsequent trends in criminal behavior in Denmark reported observing a reduction in certain sex crimes. Western countries' attitudes toward pornography since then have tended toward permissiveness, largely allowing for the possession, production, and sale of pornographic media. Over time, increased societal acceptance has resulted in the creation of increasing amounts of content depicting various atypical ("deviant") sexual acts, though countries have generally enforced prohibitions against materials portraying minors or animals.

The connection between pornography consumption and sexual offending has been examined and argued at length since the earliest Denmark study. States and other organizations hold an interest in determining individuals' risk of sexual offending, but a valid assessment of such risk relies on having scientifically established risk factors. While some risk factors, such as previous offending, are well understood, others such as pornography consumption have been persistently controversial in certain regards. There are known criminal cases in which alleged perpetrators, at times accused of especially heinous crimes against multiple alleged victims, appear to have specifically sought out violent pornography. Yet, whether the consumption of specific subsets of pornography, such as content featuring adult actors simulating violence, constitutes an independent risk factor for sexual offending is an area of limited research. [1-3]

This presentation will provide a brief overview of the ways risk factors and actuarial risk assessment instruments are utilized in contemporary forensic assessment. Its central focus will be an overview of the relationship between pornography and sexual offending with special attention given to the connections between serial violent crime and pornography depicting violence.

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Pornography; Risk Assessment; Sexual Crimes

J32   Munchausen by Proxy Syndrome: When We Dare and Why We Dare to Care

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Learning Overview: The goal of this presentation is to go through several cases of Munchausen syndrome by proxy to underline the difficulty in diagnosis and the need for both clinicians and forensic experts to collaborate.

Impact Statement: The presentation will impact the forensic science community by adding knowledge to this relatively rare phenomenon that constitutes a form of child abuse and may require the intervention of the forensic physician and forensic toxicologist from the very beginning.

The term Munchausen syndrome by proxy refers to a situation in which one or both parents procure or invent symptoms or illness that their children do not have, exposing them to continual medical examinations, including surgery that can severely injure or, in extreme cases, lead to death.[1] Usually, the victim is a young child, and the perpetrator is the mother, that is, the person to whom the child's care is almost exclusively entrusted, effectively placing her in the best position to simulate the illness. It can be considered a form of abuse and is described in *DSM-5* as a fictitious disorder caused to others in the category somatic symptom disorder and related disorders.[2]

A detailed examination of four cases is given, all with a common feature: administration of chemicals. The first case which is analyzed relates to an incomprehensible and cyclic recurrence of a comatose state in a pediatric patient, later traced to the administration of barbiturates. The second case also involves a child who was experiencing severe hypoglycemic seizures caused by the exogenous administration of insulin. The third is about a hyperkalemia episode that was later motivated by adulteration of the hydration bag with potassium salts. Finally, the last and foremost is the alleged administration of valproic acid.

Although several court cases have dealt with Munchausen by proxy, the demonstration at trial of conduct that is criminally relevant or otherwise traceable to a diagnosis is often problematic for two main reasons. First, the difficulty of making a true diagnosis is motivated by the low number of cases and the generic nature of symptoms; second, there is the difficulty of tracing the overcure back to a criminal case and tracing it back to the caregiver.[3] Only an integrated approach, based on collaboration among multiple professionals, including the role of the forensic toxicologist, can ensure the achievement of a rapid diagnosis and the protection of the victim.[4] Sampling biological fluids for chemicals to which symptomatology can be traced is often diagnostic in cases such as those described.

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Munchausen Syndrome by Proxy; Child Abuse; Intoxication

J33 Suicide Notes: Postmortem Analysis and Preventive Considerations

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Learning Overview: After attending this presentation, attendees will be aware that suicide notes can provide valuable information on suicide victims.

Impact Statement: This presentation will impact the forensic science community through the analysis of suicide notes and their possible application in preventing such a phenomenon.

A suicide note is a message written by a person who has decided to commit suicide. Information provided through the analysis of these messages is diverse and sometimes extremely valuable. The length of these messages may be like a long discussion, and at other times, they contain only a few words. Their content includes reasons for the act, information about the manner of death, forgiveness from loved ones, accusations, instructions, or last wishes. In other circumstances, they may simply contain a brief farewell.[1,2]

In this study, 858 autopsies performed by the Unit of Legal Medicine of Pavia of people deceased by suicide were collected from 1999 to 2022. Only 81 people out of 858 left a message (9.4%); in 66 cases (81.5%) it was written on a note or a letter; in 6 cases (7.4%) it was written on an SMS or in an email, and in 3 cases (3.7%) it was in the form of a voice message. In 1 case, there was only a single photo (1.2%) sent right before the suicide. In 5 cases (5/81, 6.2%), the form of the message was not specified. Suicide notes in digital forms (SMS, email, or photo) are more common among people ≤50 years old if compared with the older population. Sixty-five were male (80.2%), 15 were female (18.5%), and 1 was in gender transition (1.2%). The mean age was 49.4 years old, and the most representative age group was from 35 to 65 years old (46/81, 56.8%). The most common cause of death among these people was mechanical asphyxia (47/81, 58%). A detailed analysis of 21 suicide notes out of 66 written on paper was conducted: 7/21 related to requests (e.g., "*Let me go, it's my long-meditated and very painful choice*"); 21/21 related to expressions/wishes (e.g., "*I would have liked to become a tough guy like you ... unfortunately I didn't succeed*"); 6/21 related to personal problems (e.g., "*I cannot stand it anymore. I have a diabetic foot and a compromised knee*"). It was found that females tend to leave suicide notes written on paper (93.8%) and to use more words, compared to males. In addition, considering people with at least one psychiatric disorder diagnosis, there was a significant difference ($p < 0.05$) between those who left suicide notes (21/81, 25.9%) and those who did not (307/777, 39.5%).

In conclusion, the study of the characteristics of suicides and the thematic analysis of suicide notes allowed better understanding of the relationship between risk factors, life experiences, and the choice to commit suicide. This information can be useful from a prevention point of view. For example, considering the increasing use of digital devices to write suicide notes in recent years, especially among young people, it may be possible to use artificial intelligence to detect suicide notes through specific algorithms for early identification of individuals at risk.[3,4]

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Suicide; Prevention; Psychological Behavior

J34 Neuroscience in the Courtroom

Grace Cheney, Stanford University, Denver, CO; Octavio Choi*, Stanford University, San Carlos, CA; Gayathri J. Dowling, National Institute on Drug Abuse, Bethesda, MD; Michael Hernandez, University of Vermont, South Burlington, VT

Learning Overview: Attendees of this presentation will learn about the emerging field of neurolaw and gain insights into recent advances in neuroscience relevant to legal decision-making. This presentation will first provide a broad introduction into the types of neuroevidence used in the courtroom while emphasizing the importance of understanding the limits and potential pitfalls of neuroscience. The Project Director of the National Institutes of Health's Adolescent Brain Cognitive Development (ABCD) Study® will describe its ground-breaking scope, share key findings, and discuss its unique open science model. Next, the presentation will focus on the neuroscience of adolescent and young adult behavior and prompt reflection on the evolving conceptualization of neurodevelopmental immaturity in the law. Finally, this presentation will conclude by delving into the complexities faced by individuals with neurodevelopmental disorders within the legal framework, underscoring the need for an informed approach to promote a just legal system.

Impact Statement: This presentation will impact the wider forensic science community by introducing attendees to the field of neurolaw and educate attendees regarding the use of neuroscientific evidence in legal settings. This presentation's balanced exploration of neuroscience's potential, along with its scientific, legal, and moral limitations, will encourage responsible and thoughtful integration of neuroevidence in the courtroom, ensuring that its application is grounded in evidence-based practices. Overall, this presentation will contribute to the growing understanding of neurolaw's potential and its responsible use in the forensic science community.

Neurolaw is an emerging interdisciplinary field that examines the role of neuroscience in the law. Recent neuroscientific advances have generated an intense amount of interest in psychiatric and forensic communities, owing to their potential to elucidate mental states and capacities that are fundamental to legal decision-making. This presentation will introduce the pioneering ACBD® Study and highlight how neuroscience can be used and misused in legal settings, especially for adolescents and litigants with neurodevelopmental disorders.

Our panel will begin with Dr. Octavio Choi presenting "Introduction to Neurolaw"' to provide a broad introduction to the field of neurolaw and the types of neuroevidence being used in the courtroom. He will present the latest research highlighting neuroscience's potential to guide determinations of criminal responsibility, lie detection, and pain measurement.[1] Dr. Choi will also discuss the limits and potential of neuroscience to mislead and confuse finders of fact if used improperly in the courtroom.

Dr. Dowling, the Project Director of the National Institutes of Health's Adolescent Brain Cognitive Development will present "The Adolescent Brain and Cognitive Development (ABCD) Study: Potential Value for Forensic Sciences." The ACBD® Study is the largest longitudinal study of brain development and child health in the United States, having enrolled nearly 12,000 diverse youth from across the country ,starting at ages 9–10 and assessing them repeatedly for a decade to understand brain, cognitive, social-emotional trajectories and the factors that influence them.[2] Dr. Dowling will introduce the ACBD® Study, discuss its objectives and methodologies, which include state-of-the art neuroimaging, biospecimen collection, and comprehensive assessments of physical and mental health, substance use, culture, and environment, as well as residential history-derived data (e.g., pollution exposure, neighborhood/school characteristics, local policies) to disentangle individual and socioenvironmental contributors to health.[3] Dr. Dowling will highlight key findings from the study that have resulted from its unique open science model, allowing researchers from around the world to access its extensive dataset, fostering collaboration and transparency in research, with the ultimate goal of providing actionable information to help educators, health professionals and policymakers improve the lives of all children, today and for generations to come.

Dr. Cheney will present "Risky Business: Understanding Immaturity through Developmental Neuroscience." Teens are stereotyped as being moody, reckless, preoccupied with their friend's opinions, and, above all, immature. But what does neuroscience have to say about these stereotypes, and when do youth finally "grow out of it?" Dr. Cheney will examine the neuroscience underpinning adolescent behavior and highlight recent studies demonstrating neural and cognitive immaturity lingering well past age 18.[3] Using landmark rulings in adolescent death penalty cases, Dr. Cheney will trace the law's evolving conceptualization of neurotypical neurodevelopmental immaturity and prompt reflection on how this may (or may not) inspire juvenile justice policy revision.

Dr. Hernandez will present "Navigating the Legal System with Neurodevelopmental Disorders." Neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) pose unique challenges, affecting communication, social interaction, and decision-making abilities. For example, some individuals with autism may struggle to navigate the legal system or understand legal proceedings.[4] Dr. Hernandez will delve into empirical research, case studies, and clinical insights to highlight the complexities faced by individuals with neurodevelopmental disorders within the legal framework.

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Mental Health; Imaging; Juvenile

J35   Advancing Forensic Psychopharmacology: A Comprehensive Update for Forensic Scientists

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Learning Overview: Overall, this interdisciplinary presentation aims to equip forensic psychiatrists, pathologists, jurists, and toxicologists with the knowledge and skills necessary to navigate complex legal cases involving mental health, substance abuse, and toxicology. By fostering a collaborative and comprehensive understanding of psychopharmacology within the forensic context, attendees will be better prepared to conduct rigorous evaluations and provide expert testimony in legal proceedings, ultimately contributing to the just and equitable resolution of mental health-related legal cases.

Impact Statement: The complex interplay of psychopharmacology, forensic psychiatry, pathology, and toxicology significantly impacts legal cases involving mental health issues, substance abuse, and toxicological findings. This presentation offers a specialized and interdisciplinary update on the latest developments in psychopharmacology.

Psychopharmacology, the study of how medications affect the mind and behavior, is an ever-evolving field that plays a crucial role in the treatment of mental health disorders. This presentation offers an informative and comprehensive update on recent advancements in psychopharmacology, providing valuable insights into the latest medications, treatment strategies, and evidence-based approaches in the management of various psychiatric conditions. Attendees will explore the challenges of differentiating between therapeutic use and abuse of psychotropic medications and the potential forensic implications of polypharmacy in psychiatric patients.

Additionally, this presentation explores the intricate relationship between psychotropic medications and forensic pathology and toxicology. Attendees will gain insights into how medication interactions can impact postmortem findings, with a focus on interpreting toxicological results in cases involving both psychiatric medications and illicit substances.

The presenters will address ethical considerations related to medication management and prescription practices within the forensic setting. Attendees will explore the complexities of informed consent, patient confidentiality, and the duty to provide impartial and evidence-based testimony in legal proceedings. Ethical issues are also a frequent occurrence for pathologists in suicide investigations. When facing suspected suicide cases, pathologists must approach their examination with sensitivity, acknowledging the profound impact on the deceased's family and the possible financial repercussions. Balancing the need for accurate forensic examinations with preserving dignity while upholding patient confidentiality poses significant ethical challenges.

Forensic Psychiatry; Forensic Professionals; Forensic Analysis

K1   A Comparative Analysis of Wet Ink and Digitally Captured Handwriting: Real-World Applications

Steven N. Bunch*, East Tennessee State University, Johnson City, TN; Heidi H. Harralson, East Tennessee State University, Tucson, AZ; Larry S. Miller, East Tennessee State University, Johnson City, TN

Learning Overview: Within the context of forensic handwriting examination, after attending this presentation, attendees will be informed about the comparative differences between handwriting that is traditionally written with an inking pen on paper versus handwriting written on digital pads.

Impact Statement: This presentation will impact the forensic science community by informing attendees that Forensic Handwriting Examiners (FHEs) need supportive research regarding real-world scenarios when the best evidence is not available. Handwriting fluency decreases when writers perform handwriting tasks with no visual feedback and other constraints. Results help FHEs understand the limitations associated with challenging handwriting tasks.

Introduction: This study is a qualitative analysis and comparison of the visual data associated with wet ink handwriting and digitally captured handwriting and expands on existing research showing that digitally captured handwriting and wet ink handwriting exhibit differences.[1] This study replicated a challenging, real-world handwriting task written on a digital tablet with no visual feedback while the writer was in a standing position. The digitally captured handwriting was compared to a conventional writing task written with an inking pen on paper. This comparison is relevant because FHEs perform comparative analyses of wet ink signatures to Digitally Captured Signatures (DCSs) written under varying and diverse conditions. Our study tested the intra-writer consistency between the two tasks and identified observed differences in the graphic pattern. We examined the types of differences between the two tasks visually and qualitatively without reference to the captured computerized data associated with each digitally captured writing task. The focus of this study was to imitate a challenging, real-world application where FHEs are only provided the static graphic image of digitally captured writing.

Methods: Participants included 22 college students, which represented a convenience sample. To protect anonymity, the participants were assigned a numeric code and wrote a signature-like task (George Washington) instead of their real signatures. Three signature-like samples were collected for two handwriting tasks. In Task 1, all participants were provided the same type of ballpoint inking pen and same type of blank copy paper for writing wet ink signature-like tasks. The participants were seated while signing for Task 1. For Task 2, digitally captured signature-like tasks were collected on a single Topaz SigLite 1x5 T-S460 pressure-sensitive digital signature pad. Subjects were standing while writing during Task 2. A total of 138 signature-like tasks were collected, 69 samples for each task.

Analysis/Results: For Task 2, only the static or image output of the digitally captured writing was visually examined. The handwriting was initially examined for intra-writer consistency within each task before the comparative examination between tasks. Handwriting factors that were observed and compared included fluency, proportion, letter accuracy, presence of diacritics, legibility, spelling, spacing, beginning/ending strokes, and complexity.

In the intra-writer comparison for Task 1, not all writers were fluent in cursive writing and a few writers showed a wide range of variation even when writing with an inking pen on paper. In examining intra-writer variance in Task 2, a wider range of variation was evident, which was expected since the participants did not have visual feedback while writing. In comparing Task 1 and 2, results showed differences in the form of decreased fluency, missing diacritics, decreased accuracy, different and erratic proportions, letter entanglement, slurred letters, decrease in space between the two names, reflex of avoidance, and decreased complexity. Differences in intra-writer variation, decreased fluency, and decreased complexity were the most prominent among the handwriting factors observed between Tasks 1 and 2. Fluency and complexity are important in handwriting examination, so changes in these factors can inhibit authorship identification.[2] The size of the digitally captured writing was influenced by the dimensions of the digital pad, whereas the wet ink writing was written on blank, unruled paper. This corroborates previous research that writers adapt size to device constraints.[1,3]

Conclusion: It was found that some writers exhibited such extreme variation between tasks that writer identification could be inhibited. Observations of the 22 participant writings showed that some subjects exhibited extreme variation between the writing tasks while a few other participant writings exhibited enough stability and consistency to support identification. This research serves as an aid to FHEs so they recognize which handwriting elements may be most affected when writing is produced under unstable or challenging digitally captured writing conditions.

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Signature Identification; Digital Evidence; Handwriting

K2 Estimating Sufficiency of the Pairwise Euclidean Distance on Sets of Handwritten Documents

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Learning Overview: After attending this presentation, attendees will have learned about the output of the FLASH ID® system, a black box automated handwriting identification system. Attendees will also learn about utilizing the FLASH ID® system output to generate scores for other purposes and strategies to estimate the sufficiency of the Euclidean distance between pairs of handwritten documents.

Impact Statement: This presentation will impact the forensic science community by providing a means to estimate the sufficiency threshold for pairs of handwritten documents processed through the FLASH ID® system.

Many automated handwriting identification systems use complex, black box sets of algorithms to provide potential answers to the question at hand, which depends on the system used. The FLASH ID® system is designed to provide a ranked list of a closed set of writers in order of the most likely writer in the database to have written a questioned document. In recent work, a set of pairwise scoring rules (known as the Vector of Scores [VOS] method) has been developed that can compare pairs of questioned documents from the VOS output of the FLASH ID® system.[1] This set of rules involve the Euclidean distance, or the square root of the sum of squared distances. The overall goal is to characterize the black box error rates of the FLASH ID® system for specific sets of documents and to cluster writers. To accomplish this goal, the West Virginia University (WVU) dataset is used and will be introduced to attendees.

Subjects in the WVU dataset provided two different types of documents in their natural handwriting: the modified London Letter (repeated three times), and a "freeform" document (one to three pages long), which allowed them to write anything they wanted to, but were provided prompts to focus their attention to a coherent sample. Samples were then separated into printed or cursive writing for processing with the FLASH ID® system. Pairs of VOS from the FLASH ID® system from the WVU documents were studied to provide a characterization of the black-box error rates as the number of pages of writing contributing to the VOS increases. For each style of handwriting and document subject, the Receiver Operator Characteristic (ROC) curves were calculated using within- and between-source Empirical Cumulative Distribution Functions (ECDFs); the black box error rates are estimated from the ROC curves.

The ROC curves will be presented in a graphical manner and their interpretation provided. The writers will also be clustered using hierarchical clustering methods and presented in a graphical manner, grouping together writers who produce similar VOS. The estimated black box error rates based on the amount of writing can then be used to estimate the sufficiency threshold for rendering handwriting identification conclusions using the FLASH ID® automated system and an example will be presented. The results of the error rates by style of handwriting will be presented for both the London Letter and the "freeform" documents.

Reference:
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Handwriting; Error Rate; Black-Box

K3   The Mozart Scribe: Applying Forensic Handwriting Examination Methods to Identify Scribes in 18th-Century Music Calligraphy Manuscripts

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Learning Overview: The goal of this presentation is to demonstrate how forensic handwriting examination methods can be applied in the analysis of valuable historical documents.

Impact Statement: This presentation will impact the forensic science community by describing how a combination of forensic handwriting examination and paleographic methods can be jointly applied in the analysis of rare, valuable, and historical manuscripts, including those written in an unfamiliar script such as music calligraphy. Application of forensic methods is impactful in the analysis of historical manuscripts when manuscripts are authenticated or challenged.

Introduction: Analysis of scribal authorship in historical manuscripts is frequently carried out by researchers, historians, and paleographers. In prior research, it has been demonstrated that the combined application of forensic and paleographic examination methods is particularly useful in the study of historical manuscripts, especially when comparative evidence is minimal or difficult to verify and antiquated language and writing styles are present.[1,2]

In the case of music manuscripts, music calligraphy involves simple strokes with limited complexity. Upon initial review of music manuscripts, it is easy to disregard subtle differences that can point to more than one author, especially if an examiner is not fluent in reading or writing music calligraphy. In this presentation, music calligraphy elements and terminology relevant to forensic handwriting examination will be demonstrated and defined.

With respect to the analysis of music calligraphy, "like any written language, hand-produced musical scores, both the initial and the notator's [sic] work can be examined and compared, and identification made as to who authored the score . . . if one has original questioned material and ample exemplars and compares the two, as in any other document case, an identification can be made."[3]

Method: In a review of the scribal authorship of two 18th-century musical manuscripts purportedly written by Wolfgang Amadeus Mozart (Violin Concertos 1 and 4), an analysis of the music calligraphy between the two documents was analyzed and compared using forensic handwriting examination methods developed by the American National Standards Institute/Academy Standards Board (ANSI/ASB) and the Modular Method.[4,5]

Results: A comparative analysis of the music calligraphy between Violin Concertos 1 and 4 showed structural differences in the handwritten calligraphy forms. Characteristics evaluated in the music calligraphy such as beaming, leger lines, and clefs revealed several differences between the two manuscripts.

Conclusion: The differences observed between the handwritten music calligraphy on Violin Concertos 1 and 4 was sufficient to conclude that more than one scribe, a scribe other than Mozart, was involved in the writing of Violin Concertos 1 and 4. The forensic handwriting examination methodology relied upon in this analysis established that the music calligraphy on Violin Concertos 1 and 4 were written by two different scribes.

References:
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2.   Jarvis, M. W. B. (2007). *Did Johann Sebastian Bach write the six Cello Suites?* (Doctoral thesis). Charles Darwin University.
3.   Calvert, J. R. (1980). The identification of hand-printed musical scores. *Journal of Forensic Sciences*, 25(3), 619-623.
4.   ANSI/ASB (2022). *Standard 070, standard for examination of handwritten items (1st ed)*. Retrieved: https://www.aafs.org/sites/default/files/media/documents/070_Std_e1.pdf
5.   Found, B. & Bird, C. (2016). *Documentation of forensic handwriting method: A modular approach*. Victoria Police Forensic Services Department & Forensic Science South Australia.

Handwriting; Questioned Documents; Document Analysis

K4   **The Influence of Writing Speed in Forensic Handwriting Analysis: A Case Study on Suicide Notes**

Mardi Scheepers*, South African Police Service, Centurion, Gauteng, South Africa

WITHDRAWN

K5 A Conservation Approach to Thermal Printer Ribbon Preservation and Imaging

Tobin A. Tanaka*, Canada Border Services Agency, Gloucester, ON, Canada; Caroline Bernier, Canada Border Services Agency, Ottawa, ON, Canada; Sincere Ip, Canada Border Services Agency, Ottawa, ON, Canada; Helen Kolodziejczyk, Canada Border Services Agency, Nepean, ON, Canada

Learning Overview: After attending this presentation, attendees will understand how conservation science resources were used in the technical problem of preserving used thermal printer ribbons for imaging and forensic comparison.

Impact Statement: This presentation will impact the forensic science community by being practically oriented in the form of a technical note to inform other practitioners of materials that are available to preserve used thermal printer ribbons for forensic analysis.

The widespread use of thermal transfer printing, such as dye sublimation printing for the production of cards, has resulted in cases where suspect printers and used ribbons are submitted to the laboratory. These types of printers may be found in any type of entity that issues such cards for activities such as membership, library use, government programs, student cards, retail loyalty rewards programs, among other uses. Where higher security applications are in place, the thermal transfer printer may be used for personalization while the non-variable graphics are produced by commercial and security printing processes such as offset lithography with ultraviolet reactive ink.

Thermal transfer printers may be exploited in the production of false/counterfeit cards of all types. As thermal transfer printers rely on the use of an intermediate media (i.e., a single-use spooled color panel ribbon) to impart graphics/text to plastic cards, the used portions retain images of printed graphics/text. Typical spooled ribbons for use in thermal transfer printers have the capacity to print several hundred cards. Ribbons are usually made with a thin, flexible polyester film having several dye-based color panels.[1] Additional panels with clear topcoat protection and for certain specialized applications such as ultraviolet reactive layers may be present. Certain ribbons may have only one color panel, and some will have a series of color panels to print color on one side of a card, then only a black panel for monochrome black printing on the other side of a card. Magnetic stripes and signature strips are examples of additional specialized features that may be incorporated in ribbons.

This combination of hundreds of images with a fragile film base poses several challenges in handling and processing. Once used, the ribbons were never designed to be rolled flat and viewed by any means. Further complicating any processing is the need for repeated handling of the ribbon under different magnifications and illumination conditions when comparisons of macroscopic and microscopic printing characteristics to printed suspect cards is needed.

While imaging systems have been made to facilitate the recording of such used printer ribbons the acquisition of such systems may not be possible due to cost, as well as technical reasons.[2] As a result, the need for manual preservation and recording of such used printer ribbons is needed for seized ribbons.

The use of custom-made polyester sleeves has provided a means to achieve the goals of preservation and recording/imaging. The approach taken considered the suggested practices of archivists and conservationists to reduce potential problems that could arise during and after processing of ribbons.

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2. J. C. Stephens, I. Geiman, S. M. Rohde and M. M. Rohde, Thermal Ribbon Analysis Platform (TRAP): The Validation of a New Instrument for Recovering Images on Ribbons, *Journal of the American Society of Questioned Document Examiners*, vol. 19, no. 1, pp. 17-24, June 2016.

Questioned Documents; Document Analysis; Printing

K6 A Study of Natural Variations in Handwritten Signatures: Implications in Forensic Examinations

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Learning Overview: After attending this presentation, attendees will understand how the natural variations in handwritten signatures play an important role in the field of document examination, especially in the identification of signatures.

Impact Statement: This presentation will impact the forensic science community by identifying the natural variations on the basis of the length and height of the signatures from the repeated signatures of the same writer. This knowledge may help in the exclusion and inclusion of the executors based on the existence of natural variations in the signatures.

The existence of natural variation is a norm in a set of signatures as the execution of writing or signature is the result of the combination of brain, nerve, and hand movement.[1,2] Therefore, the same content of writing cannot be produced exactly the same again by the writer.

The signatures of a person are used as an identity on important documents.[3] Sometimes this identity may be misused by another person to gain some benefits after copying or simulating the signatures. Handwriting and forensic experts identify the differences and similarities in handwriting features to establish common or different authorship. Generally, handwriting experts extract the handwriting features from the signatures and compare these with the known signatures of the person. The variations in the writing and signatures are of two types: variation with intention or variation due to nature. Therefore, handwriting experts identify these variations in the signatures, whether by nature or by intention.

In the present study, a total of 26 participants (18 males and 8 females) were involved. After obtaining ethical approval from the Panjab University Institutional Ethical Committee and written informed consent from the participants, ten signatures were obtained from each participant on a given proforma. As a result, a total of 260 signatures from 26 participants were analyzed in the study. The participants were provided A4 white sheets and a blue ball pen to execute the signatures in a standard position (sitting). The length and height of the signatures were measured with the help of a ruler.[4]

In the present study, the natural variations in signatures were assessed by measuring the length and height of the signatures, as these characteristics sometimes form the basis of opinion regarding forged signatures. The results of the present study indicate that the length and height of the signatures were slightly changed in the set of repeated signatures. Moreover, the height of the signatures showed more consistency as compared to the length. The results of the study also indicate that the variations that exist in the repeated signatures are a part of natural variations usually occurring in the signatures of the same person. The same content of the writing cannot be produced exactly again by the writer, whether as signatures or a piece of handwriting. The study further establishes that in forensic examinations, if a sufficient number of specimen signatures/samples are available, the document expert must specify the characteristics based upon the natural variations before giving an opinion regarding the forged signatures. The present study may help forensic scientists identify the natural variations in a set of signatures of the same person as the writing characteristics of signatures executed by the same writer are showing consistency.

References:
1. Eckert, W. G. (1997) *Introduction to forensic sciences*. CRC Press, New York
2. Harralson, H. H., & Miller, L.S. (2018) *Huber and Headrick's Handwriting Identification: Facts and Fundamentals*. 2nd Edn CRC Press. Boca Raton, FL.
3. Harrison, W. R. (1958) *Suspect Documents: Their Scientific Examination*. Nelson-Hall Publishers, Chicago.
4. Matuszewski, S., & Maciaszek, J. (2008). Natural variation in length of signature components. *Problems of Forensic Sciences*, 74: 182-189

Document Examination; Handwritten Signatures; Natural Variations

K7   Above and Beyond: Examining "Hidden" Features in Travel and Identity Documents

Carolyn Bayer-Broring, MFS*, Immigration & Customs Enforcement, Derwood, MD

Learning Overview: Attendees will be given a brief introduction to the three levels of security features found in travel and identity documents, followed by a demonstration of specific features or flaws found in certain documents, and the scientific instrumentation and capabilities required to view those features.

Impact Statement: This presentation will impact the forensic science community by providing examiners with some ideas about how to better conduct examinations and how to better utilize the tools and instrumentation available in forensic labs in order to discover and better visualize those features.

In the field of domestic and international travel and identity documents, there are three levels of security features to take into consideration when designing a document, conducting an examination, or training others to utilize or examine those documents. First level (or "overt") features are those that can be seen with the unaided eye and don't need any special instrumentation or training to view. These would include features like a watermark in paper or an optical device like color-shifting ink on a page surface. Second level (or "covert") features are generally hidden features that can be viewed with simple, readily available instrumentation like a magnifier or flashlight and require a nominal amount of training to view. These would include an ultraviolet security feature or microline-printed text on a page. Third level (or "forensic") features are those that are hidden and require specialized instrumentation in order to view. Their presence is usually disclosed only on a law enforcement or need-to-know basis and requires a higher level of training and expertise to view. These would include Infrared (IR) dropout inks and optical taggants.

This presentation will demonstrate for attendees some specific instances of casework encountered in the Homeland Security Investigations Forensic Laboratory where laboratory equipment such as those utilizing different light sources, as well as X-rays, were utilized to assist with the visualization of "hidden" or covert features. Casework demonstrated will include passports utilizing chip-and-antenna technologies, where counterfeit chip-and-antenna arrays have been detected through the use of forensic-level examinations. Additionally, alterations of documents through the removal of travel stamps will be demonstrated, where instrumentation and light sources were the only way to detect that the alterations had taken place. Genuine documents will also be shown, to include those where seemingly "overt" features are found that, by using high-magnification capabilities found in the laboratory setting, turn out to actually be forensic features, containing information visible only through the use of true forensic examinations.

Questioned Documents; X-Ray; Light

K8   A Cleaner Cascade Method for EDD: Toner Developer Guides

Mark Goff*, MSP Lansing Laboratory, Lansing, MI

Learning Overview: The goal of this presentation is to demonstrate and evaluate the use of physical barriers to aid in the collection of cascade toner when using an Electrostatic Detection Device (EDD) for large documents.

Impact Statement: This presentation will impact the forensic science community by explaining that the use of physical toner developer guides reduces the time a forensic document examiner spends on EDD toner developer collection and improves the overall speed of development for multiple documents.

This presentation will demonstrate and evaluate the use of physical barriers to aid in the collection of cascade toner when using an EDD for large documents.

When processing a paper document for latent writing impressions, the user has multiple options to develop writing impressions; Toner Pad development, Aerosol Hood development "poof box," and Cascade Developer. All of these options begin with placing the document on the platen, activating the vacuum, covering the platen with imaging film, and charging the surface to develop an electrostatic image. However, the methods differ from this point. The Cascade Developer method is the most commonly used method and involves tilting the charged platen and pouring toner-covered beads along the surface into a collection tray at the back. This method is efficient in terms of time and resource usage but is not practical for larger documents such as petitions or legal documents. When processing larger documents with the cascade method, the beads will spread out and fall over the edges of the platen. This adds additional processing time because the beads are not collected by the collection tray, but instead must be cleaned from the surface supporting the EDD. Therefore, for larger documents, it can be advantageous to use the Toner Pad development or the Aerosol Hood, but each has its own drawbacks. The Toner Pad development method is a toner-impregnated pad that is wiped over the charged surface of the EDD to develop images and is a consumable supply that needs to be replaced. Toner Pad development can also leave streaks in the surface that may interfere with decipherment of latent indentations. The other development option for large documents is the Aerosol Hood, which sprays a fine cloud of toner developer over the charged surface of the platen to develop latent writing impressions. While some say this method provides better results, it is an optional accessory, is more labor intensive, requiring the placement of the aerosol hood for each document, and additional cleaning of the clear aerosol chamber.

To make the EDD cascade development method more practical for larger documents, an attachment was developed, and 3D printed, to channel the toner developer into the collection tray without spilling over the sides. This presentation will explore the development and usage of these toner developer guides.

After viewing this presentation, attendees will be provided with links to 3D print their own toner developer guides for various models of EDD and will have an understanding of the guide development process to modify the design to fit additional models they may use.

Questioned Documents; Document Analysis; Handwriting

K9 The Characterization and Identification of Historical Inks and Their Colorants

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Learning Overview: After attending this presentation, attendees will be informed about methods used to sample, characterize, and identify historical writing inks. Attendees will also learn about the trends in the properties of various inks.

Impact Statement: This presentation will impact the forensic science community by providing a new and practical approach to the study of historical ink samples.

Forgeries are common in the realm of autographed memorabilia and collectibles. While handwriting comparisons can provide insights into the stylistic aspects of signatures and writing, a chemical analysis of the ink and media (e.g.,paper, leather, paint) can provide orthogonal and objective constraints on questions of age and authenticity.

Among the components of a historical document, the identification of inks and their components present various practical analytical challenges for reasons that include: the often-short length of ink line available in writing or a signature, limitations that often exist on consumptive sampling or analysis, the relatively low concentration of ink needed to form a dark line, and the complexity of an ink's composition. Often, historical inks are based upon natural components such as extracts from galls and logwood (haematoxylum) whose composition can vary based upon the specific plant and extraction procedures. These natural components may be supplemented with a range of synthetic dyes, further complicating the chemistry of an ink sample. Furthermore, the composition of an ink on media can change as it dries and with time.

In an attempt to more systematically study these analytically challenging samples, a set of 50 historical ink samples were analyzed by a range of non-destructive and minimally consumptive analytical methods. These dried inks were collected from ledgers, stock certificates, and journals with dated entries. They range from approximately 1900 to 1970 and span six nominally attributed colors: black, brown, blue, green, purple, and red. In addition to the aged ink samples from the documents, a series of recently deposited known inks were also systematically analyzed. These include laboratory synthesized samples from historical recipes and commercially available historical inks from boutique suppliers.

The response of each ink to a combination of alternate light sources spanning Ultraviolet (UV) to white to near-infrared illumination, coupled with a range of barrier filters, were studied under a set of controlled conditions to permit the objective characterization of luminescence and absorbance properties. Images of the ink response were captured with a series of standard ink samples to ensure consistent illumination and capture conditions. The elemental composition of the inks was studied through elemental maps of ink lines from each sample through a combination of Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) and micro- X-Ray Fluorescence (micro-XRF) spectroscopy. Finally, each ink was characterized by Raman microspectroscopy using both 785nm and 532nm lasers. The data from each of these methods has been compiled to explore trends and associate the various characteristics of these historical ink samples. Finally, the Raman data has been analyzed to identify various components of the inks, which have included traditional ink colorants such as logwood, iron gall, and several synthetic dyestuffs. The results of this study of historical writing inks provided new and practical insights into the properties and composition of authentic historical writing inks.

Ink Analysis; Document Analysis; Raman Microscopy

K10 Using Variable Light Source Technology to Support World War II Service Member Identification

Sarah C. Kindschuh*, Defense POW/MIA Accounting Agency Laboratory, Bellevue, NE; Larkin Kennedy, SNA International Supporting Defense POW/MIA Accounting Agency Laboratory, Offutt Air Force Base, NE

Learning Overview: After attending this presentation, attendees will understand the practicality of using Variable Light Source (VLS) technology to examine World War II-era items and documents in support of American service member identifications by the Defense POW/MIA Accounting Agency (DPAA).

Impact Statement: This presentation will impact the forensic science community by explaining how the DPAA Laboratory uses material evidence items, including documents, to support an identification and offering a case study in which items were recovered from an archaeological context, identified using VLS technology, and used in service member identifications. Additionally, this presentation offers a bridge between the DPAA Laboratory and the Questioned Documents section, two entities whose interactions in the past have likely been limited at best. The authors of this presentation believe that their work would be significantly enhanced by interacting with and learning from Questioned Documents practitioners.

The Defense POW/MIA Accounting Agency Laboratory is well-known within the forensic community as being the world's largest forensic anthropology laboratory. However, one relatively unknown aspect of DPAA's casework is the examination of personal items and documents, classified as material evidence, recovered during the search for missing United States service members. Material evidence is used by the DPAA Laboratory to actively support the identification of individuals in addition to providing insight into the service member's daily life prior to their death. Regularly examined items include ID tags, jewelry, coins, clothing, and, in somewhat rarer cases, paper documents. This presentation will offer a case study detailing the use of the VLS to identify material evidence and link it to specific individuals whose bodies were recently recovered after a World War II loss.

This case study involves a B-17 Flying Fortress bomber lost in a 1944 crash during a bombing run in Germany. Seven of the ten crewmembers either survived the crash or were identified after World War II, leaving the remains of three individuals and hundreds of items belonging to all ten crewmembers at the scene of the crash site for 75 years until an archaeological recovery was performed in 2019. Hundreds of skeletal elements and thousands of pieces of possible material evidence were recovered from an agricultural field. Items were wet and sometimes soaked in fuel and often could not be associated archaeologically to the human remains. The authors examined each item for links to the B-17 crew members. The VLS was an integral part of this analysis, allowing for many items and documents to be associated with a specific person and returned to the next of kin. The VLS was particularly critical in the identification of approximately a dozen paper documents that were recovered from two wallets found during the archaeological excavation. However, the authors readily acknowledge that they are anthropologists, not document examiners, and that future analytical work would be enhanced through established relationships with document examiners and further familiarity with standard procedures in document analysis, particularly using the VLS, in a wider range of analytical circumstances.

After attending this presentation, attendees will have a clear understanding of DPAA's process of examining material evidence and how this evidence is used to support an identification by viewing a case study analyzing World War II-era items. Additionally, this presentation emphasizes the possible benefits of collaboration and training between DPAA analysts and Questioned Documents practitioners.

Document Analysis; Imaging; Identification

K11   Attempts to Clone Stamp Impressions via Stamps Produced by Additive Manufacturing

Tobin A. Tanaka*, Canada Border Services Agency, Gloucester, ON, Canada

Learning Overview: After attending this presentation, attendees will have had an opportunity to see cloned stamps made by an additive manufacturing process (3D printing) along with their resultant impressions.

Impact Statement: This presentation will impact the forensic science community by allowing attendees to see and test for themselves examples of cloned stamps made via an additive manufacturing process.

Stamping devices are commonly used in a wide variety of applications as: a labor-saving device, to recognize a transaction, for official purposes, and as a security measure. Hand stamps may be made from a variety of materials such as rubber, polymer, or metal and can employ different inking means such as manual stamp pad, self-inking, or pre-inked.

The process of attempting to clone a stamp impression may not be so easily accomplished as it first appears. The complexity of the graphic design of a stamp impression, the presence of any background printing on the substrate, along with the color of the substrate will have an impact on how much effort is needed to create what may be a successful duplicate impression. Further complications may arise from variation in the stamping process, which may be from several factors such as: stamp type, stamp dimensions, symmetry/asymmetry of stamp size, ink adherence to stamp, physical variation in stamping, ink interaction with substrate, surface upon which impressions are made, condition of the stamp, including wear/damage, stamping technique, and nature of the substrate such as watermarks in paper such as in passports.[1]

Vir et al. presented an initial overview of the potential for additive manufacturing, informally referred to as 3D printing, to be used to clone stamps to mimic stamp impressions on paper.[2] As this presentation was given in times of restrictions on in-person meetings, attendees were not able to see the resulting stamps and/or to make their own impressions. During this presentation, attendees will have an opportunity to examine examples of clone stamps and to make impressions.

A recap of the basics of additive manufacturing, including the requirements for file formats for rendering, types of technologies, suitability of materials, overview of costs, and insights into expertise required for operation in the context of stamp cloning will be provided. Further avenues for testing and research into this area will also be provided.

References:
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Questioned Documents; Document Analysis; Stamping Devices

K12   Bad Things Can Happen to Good Signatures

Linton Mohammed*, Forensic Science Consultants, Inc, Poway, CA

Learning Overview: After attending this presentation, attendees will be aware of possible causes of unusual features in genuine signatures and how to evaluate them.

Impact Statement: This presentation will impact the forensic science community by increasing forensic document examiners' evaluation skills for signature examinations.

Forensic signature examination is one of the most difficult areas of forensic science, as signatures are comprised of a limited amount of writing that may or may not be legible. Signatures are somewhat different from extended handwriting, as signatures are used as a means of identification, whereas handwriting is used as a means of communication. Each writer's signature varies each time they sign; however, this variation is an integral element that supports an identification or elimination. The range of variation may be narrow, moderate, or wide. The complexity, individuality, and style of the signature contributes to the determination as to whether the questioned signature is authentic or not.

A thorough signature examination requires an adequate number of specimen signatures to be used as comparison standards with the questioned signature. The examiner inter-compares the specimen signatures to determine the writer's range of variation. Examiners normally request as many specimens as possible, as the greater the number means that the evaluation of the range of variation can be stronger. It is well known to the forensic document examination profession that it is easier to identify a writer than to eliminate one. This is especially so if the specimen writer is infirm, elderly, or on medications that can affect their control of the writing instrument.

In most cases, a writer can be eliminated if the specimen writer's normal course-of-business skill level is lower than the skill level exhibited in the questioned signature. However, there are exceptions to this rule when the specimen writer possesses the artistic abilities and knowledge to elevate his writing skill to simulate a higher skill-level signature of another writer. If there are repeated, unexplainable differences between the questioned and known signatures, then, in most cases, elimination is warranted.

There are, however, instances where bad things happen to good signatures. These are one-time events that will not be replicated in the specimen writer's range of variation and thus lie outside the observed range of variation. These are generally referred to as accidentals. Additionally, there are also temporary intentional adjustments that can be made by a writer that are caused by infirmity or writing conditions. How then does the examiner decide what is a fundamental difference and what is an unexplained variation or accidental? Can an examiner eliminate a writer based on one perceived difference totally, or even to a degree of probability?

This presentation will give examples of accidentals and temporary intentional adjustments and suggest how these types of features should be assessed by an examiner to avoid a Type II error. The strength of opinions that can be expressed will be discussed.

Signature Identification; Forgery; Questioned Documents

K13   Chemometric and Spectroscopic Approaches in Questioned Document Examination

Vishal Sharma*, Panjab University, Institute of Forensic Science, Chandigarh, India

Learning Overview: Attendees of this presentation will learn about the significant role of forensic document examination in detecting and analyzing forged written and computer-generated documents. The study focuses on the analysis of handwritten and laser-printed documents obtained from various sources, employing advanced analytical techniques such as Ultraviolet (UV) -Visible, Attenuated Total Reflectance/Fourier Transform Infrared (ATR/-FTIR), and Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS).

The spectral data obtained from these analyses are used to develop chemometric models for document classification, discrimination, and characterization. The study also investigates morphological features, elemental profiles, and multivariate analysis of samples from laser printers and photocopier machines. Discrimination and classification of toners based on their origin are achieved through the evaluation of SEM images and spectra. Multivariate analysis techniques, including hierarchical cluster analysis, facilitate grouping of ink and toner samples based on their chemical composition.

The presentation further discusses the assessment of developed models, such as Principal Component Analysis (PCA) and linear discriminant analysis, through cross-validation to examine inks and toners of unknown origin. The analytical and non-destructive methods presented in the study offer valuable tools for forensic document experts. The integration of chemometric methods enhances the statistical confidence of the results, which is highly advantageous in this field. Additionally, the research highlights the potential for creating a comprehensive database of inks and toner samples, enabling the identification of unknown or suspected documents in the future. This aspect of the presentation showcases the possibilities for further research and collaboration within the forensic science community.

Impact Statement: This presentation will have a significant impact on the forensic science community by showcasing the advancements and applications of chemometric and spectroscopic techniques in forensic document examination. Attendees will gain insights into the valuable role of these techniques in detecting and analyzing forged written and computer-generated documents.

The assessment of developed models, such as PCA and linear discriminant analysis, through Cross-Validation for examining inks and toners of unknown origin provides the forensic science community with valuable insights. The presentation demonstrates how these analytical and non-destructive methods offer powerful tools for forensic document experts, enhancing their ability to accurately examine and interpret evidence.

Overall, this presentation will contribute to the advancement of forensic science by presenting practical applications of chemometric and spectroscopic techniques in document examination. It will inspire researchers and practitioners to explore and adopt these methods, ultimately leading to more accurate, reliable, and scientifically sound forensic document analyses.

Forensic document examination plays a vital role in forensic science, specifically in the detection and analysis of forged written and computer-generated documents. This study focuses on the comprehensive analysis of both handwritten and laser-printed documents obtained from diverse sources, utilizing advanced analytical techniques such as UV-Visible, ATR/FTIR, and Schottky Field Emission Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM/EDS).

By employing these techniques, spectral data is acquired and subsequently utilized to develop chemometric models that contribute to the classification, discrimination, and characterization of the examined documents. In addition, morphological features, elemental profiles, and multivariate analysis are investigated for samples derived from laser printers and photocopier machines. Through the evaluation of SEM images and spectra, effective discrimination and classification of toners based on their origin are achieved. Multivariate analysis techniques, including Hierarchical Cluster Analysis, enable the grouping of ink and toner samples based on their distinctive chemical compositions.

Furthermore, the developed models, such as PCA and linear discriminant analysis, undergo rigorous assessment via cross-validation to determine their efficacy in examining inks and toners of unknown origins. These analytical and non-destructive methods provide invaluable tools for forensic document experts, enhancing their capabilities in examining and interpreting evidence. The integration of chemometric methods adds a crucial layer of statistical confidence to the results, which is highly advantageous within the forensic science community. Moreover, this research sets the stage for the establishment of a comprehensive database containing information on various inks and toner samples. Such a database would prove invaluable in the identification of unknown or suspected documents in future investigations.

In conclusion, this study demonstrates the significant contributions of advanced analytical techniques, chemometric modeling, and multivariate analysis in forensic document examination. By adopting these approaches, forensic experts are better equipped to detect forgeries, classify documents accurately, and provide scientifically supported conclusions. The findings of this research pave the way for continued advancements in the field, fostering improved document examination practices and, ultimately, enhancing the reliability and validity of forensic investigations.

Questioned Documents; Chemometrics; Ink Analysis

K14 FDE Forum

Carl R. McClary*, ATF, Sumter, SC; Karen J. Nobles, MFS, Nobles Forensics, Pensacola, FL; Samiah Ibrahim, BSc, ForensInk, Ottawa, ON, Canada

Learning Overview: After attending this presentation, attendees will have access to new and different ideas, opinions, and methods to consider when approaching questioned document problems in the laboratory. These ideas and methods will represent experiences from different countries and cultures.

Impact Statement: The impact on the forensic science community would be the benefit to the attendees of an open exchange of ideas and viewpoints. As scientists, we often learn as much from another's thought processes and experiences as we do from principal trainers or other sources. Younger examiners and trainees can benefit from the experiences of more experienced examiners and those experienced examiners can benefit from hearing new and novel viewpoints.

The concept of the Forensic Document Examiner (FDE) Forum is not new to the Questioned Document community and was first utilized at the Southeastern Association of Forensic Document Examiners (SAFDE) meetings in the early 1990s as a method of opening discussion on certain topics of interest to the members. At these meetings, there was discussion on subjects upon which there may have been disparate opinions. Through forums of this type, there have been many lively discussions on controversial topics. Not all discussions must necessarily be centered on difficult issues facing forensic document examiners. Discussions may also focus on the attendee's thoughts and interpretations of quotes from recognized texts in the field. Other discussions could begin with the conclusions and opinions of some of these famous authors in criminal and civil cases of notoriety, both past and present. There may be opportunities to hear opinions from attendees on broader forensic themes from other disciplines. These might range from mitigation of bias, evidence handling, cross-contamination prevention, or crime scene issues. Forensic terminology could be discussed that may be common usage for all disciplines or specifically to forensic document examination.

This type of forum, one which is presented with international viewpoints, is an excellent way to poll attendees about their preferences on examination methods, conclusions, court testimony, or the current standards in the field. The inclusion of those who are in training or who may have just completed their training provides the attendee with a myriad of experience levels. This involvement can help the experienced practitioner learn what may have evolved in the culture of their discipline while providing the trainee with the experience of hearing from more senior colleagues. Previous forums have been successful due to input from attendees before the conference and the authors are requesting questions in advance.

Questioned Documents; Methods; Conclusions

K15 Forensic Digital Document Examination: Moving Forward in a Post-Pandemic Society

Timothy Campbell*, Canada Border Services Agency, Ottawa, ON, Canada

Learning Overview: After attending this presentation, attendees will be aware of the various forms that modern digital documents may take, the occurrences that they can undergo after creation, including manipulation and wholesale counterfeiting, and how the existing methods and tools that they already utilize as a Forensic Document Examiner (FDE) for physical documents can be used in the course of a similar digital document examination and supplemented with new ones.

Impact Statement: There has been a marked increase in the presence of digital documents in all aspects of an FDE's workflow, and this presentation will impact the forensic science community by discussing those that apply specifically to the examination of digital document evidence. This is specifically within the FDE discipline (Questioned Documents Section) with a clear delineation from the digital evidence discipline (Digital & Multimedia Sciences Section), aiming to fill this void between the two groups of examiners.

Digital documents are nothing new; they are merely a technological extension of the traditional paper-based means of communication between people and/or points in time. However, the internet has given rise to a preponderance of digital communication and commerce, including the negative aspects that follow, such as the post-creation manipulation of information in existing digital documents, and their counterfeiting, *ex nihilo*.

While FDEs have traditionally been the experts to be involved in the physical realm of documents, digital evidence experts have done so within the digital realm, but not entirely so. A nexus exists and FDEs are seeing a marked uptick in the submission of digital documentary evidence that began prior to the pandemic, but even more so since the temporarily mandated segregation of society necessitated the continuance of business transactions, academic learning, and professional activities through the digital realm.

While this shift has provided a measure of convenience in a time of strife, it came at the cost of lowered security and reliability of information in some forms, such as poor quality imagery being accepted in lieu of original identification materials or the deliberate alteration and counterfeiting of personal credentials. Likewise, signatures saw a great increase in digital use, but the quality and authenticity left much more to be desired than only a few years prior. With ISO 32000, PDFs have proliferated and become one of the standard means of information exchange and, therefore, a large part of the digital evidence an FDE encounters.

The time is ripe for the Forensic Document Examination community to discuss the various issues that have arisen from the generally accepted use of such documents, including the forms they may take, the occurrences within the life cycle of a digital document, and the tools and methods the FDE may already have in their forensic toolbox, as well as some new ones, in order to properly and reliably conduct such an examination.

Please note that no network, file system, social media, or mobile device analysis will be discussed, only documents as they are of interest to FDEs.

Document Analysis; Digital Evidence; Forgery

K16   Quantifying Bayes Factors for Forensic Handwriting Evidence

Anyesha Ray*, Ames, IA; Danica M. Ommen, Iowa State University/CSAFE, Ames, IA

Learning Overview: After attending this presentation, attendees will have a greater understanding of how statistical analysis, specifically the Bayes Factor obtained using objective features extracted from handwriting evidence, can support a forensic document examiner's subjective writership determination.

Impact Statement: Bayes Factors are highly recommended as a statistically sound method of interpreting forensic evidence, and this presentation will impact forensic document examiners by providing specific instructions on how to compute this statistic for determining the source of handwriting evidence.

Questioned Document Examiners (QDEs) are tasked with analyzing handwriting evidence to make source (or writership) determinations. The Center for Statistics and Applications of Forensic Evidence (CSAFE) has previously developed computational methods to automatically extract quantifiable handwriting features and statistical methods to analyze handwriting evidence to aid QDEs.[1-3] The method developed by Crawford et. al uses a K-means clustering algorithm and Bayesian hierarchical model to perform closed-set writer identification.[2] This means a questioned document is assigned to its most likely writer from a set of known writers but does not allow for the possibility of the questioned document to be written by someone not included in the set. Another method developed by Johnson and Ommen utilized machine learning techniques and Score-based Likelihood Ratios (SLRs).[3] SLRs have been criticized for a variety of shortcomings, including a lack of coherence and ability to incorporate the rarity of the features. Our goal is to develop a method that supports feature-based open-set writer identification while avoiding these issues. We implement an approach to quantify the value of forensic handwriting evidence using Bayes factors and Markov Chain Monte Carlo (MCMC) computational techniques like those described in Collins and Ommen.[4] There are two paths to consider, depending on the forensic question: the common source and the specific source identification problems. We demonstrate the approach for each identification problem using documents from the CSAFE Handwriting database, which consists of documents of various lengths from over 240 writers: the London Letter is the longest, followed by an excerpt chosen from the book *The Wonderful Wizard of Oz*, and the phrase "The early bird may get the worm, but the second mouse gets the cheese" is the shortest.[5] Handwriting features are extracted using the "handwriter" system, clustered using K-means, and subsequently used to quantify the Bayes factor.

The Bayes Factor allows us to determine which opposing hypothesis to support: the prosecution hypothesis (the questioned document was written by the suspect) or the defense hypothesis (the questioned document was written by someone else in the alternative source population). We have three pieces of evidence u_i is information we gather from the unknown or evidence (e.g., material we gather from suspect), s_j is information gathered from the control document or specific source (e.g., material we gather from suspect), and A_{ij} is information from writers in alternative source. (e.g., documents collected from everyone else related to crime). For the simulation, we set up three scenarios. One where the prosecution hypothesis is true and two where the defense hypothesis is true. For the defense hypothesis, we have a simulation where we calculate Bayes Factors for writers that are similar to each other and another where they are different from each other. The performance of the methods is assessed using cross-validation and rates of misleading evidence (among other measures).

References:
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[2.] A. Crawford, *Bayesian hierarchical modeling for the forensic evaluation of handwritten documents*, PhD thesis, Iowa State University, 2020.
[3.] Madeline Quinn Johnson and Danica M. Ommen. Handwriting identification using random forests and score-based likelihood ratios. *Statistical Analysis and Data Mining: The ASA Data Science Journal*, 15(3):357–375, 2022.
[4.] Collins GL, Ommen DM. *Quantifying the Value of Forensic Handwriting Evidence using Open-Source Feature Extraction* [thesis]. 2022.
[5.] Amy Crawford, Anyesha Ray, Alicia Carriquiry, James Kruse, and Marc Peterson. *CSAFE Handwriting Database*. 11 2019.

Statistics; Questioned Documents; Handwriting

K17 Quantifying Writer Variance Through Rainbow Triangle Graph Decomposition

Alexandra Arabio*, Center for Statistics and Applications in Forensic Evidence, Ames, IA; Alicia Laura Carriquiry, CSAFE, Ames, IA; Danica M. Ommen, Iowa State University/CSAFE, Ames, IA

Learning Overview: After attending this presentation, attendees will have a further understanding of the concept behind rainbow triangle graph decomposition and how it is able to increase quantifying the analysis of handwriting.

Impact Statement: This presentation will impact the forensic science community by demonstrating a new method for analyzing handwriting that could be used in junction with current practices to be able to better quantify results.

Handwriting comparative analysis is based on the principle that no two individuals can produce the same writing and that an individual cannot exactly reproduce his/her handwriting. This project aims to assess and quantify the natural variations produced by a distinct writer. In an attempt to support traditional examination with objective measures, this project provides results from a study where features of handwriting are examined through point decomposition and rainbow triangulation. Using this method to examine handwriting samples, more specific information can be obtained from each exemplar and can be standardized to be compared both within a writer and between different writers.

The characteristics or landmarks of each handwriting sample get marked as a different color. These characteristics include the minimal x-axis value when the sample is plotted, the maximal x-axis value, the minimal and maximal y-axis values, any stroke that overlaps (location where 2+ pixels have the same [x,y] coordinates), and any terminal locations of a writing stoke. Triangles are then formed decomposing the nodes from the origin (0,0) coordinate, then these segments are enclosed to form triangle sets systematically. Triangles provide information on angles, edge slopes, edge lengths, and areas that all prove useful for quantitative analysis and when trying to compare triangles in terms of similarity and possible congruency or similarity. By forming rainbow triangles over these samples, it is possible to gauge the variation within a single writer and to compare these quantitative values to other samples of unknown sources. Rainbow triangles are formed so that each vortex or node within a triangle set has a unique color, and each edge is unique to its triangle so that it is not to be used to form a different triangle in another set. Using this information, the study aims to form a quantitative analysis of handwriting samples and to calculate how similar or dissimilar two samples are from one another. One of the study's main goals is to form these triangles from multiple samples from several different writers and to group, identify, and accurately determine what samples came from which writer. Finally, multiple summary statistics are explored to determine whether any can be used to discriminate between inclusions and exclusions using data where ground truth is known, such as a true match. This project hopes to impact the forensic community by demonstrating a new method for analyzing handwriting that could be used in junction with current practices to better quantify and support results regarding the source of a questioned document.

Through the complete decomposition of the origin, rainbow triangles are formed over the sample. By forming Kneser Graph triangles over these samples, it is possible to gauge the variation within a single writer and to compare this quantitative value to other samples of unknown sources. Using this information, the study aims to form a quantitative analysis of handwriting samples and to calculate how similar or dissimilar two samples are from one another.

Segment Decomposition; Variability; Probability

K18   The Collection and Characterization of Counterfeit State Identity Cards and Driver's Licenses at the Homeland Security Investigations Forensic Laboratory

Luther S. Schaeffer, MSFS*, Homeland Security Investigations Forensic Laboratory, McLean, VA; Elaine X. Wooton*, Homeland Security Investigations Forensic Laboratory, McLean, VA

Learning Overview: After attending this presentation, attendees will be aware of the latest trends observed in the illicit counterfeit identity card market based upon seizures received and examined by the Homeland Security Investigations Forensic Laboratory.

Impact Statement: This presentation will impact the forensic science community by increasing awareness of the impact counterfeit identity documents have on society as a whole as well as trends observed in the production of these illicit documents.

The Homeland Security Investigations Forensic Laboratory's mission is to protect United States national security by providing expert-quality forensic, scientific, intelligence, and investigative support services to the Department of Homeland Security (DHS), Immigration and Customs Enforcement (ICE), Homeland Security Investigations (HIS), and other foreign and domestic agencies. The activities highlighted in this session will include the collection, examination, and characterization of seized counterfeit state-issued credentials (i.e., driver's licenses) conducted at the forensic laboratory. Currently, the laboratory holds approximately 250,000 counterfeit driver's licenses, and that number continues to climb. Staff at the HSI Forensic Laboratory, located in operations, questioned documents, and research and development sections, coordinate activities related to the evaluation of the online marketplaces as well as the physical items received in order to better understand and, ultimately, disrupt the flow of the counterfeit items into consumer's hands.

The United States counterfeit driver's license market is massive, with societal costs estimated to be approximately 250 billion dollars annually. The marketplaces for counterfeit driver's licenses are estimated to bring in more than 500 million dollars annually, which can then be used to improve their products, start new marketplaces, or engage in other illicit activities.[1] The effort of the HSI Forensic Lab is to develop a thorough understanding of the materials used in the production and construction of the cards, as well as fully document the methods of print production used. By amassing this information, the lab can then identify "families" of the IDs and track where they are coming from and what tools the counterfeiters may have so that issuing authorities can respond to and prevent future vulnerabilities by avoiding easily counterfeited security features, making changes to their existing design, and/or adding new features to their IDs. This presentation will provide a summary of the current state of the illicit marketplaces found on the open web, the methods of printing, assembly, and production used to simulate genuine documents, as well as characterization of the materials used to construct these documents.

Rerference:
1.   *Securing Drivers Licenses*, Document Security Alliance, https://www.documentsecurityalliance.org/forms/Securing-Drivers-Licenses.pdf. Accessed 07/26/2023.

Document Analysis; Printing; Polymers

K19   The Discrimination and Characterization of Computer-Generated Printed Documents Using Energy-Dispersive X-Ray Fluorescence Spectroscopy Coupled With Chemometrics

Nhea Verma*, Central Forensic Science Laboratory, MHA, GoI, Shimla, Himachal Pradesh, India; Vishal Sharma, Panjab University, Institute of Forensic Science, Chandigarh, India; Dinesh K. Sahu, SFSL Chattisgarh, Raipur, Chhattisgarh, India

Learning Overview: This presentation will demonstrate the methodology for examination of computer-generated documents in a comprehensive way based on their spectral behavior and chemometric analysis using analytical methods in a completely non-destructive manner. This presentation will allow attendees to have better understanding of the use of multivariate statistical analysis for the extraction of important spectral features necessary for discrimination and characterization studies. This presentation will also include highlights about the shift in the dynamics of crimes in the recent past, which will allow attendees to identify the pointers that play a significant role in adopting a certain methodology for examination and analysis of exhibits in real case examinations. Also, the representation of data in the form of graphs, chats, etc. in this presentation will prove to be their best source of information regarding the forensic document examination.

Impact Statement: This presentation will impact the forensic science community by introducing attendees to information about how documents are frequently encountered as the common tools of fraudulent manipulation, the objects of counterfeiting, and the ways of concealing incriminating truths in the form of disputed signatures, threatening letters, counterfeit currency, false medical documents, etc. Also, attendees will learn the capabilities of multivariate statistical analysis for the extraction of important spectral features necessary for discrimination and classification of exhibits that are commonly received in forensic science laboratories.

The present study focuses on the examination of computer-generated documents (black toners) in a comprehensive way based on the spectral behavior and chemometric studies of these samples when analyzed with analytical methods. The idea behind the present approach is to characterize, discriminate, and classify the printed documents obtained from various sources of laser printers and photocopiers. The complex nature of toner powders due to the presence of large variety of binders, colorants, charge control agents, magnetic agents and other additives have posed a great challenge to the identification and examination of the forged and counterfeit documents that have increased in the number of crimes with the availability of advanced technology at very affordable and accessible terms.[1-3] The suspected or fraudulent documents include false medical certificates, university degrees, property papers, cloned checks, counterfeit currency, forged bills, etc. and are frequently received in forensic science laboratories for the identification and examination of their source of origin.

Given the size and complexity of document examinations, there is a need for practitioners to formally and strategically determine a course of conduct that allows them to undertake the most robust and efficient examination possible. The detection of computer-generated document forgeries has always been a difficult and time-consuming task for forensic document examiners.[4] With the aim to support the aforesaid examination processes, Energy-Dispersive X-ray fluorescence spectroscopy (EDX-8000) is explored as a recent tool to analyze black toners obtained from laser printers and photocopier machines. Thirty samples each from the laser printer and photocopier machines were procured and studied for elemental profile and multivariate analysis. The acquired data was evaluated to discriminate and classify the toners having a different source of origin. Multivariate analysis was applied to develop a model of classification to successfully classify the printed documents on the basis of the similarities and differences in their composition. Hierarchical Cluster Analysis (HCA) discriminates the printouts in the forms of groups based on their chemical composition. The laser printer and photocopier printed documents were grouped into respective clusters based on their elemental composition. Cross-validation was further conducted to assess the capabilities of developed Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) models for the examination of printouts from unknown origins.[5-7]

References:
1. Verma, N., Sharma, V., Kumar, R., Sharma, R., Joshi, M.C., Umapathy, G.R., Ohja, S. and Chopra, S., 2019. On the spectroscopic examination of printed documents by using a field emission scanning electron microscope with energy-dispersive X-ray spectroscopy (FE-SEM-EDS) and chemometric methods: application in forensic science. *Analytical and bioanalytical chemistry* 411, 3477-3495.
2. Verma, N., Kumar, R. and Sharma, V., 2018. Analysis of laser printer and photocopier toners by spectral properties and chemometrics. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 196, 40-48.
3. Marshall G. Recent progress in toner technology. *Soc Imaging Sci Technol*; 1997.
4. Scharfe M. *Electrophotography and optimization*. Letchworth, Hertfordshire, England: Research Studies Press Ltd. New York: John Wiley & Sons INC; 1984.
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6. Kumar, R. and Sharma, V., 2018. Chemometrics in forensic science. *TrAC Trends in Analytical Chemistry*, 105 191-201.
7. Totty R. The examination of photocopy documents. *Forensic Sci Int.* 1999; 46: 121–6.

Fraud; Questioned Documents; Statistics

K20   The Hitler Diaries Revisited

Thomas W. Vastrick, BS, Apopka, FL*

Learning Overview: After attending this presentation, attendees will be informed about the forensic evidence in the matter of the publication 40 years ago of the alleged existence of substantive handwritten diaries by Adolph Hitler. Attendees will learn the background of the diaries' creation and the misinformation promoted by the highly publicized sensationalism that was part of the diary publicity.

Impact Statement: This presentation will impact the forensic science community by educating younger scientists as to some of the pitfalls that come with highly sensational cases and what can be done to limit the spread of disinformation. In addition, this presentation will educate the forensic science community so it is better prepared to address any questions that may arise concerning this issue.

It was 40 years ago (April 22, 1983) that a German publication, *Stern Magazine*, advised the world of the existence of 60 volumes of handwritten diaries from Adolph Hitler covering the years 1932 through 1945. The news was sensationalized around the world. However, the claims by *Stern Magazine* were also met with almost immediate skepticism. In support of their position, *Stern Magazine* noted that three independent forensic document examinations were conducted, and the writing was verified. This information was not accurate and, ultimately, exposed the fraud instigated by certain individuals with monetary incentives for acceptance of these diaries as real. One was Konrad Kujau, a.k.a. Conrad Fischer, an artist with a background in painting and poems purportedly authored by Adolph Hitler. Another was Gerd Heidemann, a reporter for *Stern Magazine* who owned one of Hermann Goering's boats and, for a time, was in a romantic relationship with Edda Goering (Hermann's daughter). The third player was Thomas Walde, Head of Contemporary History for *Stern Magazine*. Both Heidemann and Walde held contracts for profit sharing of the diaries.

The three document examiners consisted of Mr. Ordway Hilton, document examiner in private practice in New York City; Max Frei-Sultzer (Frey-Zuler), an examiner with the Zurich Police Forensic Science Department; and a third unnamed examiner "in the employ of the German police." These examiners were each supplied with one page of the diaries. This is a significant omission in that it was common knowledge that Hitler suffered physical deterioration, particularly in the last few years of his life. Further, the alleged known specimens were comprised of documents from the personal collections of Mr. Heidemann and Mr. Walde that had also been written by Mr. Kujau. Mr. Hilton explained that his conclusion was that the questioned writing on the one page of the alleged diaries was written by the same person that wrote the submitted known specimens. This conclusion was accurate. The only problem was that neither had been written by Hitler, even though that was the promotion of the results. One lesson learned from this situation was the importance of insisting on independent known specimens. Indeed, current published industry standards specifically caution examiners about using solely specimens provided by stakeholders in litigation.

As we reach the 40th anniversary of *Stern Magazine's* announcement, some of the misinformation is finding its way back into journalistic streams.[1] The article will be dissected regarding its accuracies and inaccuracies.

Reference:
[1.]   Tirchie, Stuart. The fake Hitler Diaries are 40 years old, but we still haven't learnt from the mistakes. inews.co.uk, April 22, 202

Forgery; Fraud; Questioned Documents

K21   A Framework for the Creation of QuompenDium—The Mega-Site for All Things QD

Zain Bhaloo, MSc*, Canada Border Services Agency, Ottawa, ON, Canada

Learning Overview: Attendees to this presentation will learn about the proposal to create QuompenDium, a "compendium" for the Questioned Documents (QD) community that would enable examiners direct, quick, centralized, and reliable access to our daily common-use tools, references, information, and much more.

Impact Statement: This presentation will impact the Forensic Document Examiner (FDE) community by laying out the framework for the creation of the QuompenDium that would one day be a jumping-off point to relevant information required by QD examiners.

As the amount of information available online for any given topic grows, so too does the number of places in which this information can be found. Access to this information can be time-consuming, convoluted, and scattered. Even though access to the information online is generally open, it requires FDEs to scour multiple locations, and often these repositories are incomplete, which means not all laboratories and examiners have access to the same important information. These inefficiencies, combined with a lack of places to access important historical and archival information, spotlight the necessity for a centralized location that is globally accessible, globally relevant, and updated regularly that FDEs can call on quickly and easily to get the information they need.

It is for these many reasons that we propose the creation of the QuompenDium—the FDE's mega-site for all things FDE. It is a "one stop shop," a compendium, for the FDE community that would enable FDEs direct, quick, centralized, and reliable access to our daily common-use tools, references, information, and much more. It would eventually allow access to forums for discussion, standards, organizations and bodies, FDE labs and examiners, conferences, references, news, legal documents, and even specialized subject matter expert areas. The QuompenDium would be updated regularly and on demand with new and verified links, new reference papers, weekly FDE-related news, upcoming events worldwide, access to newly published standards, and much more.

This presentation will lay out the framework for the creation of the QuompenDium that would one day be a jumping-off point to relevant information required by FDEs and will demonstrate it not only as a means to access information but also as a means to improve the timeliness and method of delivery of the information. The presentation will also include built-in time for comments, concerns, suggestions, and requests from the FDE community present to allow for as fully complete a compendium as is possible.

Questioned Documents; Reference Material; Standards

K22   A Proposal to Update SWGDOC Standards During the Transition Period: A Discussion

Thomas W. Vastrick, BS*, Apopka, FL

Learning Overview: After attending this presentation, attendees will be informed about the current status of published industry standards. The still-valid Scientific Working Group for Forensic Document Examination (SWGDOC) standards not currently retired through the accreditation of standards by the American National Standards Institute/Academy Standards Board (ANSI/ASB) are more than a decade old. As such, the need to update or re-accept these standards is long overdue. This presentation will be an open discussion and attendee participation will be encouraged.

Impact Statement: This presentation will impact the forensic science community by encouraging the immediate update of numerous published industry standards within the forensic document examination community while we are in a transitory state with our standards.

In 2012, SWGDOC ceased its relationship with the American Society for Testing and Materials (ASTM) International. Shortly after that, the United States Department of Commerce initiated a project to unify SWGs under one umbrella entity in order to standardize the standards to the extent possible. Ultimately, the Organization of Scientific Area Committees (OSAC) and the Academy Standards Board (ASB) have been created in order to develop and gain accreditation of published industry standards. This is a long process. While we are in a transitory period, we rely on the newly published standards as they are developed and the SWG standards in areas yet to be updated. One negative result of this transitory period is that certain standards are becoming dated. This can be, and should be, addressed. How can we update our previous standard without creating, essentially, a third entity involved in standards development? One way is to approve updates en masse without any changes for standards that have maintained their relevancy over time. In addition, there is the opportunity to address any significant tweaks that need to be made based on issues that may have arisen over the past ten years or so.

This presentation will be in the form of an open discussion within the forensic document examination community to assess what can be done concerning SWGDOC standards that have yet to be retired. Can SWGDOC standards not retired by the accreditation of ASB standards of the same subject be themselves updated? Can any or all of these SWGDOC standards be voted upon to be accepted as is should it be deemed that they are still fully relevant? This discussion will address the SWGDOC standards still applicable and discuss their content so that some consensus may be found. In addition, this discussion will also attempt to develop a procedure in order to achieve these goals. Upon completion, it is hoped that this information will result in SWGDOC updating their current existing standards.

Document Analysis; Standards; Forensic Science

K23   An Update on Standards Development Activities Related to Forensic Document Examination

Kevin P. Kulbacki, MFSF*, KDX Forensic Consulting, LLC, Chicago, IL

Learning Overview: After attending this presentation, attendees will better understand the status of the current and upcoming Forensic Document Examination (FDE) standards being developed by the Organization of Scientific Area Committees (OSAC) FDE Subcommittee and the Academy Standards Board (ASB) FDE Consensus Body.

Impact Statement: This presentation will impact the forensic science community by creating greater awareness of the current standards development activities related to FDE. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

During this presentation, an update will be provided on the standards development activities related to Forensic Document Examination. This will begin with an introduction to OSAC and ASB, and an overview of the standard development process as it relates to both organizations.

Second, this presentation will discuss OSAC Proposed Standards at various stages of development. The OSAC FDE Subcommittee is currently working on new Draft Proposed Standards as well as on submitting current ASB standards to the OSAC Registry.

Third, this presentation will discuss Draft ASB Documents (including Best Practices, Standards, and Technical Reports) at various stages of development at ASB. The ASB FDE Consensus Body is currently working on multiple new documents, some of which have already gone through rounds of public comments.

Fourth, this presentation will discuss completed ASB Standards. As of the writing of this abstract, the ASB has published seven Forensic Document Examination Standards, namely the Standard for Examination of Documents for Indentations, the Standard for the Examination of Documents for Alterations, the Standard for Examination Stamping Devices and Stamp Impressions, the Standard Scope of Expertise in Forensic Document Examination, the Standard for Examination of Handwritten Items, the Standard for the Preservation and Examination of Charred Documents, and the Standard for the Preservation and Examination of Liquid-Soaked Documents. The ASB Forensic Document Examination Consensus Body is also currently working on eight additional documents.

Finally, this presentation will highlight efforts by OSAC and ASB for supplemental training related to all standards that go through this process, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

The categorization of the in-progress documents listed herein is accurate as of the writing of this abstract and may be subject to change prior to the presentation, which will reflect the most accurate information at that time.

Standards; Forensic Document Examination; OSAC and ASB

K24   New ASB Standards for Forensic Document Examiners: How They Differ From the Standards They Superseded and What You Need to Know to Use Them

Mark Goff, BA*, MSP Lansing Laboratory, Lansing, MI; Stephanie Kingsbury, MFS*, U.S. Postal Inspection Service, National Forensic Laboratory, Dulles, VA

Learning Overview: The goal of this presentation is to compare and contrast legacy Scientific Working Group for Forensic Document Examination/American Society for Testing and Materials (SWGDOC/ASTM) standards with recently published Academy Standards Board/American National Standards Institute (ASB/ANSI) forensic document examination standards. After attending this presentation, attendees will have a better understanding of the changes to standards they were familiar with and what changes they may need to make in their examination procedures to comply with the new standards.

Impact Statement: This presentation will impact the forensic science community by increasing awareness of changes to standardized practices within the field of forensic document examination. Forensic managers will have a resource to use when evaluating new standards for implementation within their respective laboratories.

The Forensic Document Examination (FDE) field has a long history of standardization that started in the 1990s and has continued through ASTM, SWGDOC, and, most recently, ASB/ANSI. The legacy ASTM/SWGDOC standards, while pioneering in their inception, progressed through iterative changes over many years and suffered limited updates over the past ten years. With the major changes in perception of forensic science over the past 15 years, the development of the Organization of Scientific Area Committees for Forensic Science (OSAC) and the change from ASTM/SWGDOC to the Academy Standards Board (ASB), many of the updated ASTM/SWGDOC standards experienced generational changes that may be vastly different than the previous standards.

This presentation will seek to highlight some of the changes to previously published standards to ensure that the forensic document examiner has a complete understanding of the current state of FDE standards. Some standards such as the *Standard for the Preservation and Examination of Charred Documents*, *Standard for the Preservation and Examination of Liquid Soaked Documents*, *Standard for Indentation Examinations,* and the *Standard for the Examination of Documents for Alterations* underwent more minor changes.[1-8] However, some of the legacy standards experienced larger changes, such as; *Standard for Examination of Rubber Stamp Impressions* becoming the *Standard for Examination of Stamping Devices and Stamp Impressions*, the *Scope of Work in Forensic Document Examination* changing to the *Scope of Expertise in Forensic Document Examination*, with expanded content that include a listing of required hours of training, and the *Standard for Examination of Handwritten Items* that now includes addressing screening out potential writers, and a requirement to consider alternative interpretations, to list a few.[9-14] This presentation will even cover changes and updates to the standard that never dies, the *Standard for Examination of Mechanical Checkwriter Impressions and Machines,* and some proposed changes to standards that are currently in development.[15,16]

References:
1. ANSI/ASB Standard for the Preservation and Examination of Charred Documents, 2022.
2. SWGDOC *Standard for Preservation of Charred Documents*, 2013.
3. ANSI/ASB *Standard for the Preservation and Examination of Liquid Soaked Documents*, 2022.
4. SWGDOC *Standard for Preservation of Liquid Soaked Documents*, 2013.
5. SWGDOC *Standard for Indentation Examinations*, 2013.
6. ANSI/ASB *Standard for Examination of Documents for Indentations*, 2019.
7. NSI/ASB *Standard for the Examination of Documents for Alterations*, 2020.
8. SWGDOC *Standard for Examination of Altered Documents*, 2013.
9. SWGDOC *Standard for Examination of Rubber Stamp Impressions*, 2013.
10. ANSI/ASB *Standard for Examination of Stamping Devices and Stamp Impressions*, 2020.
11. SWGDOC *Standard for Scope of Work of Forensic Document Examiners*, 2013.
12. ANSI/ASB *Scope of Expertise in Forensic Document Examination*, 2022.
13. ANSI/ASB *Standard for Examination of Handwritten Items*, 2022.
14. SWGDOC *Standard for Examination of Handwritten Items*, 2013.
15. ANSI/ASB *Standard for Examination of Mechanical Checkwriter Impressions and Machines*, 2023.
16. SWGDOC *Standard for Examination of Mechanical Checkwriter Impressions*, 2013.

Questioned Documents; Document Analysis; Standards

L1   Comparing the Performance of LC-QqQ-MS vs. LC-QTOF-MS for Targeted Analysis of NPS with a Test Mixture of 40 NPS and Metabolites in Whole Blood, Urine, and Oral Fluid

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Learning Overview: After attending this presentation, attendees will have a scientific comparison of two effective methods of targeted analysis for Novel Psychoactive Substances (NPS) using a complex mixture of substances in relevant biological matrices.

Impact Statement: This presentation will impact the forensic community by presenting scientific evidence showing which instrument and method is most effective in each circumstance of targeted analysis of NPS in a biological sample.

Background/Introduction: To combat the rising use of NPS, laboratories have become more sophisticated in screening and identifying compounds. While immunoassays are the preferred technique for screening common drugs, chromatographic separation coupled with mass spectrometry is the preferred method for detecting NPS due to its selectivity, specificity, and sensitivity. Liquid Chromatography-Mass Spectrometry (LC-MS) configurations include those with either low or high resolution via Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-QqQ-MS) and Liquid Chromatography Quadrupole Time Of Flight Mass Spectrometry (LC-QTOF-MS), respectively. However, because there is a lack of consensus on the relative screening performance for these methods, a rigorous comparison is necessary for their use in the forensic analysis of NPS.

Objective: This work compares the effectiveness of LC-QqQ-MS and LC-QTOF-MS with Data-Dependent Acquisition (DDA) to analyze multiple NPS. In this project, a 40-compound test mixture of NPS containing various structures and pharmacological classes was used to spike authentic matrices of whole blood, urine, and Oral Fluid (OF). The mixture included closely eluting NPS analytes, isomers, and metabolites that have a wide range in polarity to allow for the assessment of selectivity and other identification algorithms from the data obtained by each instrument. The performance of both acquisition methods was compared using selected figures of merit, including LOD, dynamic range, linearity, selectivity, precision, carryover, and matrix effects.

Methods: Individual standard solutions of NPS were spiked in methanol to create a mixture with a final concentration of 200 ng/mL for each compound. The mixture was diluted with blank matrix to yield concentrations of 0.5, 1, 2, 5, 10, 20, and 50 ng/mL to determine the parameters above for the targeted MS studies. Two extraction methods were used: dilute-and-shoot for urine and crash-and-shoot for whole blood and OF. The LC-QqQ-MS used a dMRM mode to measure selectivity, whereas specific MRM data was acquired to identify each compound based on their retention time and transitions, including specific collision energies, fragmentor voltages, and product ion selections for each compound. The LC-QTOF-MS used a targeted MS/MS mode that specifically targeted the precursor, retention time and collected fragment ion data using a range of collision energies. Positive electrospray ionization was used for all NPS compounds with both instruments.

Results: The performance of these targeted methods was evaluated by their sensitivity, linear range, precision, matrix effects, and specificity in whole blood, urine, and OF. The performance of each instrumental platform for each analyte was evaluated using statistical significance testing and a scoring system based on all measured figures of merit. For example, a comparison of data for the NPS 2,5-DMMA in urine indicated that LC-QqQ-MS would be the best approach for screening and confirmation of this analyte because of the higher score for this drug (9 out of 10) compared to the QTOF (4 out of 10). These values were based on a scoring system designed to provide a numerical approach to typically qualitative figures of merit. The higher score was based on a lower LOD, a linear regression of 0.99 or higher, the precision below 10% CV, the matrix effects with a deviation less than 10%, and lastly, sensitivity with specificity providing a true positive and true negative.

Conclusion/Discussion: When completed for all 40 compounds, this study identified which of these targeted methods was more effective for general forensic toxicological screening of a wide range of NPS in each of three essential specimen matrices.

Targeted Methods; New Psychoactive Substances; Forensic Toxicological Screening

L2   4-Substituted AH-7921 Analogs: Isomer Selection During Synthesis and Pharmacological Profile

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Learning Overview: After attending this presentation, attendees will be informed that AH-7921 is a scheduled narcotic and the activity of 4-substituted analogs has recently been reported. The pharmacological profile and potential for harm will be presented along with spectral characteristics. Synthesis of these analogs results preferentially in one of two possible geometric isomers. Isomeric selectivity was assessed using Nuclear Magnetic Resonance (NMR) spectroscopy for analogs with large to small substituents on the cyclohexane ring.

Impact Statement: This presentation will impact the forensic science community by presenting data that will aid in the identification of the materials when encountered. In addition to the toxicity expected for opioids, the pharmacological profile also identifies a greater potential for harm from serotonin syndrome than opioids such as morphine.

4-Substituted AH-7921 analogs have only recently been described in detail.[1] The analog *trans*-3,4-dichloro-N-[[1-(dimethylamino)-4-phenylcyclohexyl]methyl]-benzamide (4-phenyl-AH-7921) has been shown to be preferentially created at an intermediary step in its synthesis. Specifically, the formation of a-aminonitriles from substituted cyclohexanones results in unequal amounts of the two possible geometric isomers due to diastereofacial selectivity. This phenomenon has been reported for the nucleophilic attack of cyclohexanones and results from the angle of attack on the ketone by the nucleophile.[2,3] In this reaction, cyanide ion, the nucleophile, attacks an intermediate iminium ion in a similar manner as is seen with cyclohexanones. Also, the degree of selectivity observed increases with the degree to which the substitution can 'lock' the conformation of the cyclohexyl ring. The ratio of *trans:cis* isomers seen for the reaction of substituted cyclohexanones with dimethylamine hydrochloride and potassium cyanide in water were 92:8 for 4-phenyl, 81:19 for 4-*t*-butyl, and 62:38 for 2-methyl. The size of the amino group substituents did not have a large effect on the selectivity.

The ^{1}H-NMR, MS, IR, and Raman spectra will be presented.[1] In rats, 4-phenyl AH-7921 exhibited analgesic activity in the acetic acid writhing test. The pharmacological profile was provided by a battery of *in vitro* binding assays for 43 Central Nervous System (CNS)-relevant receptors. 4-Phenyl AH-7921 is a potent mu-opioid receptor and kappa-opioid receptor ligand (60nM and 34nM, respectively) and has a 4nM affinity for the Serotonin Transporter (SERT), which is a higher level of potency at this receptor than most other opioids. Blocking the serotonin transporter, such as serotonin reuptake inhibitors like paroxetine, results in increased extracellular concentrations of serotonin and increased serotonergic neurotransmission. Such high affinity for the serotonin transporter raises the possibility that this compound may be more prone than other opioids to exacerbate serotonin syndrome. This side effect has been documented for other opioids that have affinity for this transporter.[4]

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Novel Synthetic Opioids; Toxicity; Spectroscopy

L3   A Rare Case of Fatal Levamisole Toxicity

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Learning Overview: This presentation discusses a case of accidental levamisole ingestion causing death. There is only one other known published case report of levamisole causing death, which was not associated with cocaine toxicity. In that instance, levamisole was injected with intent to self-harm. This case highlights the rare, yet potentially fatal outcome of accidental levamisole ingestion.

Impact Statement: This presentation will impact the forensic science community by informing attendees that levamisole has often been seen in association with cocaine as a cutting agent and has been implicated in several cocaine-related deaths. This is believed to be the first case report of accidental levamisole ingestion causing death in the absence of cocaine.

This is the case of a 64-year-old male who mistakenly ingested a levamisole solution ("Prohibit Soluble Drench Dewormer for Cattle and Sheep") thinking it was regular bottled water after doing some work outside. He and his wife had sheep that were presented at livestock shows and they kept the solution in a spare refrigerator. The levamisole was mixed with water and was kept on the inside of the refrigerator door, with other livestock medication on the same shelf. The solution had been prepared approximately 2 months prior and had a 3-month shelf life. His wife reported that he immediately spit out the remaining liquid from his mouth and started feeling ill 20-30 minutes later, including attempts to vomit with cold sweats and vision changes. He initially declined an ambulance, and his wife subsequently drove him to a local fire station, where he became unresponsive while being loaded into an ambulance. Standard advanced life support efforts were begun, and he was transported to a local emergency department where he was noted to be bradycardic and hypotensive and then developed ventricular dysrhythmia before asystole. Death was pronounced 35 minutes after arrival at the hospital without evidence of trauma or foul play.

Autopsy and microscopic examination findings included an enlarged heart and focal mild-moderate coronary artery disease. Initial toxicology (femoral blood obtained one day postmortem) testing was positive for home medications and medications administered during resuscitation, including diphenhydramine, bupropion, trazodone, ketamine, and atropine. Vitreous electrolytes were unremarkable. Additional testing of femoral blood by a reference laboratory revealed levamisole at 19 mcg/mL (19 mg/L).

While levamisole's uses have been multifaceted[7] throughout the years, it is now primarily used in veterinary sciences[5,7] as a deworming agent. Levamisole primarily treats cases of ascariasis and hookworm infections,[8] but has also been used as a dermatological agent[9] to treat certain skin infections. However, in the early 2000's, levamisole became known as a cocaine adulterant.[10] While it is not definitively known why this occurred, some speculate that since levamisole metabolizes into aminorex, which is an amphetamine derivative,[10] the combination with cocaine allowed for potentiating and prolonging cocaine's effects. Levamisole was also noted to be a cutting agent for cocaine, bulking up the drug to increase profits for dealers.[13] The DEA reported in 2009 that "levamisole (Ergamisol) was found in as much as 69% of seized cocaine."[10]

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Overdose; Toxicity; Cause of Death

L4   A Mass Disaster Caused by Gas Leaks: A Case Report and a Forensic Workflow Proposal

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Learning Overview: After attending this presentation, attendees will be able to adopt a new workflow in cases of mass disaster due to a gas leak, which is based on a multidisciplinary approach between forensic pathologists and toxicologists.

Impact Statement: This presentation will impact the forensic community by showing a new multidisciplinary approach between forensic pathologists and toxicologists for death investigations.

Explosion-related deaths are uncommon events that require skills and communications so that a proper death investigation can be carried out [1]. Accidental home explosions are a small subset of explosions that can be associated with gas leaks, especially from Liquified Petroleum Gas (LPG). This group of products includes saturated hydrocarbons - propane and butane, which can be stored and transported separately or as a mixture. Fatal accidents occur despite safety guidelines [2-4]. It is based on an appropriate crime scene investigation in collaboration with the police, an accurate autopsy, and an accurate toxicological analysis with cadaveric and environmental comparison of the LPG concentration.

In March 2018 in Catania, some people smelled a strong smell of gas coming from a habitation and warned the firefighters. A team of firefighters made up of four firefighters and a driver performed the inspection of the building and, through the explosimeter, ascertained a high concentration of gas. Since the front door was locked with a padlock, an explosion was required in an attempt to access the house. The explosion caused the destruction of the house and the death of two of the four firefighters. During the crime scene investigation, it was noted that there was an additional cadaver inside the house. A gas cylinder with an open valve was seen and seized.

A complete autopsy was performed of the three cadavers (two firefighters and one cadaver found in the apartment). The cadavers of the firefighters showed blast injuries in different body regions (rupture of the heart, hemorrhages of the brain, hemothorax, multiple pulmonary contusions) with fractures of the skull and chest. The death of the two firefighters was attributed to the strong explosion.

The body found in the house was burned. He underwent post-mortem CT, which excluded body fractures. The autopsy showed intense soot in the upper airways.

A toxicological analysis was performed through GC-MS analysis. Forensic toxicology results in the cadaver showed the absence of carbon monoxide and the presence of propane and butane at a non-fatal concentration. The same toxicological analysis was also performed on the gas cylinder and showed a very high concentration of the same gases present in the cadaver. Thanks to the help of toxicology it was possible to establish that the cause of death was due to flash-fire death.

The results of this study underline the importance of applying a forensic protocol that includes an autopsy and a toxicological analysis of the amount of LPG present in the cadaver, as well as the environmental analysis of LPG at the crime scene. In fact, thanks to the combination of a thorough autopsy and a careful toxicological examination, it was possible to trace the exact cause of death of all three corpses found in this gas leak mass disaster. We suggest adopting a similar approach in cases involving death from explosions involving forensic pathologists and toxicologists.

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Intoxication; Mass Disaster; Intoxication

L5   Postmortem Redistribution in Forensic Toxicological Investigations

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Learning Overview: After attending this presentation, attendees will appreciate the utility of the toxicologist's assessment in medicolegal investigations. Postmortem redistribution (PMR) is well known among toxicologists but is less known among coroners, who often find themselves interpreting toxicological data on their own.

Impact Statement: Proper sampling and data interpretation are critical to establishing a fatal case of overdose or poisoning. This presentation will impact the forensic science community by informing attendees that PMR is a critical phenomenon that must be taken into consideration during forensic investigations. More studies are needed to accurately explain the PMR phenomenon and how it could influence the toxicological interpretation.

PMR is a critical phenomenon for forensic investigation. Drugs, substances of abuse, and their metabolites, even postmortem, seem to migrate from one part of the body to another; thus, making the interpretation of the results obtained in the forensic-toxicological investigation complex.[1] So far, the PMR is described as a process influenced by a transmembrane passive diffusion due to difference of gradient between blood and tissues.[2]

In the present study, scientific papers on PMR have been reviewed, paying attention to the methodology and the obtained results.[3-5] All the examined published studies are based on the ratio between the concentration found in central/cardiac blood and that in peripheral/femoral blood. This ratio is used to determine whether a substance is subject to PMR. In our review, it emerged that no methodology applied up to now has exhaustively managed to explain the phenomenon of PMR and the reported data are often conflicting. PMR does not appear to occur in all cases, even involving the same substance. Furthermore, for each substance, the number of considered cases was not suitable for a statistical evaluation. The careful analysis of the published results also raises many questions on the mechanism of simple passive diffusion widely assumed so far.

Moreover, it was investigated how much PMR could influence the toxicological investigation, comparing more than 200 cases of drugs detected in central blood with the therapeutic, toxic and lethal reference concentrations. A comparison with femoral blood was not included in our study. Considering the 241 data points obtained for central blood, 91.3% of concentrations were within the therapeutic range, with 2.9% higher but non-toxic, 4.1% toxic, and 1.7% lethal. Toxic and lethal concentrations were in accord with circumstantial data

PMR is probably influenced by multiple factors not necessarily intrinsic to the substance, none of which has so far been adequately investigated. Some of the possible variables underlying PMR will also be discussed in the presentation. The data obtained from central blood samples appear to provide adequate information to discriminate cases of normal drug intake from those of overdose and poisoning

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Postmortem Redistribution; Forensic Toxicology; Death Investigation

L6   My Way: A Case of Lethal Intoxication of an Animal Euthansia Drug in a Veterinary Doctor

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Learning Overview: After attending this presentation, attendees will have more knowledge on the lethal action of a combination of embutramide, mebezonium iodide, and tetracaine hydrochloride, a drug used for animal euthanasia.

Impact Statement: This presentation will impact the forensic science community by presenting the importance of implementing research to better comprehend the lethal combination of embutramide, mebezonium iodide, and tetracaine hydrochloride in human beings and its effects on the human body as abuse drug or in case of accidental intoxication.

Narcotic substances are often used in suicide attempts. The authors present an uncommon case of suicide committed by Tanax®, a drug typically used for animal euthanasia; Tanax® is a combination of embutramide, mebezonium iodide and tetracaine hydrochloride.

The case presented concerns a 50-year-old veterinarian found dead in his home. The body was found on a sofa in front of the door. No injuries were revealed, only a needle that was inserted into the left elbow vessel. The needle was connected through an outflow tube to a vial of Tanax® tied to a floor lamp, which was used as an infusion pole. On the left arm there was also a haemostatic lace. In the apartment, there was also a vial of Fentadon®(Fentanyl) used in veterinary medicine as an analgesic opioid, an open bottle of whiskey next to the body, and a farewell message left to his relatives that confirmed the suicidal intention.

A blood sample was taken from femoral vessels and the toxicology examination was performed using Liquid Chromatography Tandem Mass Spectrometry (LC-MS-MS) analysis.

The toxicology screen confirmed the presence of embutramide and mebezonium iodide in the blood at lethal concentrations. In the organism, these substances act as curariform drugs paralyzing the skeletal muscles and rapidly inducing respiratory collapse due to paralysis of the diaphragm and intercostal muscles. Tanax® also has a strong narcotic effect that causes paralysis of the breathing centres in the central nervous system[1].

In the literature, few deaths related to Tanax® are described, but none with as high blood concentrations as were observed in this case (embutramide: 53,4 mg/L and mebezonium iodide 33,1 mg/L).

The present case underlines the need for a greater comprehension of lethal Tanax® concentrations for humans and an increased awareness of effects on the human body. Tanax® also includes embutramide, which is derived from Gamma Hydroxybutyrate (GHB), a potential drug of abuse used for its narcotic and aesthetic effects.

A deeper knowledge on this drug is also necessary to determine how to act in cases of an accidental intoxication, a real eventuality among veterinary workers

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Intoxication; Suicide; Veterinary Medicine

L7   A Ten-Year (2013–2022) Retrospective Analysis of Road Traffic Deaths in the Northern Italian Region of Campania

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Learning Overview: After attending this presentation, attendees will better understand the Italian trend in alcohol and drug use among victims of road traffic accidents.

Impact Statement: This presentation will impact the forensic community by providing data collected from the postmortem toxicological analyses performed on biological samples from 228 road accident victims in a Southern Italian region over the past ten years (2013–2022). The goal of this study was to investigate the prevalence of alcohol and licit/illicit drug consumption among victims of road traffic accidents.

Driving under the influence of alcohol (DUI) and/or licit/illicit drugs (DUID) is largely considered a risk for road safety. In Italy, DUI and DUID is regulated by articles 186 and 187 of the Italian Road Traffic Law, which was recently updated with the introduction of more severe penal sanctions. The legal limit of Blood Alcohol Concentration (BAC) is 0.5g/L, while zero tolerance for illicit drugs has been established. Based on the 2021 report of the National Institute for Statistics (ISTAT), 151,875 road accidents with 2,875 dead victims occurred in Italy among which 9.014 in Campania with 214 dead victims. The aim of the study was to collect data useful for the improvement of toxicological analyses and preventive policies. A retrospective analysis of deaths related to road traffic accidents from 2013 to 2022 in Campania was performed based on post-mortem toxicological findings taken from biological samples collected at autopsy.

Dead victims from fatal road accidents (n=228) were accounted in total from 2013 to 2022: 82 from 2013 to 2018 and 146 from 2019 to 2022. Most traffic accidents involved cars and motorcycles (87,7%) and a few involved bicycles, trucks and trains. 106 out of 228 cases (46,5%) tested positive for alcohol and/or drugs. Among the positive cases, 104 males (98,1%) and 2 females (1,9%) were accounted. Most of the victims belonged to the 40-49 years age-group (28,3%). Victims were mostly positive for licit/illicit drugs (40 out of 106 cases - 37,7%), followed by alcohol (39 out of 106 cases - 36,8%). 27 out of 106 cases (25,5%) were positive for alcohol and drugs in combination, among which cocaine was the most detected drug (18 out of 27), followed by delta-9-THC (11 out of 27) and benzodiazepines (4 out of 27). 21 cases of polydrug use were detected (19,8%), among which 9 cases were in combination with alcohol. In polydrug use, the most common combination was cocaine and D9-THC (8 out of 21 cases). Regarding the distribution of drugs detected among the 67 DUID cases, cocaine was found in most of cases (30 out of 67), followed by delta-9-THC in 22 cases, benzodiazepines in 16 cases, and other pharmaceuticals. BAC >1,5 g/L was found in most of the DUI cases positive for alcohol alone (28 out of 39 cases) or in combination (13 out of 27 cases). According to the position of the victim inside the vehicles, 74 of 106 victims (69,8%) were drivers mostly driving motorcycles (39 cases), followed by cars (28 cases) and bicycles (5 cases). Six victims were passengers and 21 were pedestrians mostly struck by cars. Most accidents occurred at night (58 cases) and during the weekends. Multiple injuries to the whole body was the most common cause of death (82 out of 106 cases - 77,3%), followed by thoraco-abdominal injuries (18 out of 106 cases, 17%); hemorrhagic shock and head/neck injuries. Most victims died quickly after the impact crashes (81out of 106, 76,4%) while 17 victims died in the hospital after few hours (16%).

In conclusion, the abuse of alcohol and drugs is a major public safety problem in Europe and the USA, where DUI and DUID cases represent approximately 34% and 31% of all road deaths, respectively.[1-2] According to these data, legal sanctions in DUI and DUID cases seem to have a poor impact. Road-crashes remain the eighth leading cause of death.[1-6] Therefore, a multidisciplinary approach, involving educational and preventive strategies should be promoted.

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Driving Under the Influence of Alcohol (DUI); Driving Under the Influence of Drugs (DUID); Road Traffic Deaths

L8   Trends in Neonatal Deaths From Fentanyl

Emily Fenton*, NMS Labs, Lansdale, PA; Kari M. Midthun, NMS Labs, Horsham, PA; Brianna Peterson, NMS Labs, Horsham, PA

Learning Overview: The goal of this presentation is to analyze trends in neonatal deaths. Data mining techniques were applied to focus on fentanyl-related neonatal deaths from cases received and reported by NMS Labs from January 2019 through July 2023.

Impact Statement: This presentation will impact the forensic science community by presenting findings that suggest that the neonatal opioid and polydrug crisis is rampant, mirroring trends seen among adults. There must be a greater emphasis on harm reduction strategies, including parental treatment for drug abuse.

Fentanyl is a prescription opioid used as an anesthetic/analgesic. It is reported to be 80 to 200 times as potent as morphine and has a rapid onset of action as well as addictive properties. Signs associated with fentanyl toxicity include severe respiratory depression, muscle rigidity, hypotension, coma, and death. Over 150 people die every day from overdoses related to synthetic opioids like fentanyl.[1] In this study, trends in neonatal deaths were analyzed. Data mining techniques were applied to focus on fentanyl-related neonatal deaths from cases received and reported by NMS Labs from January 2019 through July 2023. Cases were limited to infants, ages 0-17 days old, where maternal drug use contributed to death, either through *in utero* demise or death occurring within days of birth. Our analysis included the total positive drug findings with the concentrations of fentanyl, norfentanyl and 4-ANPP found in neonatal deaths and additional information including case history and age. Of the 115 cases identified, 98 total neonatal death cases, or approximately 85%, involved polydrug combinations. There were 44 total cases involving methamphetamine, accounting for the highest polydrug finding in fentanyl-related neonatal deaths. Cocaine or benzoylecgonine, the major metabolite of cocaine, accounted for 14 total cases, or approximately 12%. The animal sedative, xylazine, was identified in 6 cases. Naloxone was only used in 2 of 41 neonatal death cases identified by screening panels provided by NMS Labs. The highest concentration of fentanyl in liver tissue found in a neonatal death case was 1500 ng/mL, with a range of 4.0-1500 ng/mL and a mean of 194 ng/mL. The highest concentration of fentanyl in blood found in a neonatal death case was 280 ng/mL, with a range of 0.28-280 ng/mL and a mean of 20 ng/mL. The primary limitation of this research is that it relied on the accuracy and completeness of the investigations conducted at the time of death, including record of age and case history. Findings from this study suggest that the neonatal opioid and polydrug crisis is rampant, mirroring trends seen among adults. There must be a greater emphasis on harm reduction strategies, including parental treatment for drug abuse.

Reference:

[1].   NCHS, National Vital Statistics System. Estimates for 2020 are based on provisional data. Estimates for 2015-2019 are based on final data (available from: https://www.cdc.gov/nchs/nvss/vsrr/drug-overdose-data.htm).

Fentanyl; Neonatal; Polydrug

L9   Collecting and Analyzing Toxicological Evidence From Compromised Human Remains: An Interdisciplinary Approach

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WITHDRAWN

L10 A Complete Review of Propofol Misuse in the Health Care System and as a Drug of Abuse

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Learning Overview: After attending this presentation, attendees can expect to gain a heightened awareness of the potential of propofol misuse and the significance of cases of propofol addiction in both healthcare workers and laypersons. Attendees will learn about the infamous case of Michael Jackson, known as the "King of Pop," whose cause of death was from "acute propofol intoxication." Additionally, this presentation will discuss instances of addiction involving healthcare workers. Attendees can expect to be well-informed about the significant impact of propofol misuse on both individuals and the broader healthcare community.

Impact Statement: The presentation will impact the forensic science community by highlighting the field of anesthesia in regard to crime scenes and toxicology analysisof propofol abuse cases and raising the question of whether propofol should be classified as a controlled substance on a global scale. By examining the misuse of propofol through forensic investigation such as the position of the needle injection to assist in determining whether it is a suicide or a murder, this presentation seeks to provoke thoughtful discussions and considerations about its potential worldwide control as a means to safeguard patient safety and advance the field of anesthesia.

Propofol is a strong anaesthetic agent used widely among healthcare workers. Propofol is commonly used for sedation because it impacts the activity of neurons in individuals. This "milk of amnesia" has been used in surgery for patient sedation. However, propofol has a high potential to be a drug of abuse since it reportedly produces recreational euphoria, depression relief, and increased sexual desire.[1] Furthermore, studies showed that healthcare workers have a high potential for abusing propofol. Propofol is a potent drug, and when administered in high doses over short periods, it can lead to severe side effects or even fatal outcomes. In addition, the disadvantages of propofol can result in discomfort and complications such as injection site pain and the development of propofol infusion syndrome.

Even though propofol is mostly analysed for its mechanisms toward the body but its potential of being a drug of abuse has rarely been taken into consideration. Moreover, propofol addiction pharmacodynamics is not a thoroughly researched topic[3] Propofol is not registered under the Controlled Substances Act (CSA). The only country that considers propofol a controlled substance is South Korea; hence, it's illegal to use propofol other than in surgeries. Other countries do not consider propofol as a controlled substance.[4] Due to the limited amount of research, every country that consisted of propofol cases will be reviewed.

Cases of propofol addiction will be presented to assess the potency of propofol's effects on the body and its potential to lead to addiction. Several studies on healthcare workers that self-administrated the propofol causing their addiction will be examined. Moreover, the infamous case of Michael Jackson's death due to propofol overdose will be highlighted. Furthermore, studies have indicated the use of propofol in cases of homicide and suicide. How can one distinguish between these two scenarios?[5]

The number of studies on propofol addiction and abuse were very limited. Compared to other drugs of abuse, propofol was less thoroughly researched in the context of addiction because of its primary therapeutic usage as an anaesthetic agent.

Propofol has not been classified as a restricted substance, due to its lack of physical dependence. As a result, little focus has been paid to the drug's potential for addiction.[6] Hence, it is crucial to promote awareness of propofol addiction.

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Propofol; Anesthesia; Drug Abuse

L11   **A Retrospective Analysis of Results From U.S. Court-Ordered Mandatory Drug Testing of Oral Fluid and Hair and the Impacts of Expanding the Testing Scope for Fentanyl and Fentanyl-Related Compounds**

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Learning Overview: After attending this presentation, attendees will be able to list the most prevalent drugs in a Court-Ordered Mandatory Drug Testing (COMDT) population and describe the impact of including fentanyl and fentanyl-related compounds in COMDT oral fluid and hair testing protocols.

Impact Statement: This presentation will impact the forensic science community by introducing this observational study that seeks to provide timely, evidence-based intelligence on growing rates of drug use and patterns of substance use in individuals enrolled in COMDT programs. This presentation also examines testing of fentanyl and fentanyl-related compounds in COMDT oral fluid and hair testing protocols.

Court-Ordered Mandatory Drug Testing (COMDT) is frequently conducted in correctional settings to determine abstinence compliance or usage history (e.g., probation, parole, custody). Approximately 65% of the U.S. prison populations have an active Substance Use Disorder (SUD) [1]. Oral fluid (OF) and hair are advantageous matrices for COMDT testing due to their ease of collection and the longer window of detection hair provides. However, COMDT testing is currently limited in scope for analytes of interest and prevalence data using OF and hair testing are sparse.

For this study we analyzed hair specimens collected for COMDT between November 2020 and February 2021. The hair specimens underwent direct to confirmation testing using LC-MS/MS for fentanyl and fentanyl related compounds, in addition to traditional drug testing analytes. Hair specimen positivity rates in COMDT were calculated with and without inclusion of fentanyl and fentanyl related compounds to determine the effect on overall positivity rate when fentanyl targets were included in the hair testing protocol.

We also conducted a retrospective analysis of 5-years of COMDT data from oral fluid and hair collected from 2015 to 2019 in nationally represented COMDT programs. A random, national COMDT sampling of 959,237 OF and 65,645 hair test results were analyzed. Specimens in the historical dataset were tested for misused substances by screening with immunoassay and confirmatory testing was performed on a subset of OF and all hair positive specimens by LC-MS/MS or -HRMS. Direct statistical inferences were determined for the larger population of all COMDT specimens. Similarly, the prevalence of positive drug tests among the analysis pool were estimated. Multiple specimens from the same individual, when available, were assessed using longitudinal analysis with a pseudo-design structure.

COMDT drug prevalence data can be compared to other prevalence data such as U.S. workforce testing. Amphetamines, opioids, cocaine, benzodiazepines, cannabinoids, and phencyclidine were detected. The screening positivity rate in oral fluid was 34.0% with 7.8% total confirmed, whereas the hair overall positivity rate was 56.0% (36,778 positive of 65,645). This is up to 5 times higher than oral fluid and hair in a U.S. workforce population during a similar time period (10.2% and 10.9%, respectively) [2]. A total of 4,404 tests were fentanyl positive (6% of OF positive tests). Of all the positive fentanyl tests, 1,587 (36%) were fentanyl alone. The remaining 2,817 (64%) were in combination with other drugs. Recurring drug combinations were also determined.

This is the first large-scale, recent drug prevalence study in a US COMDT population, and the first study on the effect of expanding the scope of testing to include fentanyl related compounds, especially in hair testing. Understanding long-term patterns of use and confirming compliance and periods of abstinence are important observations for COMDT populations. The use of hair and oral fluid for drug testing offers benefits over urine testing including longer detection windows, less invasive collection techniques, and more difficulty adulterating or substituting specimens. Detection of drugs in hair and oral fluid can also provide more focused and accurate data among special population surveillance. This study indicates that SUDs in COMDT populations (i.e., special population) can be more prevalent than workplace drug testing populations (i.e., general population). These results can serve as an early warning to help legal systems and public safety programs to support treatment for SUDs.

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Court Ordered Mandated Drug Testing (COMDT); Oral Fluid; Hair

L12 Drops of Insanity? Tropicamide Recreational Usage: Early Warning Alarms in Forensic Science

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Learning Overview: The goal of this presentation is to explain the rising trends in ophthalmic drug abuse. Following this presentation, attendees will see how the global trend of ophthalmic drug overuse and misuse endangers public health. Tropicamide, a prescription medicine, is primarily used for pharmacologic dilation in the context of eye care, such as treating open-angle glaucoma, preventing posterior synechiae, constricting blood vessels, and facilitating ocular procedures. Although it is not commonly associated with widespread abuse or addiction, it has the potential for misuse. This substance is also sometimes used recreationally in combination with other psychoactive substances, like alcohol, marijuana, and opioids, to achieve altered states of consciousness and mental disorientation. This alarming development presents a growing public health hazard, necessitating heightened awareness and vigilance within the forensic and ophthalmology community.

Impact Statement: This presentation will have a multifold impact on the forensic science community. It has wide-reaching implications, extending beyond the realm of health care to intersect with legal and socio-cultural aspects. The issue of prescription drug misuse, exemplified by tropicamide, challenges not only public health, but also regulatory systems and societal norms as incidents of criminal activity involving drug abuse have seen a rise. Criminals frequently sought to utilize drugs that weren't used as frequently and wouldn't show up in a standard narcotic drug test panel to avoid being caught. The importance of identifying and combating the abuse of lesser-known substances such as tropicamide is accentuated by its international prevalence, particularly in regions where it is not typically included in standard drug-testing protocols. The study calls for a global initiative to understand, detect, and curtail the misuse of prescription medications, highlighting the urgency of expanding research into countries like India where available information is limited.

Tropicamide is a prescription medicine that provides pharmacologic dilation that can be used to treat open-angle glaucoma, prevent posterior synechiae, constrict blood vessels, and perform ocular procedures. It is not commonly connected with widespread abuse or addiction; yet, it has the potential for misuse. Tropicamide is also used recreationally with other psychoactive substances such as alcohol, marijuana, and opioids to achieve states such as unconsciousness and mental disorientation. This drug can build up in tissues and physiological fluids, and an overdose can be fatal. It is also known as a "seven-monther" – the time it takes to kill a young person without significant pre-existing conditions.[1-2] Tropicamide can build up in tissues and bodily fluid, and renal, hepatic failure or cardio toxicity caused by tropicamide overdose is a leading cause of death. Over the preceding ten years, numerous recorded incidents of tropicamide abuse via intravenous injection have occurred in several countries[1,3], particularly in Turkey, Italy, France, Tajikistan, and Kazakhistan[4-8].

Previously, the anticholinergic pharmacological epidemic was thought to be restricted to the former Soviet Union. A recent study shows that the epidemic spreads far beyond Eastern Europe and Western Europe[9-11], with Italy and France overselling the medicine. However, such abuse is reportedly uncommon in India, with limited information on tropicamide abuse specifically. The present narrative review is carried out to enlighten the forensic science community about the emerging trends of tropicamide drug addiction, a new hazard to public health. Furthermore, the review is to emphasize the importance of a single gold standard test in establishing the presence and study of tropicamide during forensic investigations in drug-facilitated investigative processes. To achieve this, the articles were chosen based on two criteria, i.e. tropicamide drug abuse and anticholinergic drug misuse. The review concentrated on the role of tropicamide in drug misuse; healthcare professionals, law enforcement, and the community must be aware of the potential risks associated with its misuse.

In conclusion, tropicamide, despite its primary medical use, has experienced instances of recreational and illicit utilization, presenting significant health risks. This review calls for continued vigilance and awareness regarding the misuse of tropicamide. Furthermore, it emphasizes the need for standardized testing methods, such as Gas Chromatography-Mass Spectrometry (GC-MS), in detecting tropicamide, especially in cases where traditional toxicological screenings yield negative results for blood and urine samples[6].

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Drug Abuse; Tropicamide; Fatal Death

L13   Biofluid Sampler: A Promising Sampling and Sample Preparation Platform for Forensic Toxicological Investigations

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Learning Overview: This presentation will report a novel sampling and sample preparation platform designed to collect high volume of biological fluids (whole blood, urine, saliva, cerebrospinal fluid) without any modification for downstream gas or liquid phase chromatographic analysis. After attending this presentation, attendees will better understand the importance of this novel sampling and sample preparation, their potential applications, and underlying advantages over classical sample preparation techniques.

Impact Statement: This presentation will impact the forensic science community by demonstrating the advantages of Biofluid Sampler (BFS) over classical sample preparation technologies to simplify bioanalytical sample preparation workflow, improve the overall data quality, significantly reduce overall sample preparation cost, and increase the productivity of the forensic toxicology laboratory.

Among all biofluids (blood, urine, saliva, sweat), whole blood is the most information rich sample matrix. Although blood is the primary sample in most forensic toxicological investigations, it is usually converted into plasma prior to downstream processing as the classical sample preparation techniques cannot process whole blood. When blood is converted into plasma, a significant loss of the exogenous analytes could occur. The concentration of analytes in plasma also remains in equilibrium between the aqueous part and the dissolved proteins. As such, when plasma undergoes protein precipitation prior to analyte extraction, another round of analyte loss happens. To minimize the loss of analytical information during pre-treatment of whole blood, we propose a new device called Biofluid Sampler[1].

Biofluid samplers (BFS) are prepared using 100% cellulose as the substrate and sol-gel sorbent coating technology to create chemically bonded, sponge-like porous sol-gel sorbents. Biofluid sampler is an offshoot of Fabric Phase Sorptive Extraction (FPSE). However, BFS utilizes two circular disks of varying diameter stitched together. If the analysis demands, two different sol-gel sorbents coated FPSE membranes can be used in crafting a BFS. Whole blood (10-1000 µL) can be added directly onto the BFS. The BFS, after adding whole blood onto it, is allowed to air dry. Subsequently, the BFS is exposed to organic solvent for eluting the analytes. As such, protein precipitation and analyte elution occur simultaneously. The eluant can be injected to the chromatographic technique for profiling.

Although BFS has enormous potential in forensic toxicology, its full potential is yet to be exploited. Compared to commercially available Dried Blood Spot (DBS) cards, BFS offer numerous advantages including expanded sample loading capacity (10–1000 µL), exploitation of intermolecular interactions to effectively capture and retain analytes possessing widely varying polarity and physicochemical characteristics, and elimination of transporting biohazardous whole blood with expensive shipping arrangements. The application potential of the BFS in mail-in-analysis has been tested using three selected NSAIDs and the performance of BFS has been compared to classical DBS cards. The performance of BFS clearly demonstrates the advantages of the new device over DBS cards as a viable whole blood sample collection platform. It is important to mention that DBS cards utilize only surface adhesion to retain the analytes, whereas BFS exploits many functional groups that exert strong intermolecular interactions towards the analytes and prevent analyte loss, even when stored in ambient laboratory/shipping conditions.

BFS is very innovative as (i) it allows sampling and sample preparation of whole blood without any modification; (ii) after sampling, it can be shipped without any stringent shipping condition for instrumental analysis; (iii) it is poised to provide inexpensive sample preparation support to the global community of forensic toxicologists across the world.

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Sample Storage; Absorption; Chromatography

L14   Quantitation of Delta-8-Tetrahydrocannabinol in Postmortem Specimens by the Method of Standard Addition

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Learning Overview: After attending this presentation, attendees will have learned the utility of the method of standard addition when performing quantitation of delta-8-tetrahydrocannabinol (delta-8-THC) in postmortem specimens.

Impact Statement: This presentation will impact the forensic science community by presenting data that demonstrates the application of the method of standard addition to postmortem cases for the quantitation of the cannabinoid delta-8-THC.

Delta-8-tetrahydrocannabinol (delta-8-THC) is a cannabinoid with similar structural and pharmacological properties as delta-9-tetrahydrocannabinol (delta-9-THC); the structural difference between the two compounds being the location of the double bond. The 2018 Farm Bill has led to an increased availability of delta-8-THC and other related cannabinoids to the public and subsequently an increased prevalence in forensic casework at the Alabama Department of Forensic Sciences (ADFS). Currently, hemp-derived delta-8-THC is readily available for purchase, as it is not considered illegal in the state of Alabama.

Currently, in the toxicology section of ADFS, delta-8-THC is reported as a qualitative target only; however, recently, requests to quantitate this compound have increased. To investigate an alternative method of quantitation, the method of standard Addition (MSA) was used. Whereas with a "traditional" external calibration curve model, a blank matrix must be acquired, MSA uses the actual case specimen as the fortified matrix to create analytical calibrators. In postmortem cases where additional specimen types, such as liver, may be submitted for analysis, having a matrix-matched blank may not always be possible, making MSA an attractive strategy. An additional benefit of using MSA when dealing with postmortem specimens is that when used, MSA overcomes the problem of matrix effects.

Cases were chosen that were previously reported to be delta-8-THC positive to investigate the feasibility of using MSA for quantitation in blood with deuterated delta-8-THC as an internal standard. Calibrator concentrations were unique to each individual case and the concentrations were spaced around the estimated concentration of the sample. For example, when the estimated sample concentration was 7 ng/mL, the corresponding calibrator concentrations were: 1, 3, 7, and 14 ng/mL. Analysis for delta-8-THC was performed using a liquid-liquid extraction procedure utilizing a mixture of 9:1 hexane/ethyl acetate. The organic layer was transferred and evaporated under nitrogen before reconstitution of the extracts with 50:50 mixture of mobile phases A and B. Mobile phase A was water with 0.1% formic acid and mobile phase B was 0.1% formic acid in methanol. Separation and detection of the delta-8-THC was done using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) with an Agilent 6470B Triple Quadrupole LC/MS instrument. The separation was performed on an Agilent Poroshell 120 EC-C18 analytical column (2.1 x100 mm, 2.7µm). The flow rate was 0.5 mL/min with a gradient separation from 70% - 99% mobile phase B in 10 minutes. Positive electrospray ionization was utilized with delta-8-THC and deuterated delta-8-THC detection using dynamic multiple reaction monitoring.

The MSA technique produced excellent linearity with R^2 values greater than 0.990 in all cases and each calibration curve contained four points in addition to the unfortified case sample matrix. Both of these factors (the R^2 value and the number of points) meet the criteria outlined for MSA analysis in ANSI/ASB Standard 054[1]. The calculated blood concentrations for delta-8-THC were 4.1, 7.8, and 2.5 ng/mL in three postmortem cases. As demonstrated, MSA's ability to quantitate in postmortem blood without the confounding factor of matrix effects makes it a worthwhile analytical strategy and should be considered when performing quantitation in postmortem samples.

Reference:
[1]   ANSI/ASB Standard 054, *Standard for a Quality Control Program in Forensic Toxicology Laboratories*, 1st Edition, 2021. Accessed at: https://www.aafs.org/sites/default/files/media/documents/054_Std_e1.pdf.

Tetrahydrocannabinol; Postmortem; Method Development

L15 Fabric Phase Sorptive Extraction: A Green Sample Preparation Strategy for Forensic Toxicology

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Learning Overview: The goal of this presentation is to report a novel sample preparation platform, Fabric Phase Sorptive Extraction (FPSE), which combines classical equilibrium-driven sample preparation techniques (e.g., solid phase microextraction) and exhaustive sample preparation techniques (e.g., Solid Phase Extraction [SPE]). FPSE membranes are capable of selectively extracting the target analytes directly from unmodified biological samples, including whole blood, urine, saliva, and cerebrospinal fluid without requiring any modification for downstream gas or liquid phase chromatographic analysis. After attending this presentation, attendees will better understand the importance of this novel sample preparation technique, its potential applications, and underlying advantages over classical sample preparation techniques.

Impact Statement: This presentation will impact the forensic science community by demonstrating the advantages of FPSE over classical sample preparation technologies to simplify bioanalytical sample preparation workflow, improve overall data quality, significantly reduce sample preparation costs, and substantially increase the productivity of the forensic toxicology laboratory.

Most forensic toxicological investigations involve analysis of different biological fluids including whole blood, urine, saliva, and cerebrospinal fluid. Whole blood is the preferred biological sample as it is integral part of our circulatory system and it carries the most analytical information of the human body compared to other biological fluids. However, most sample preparation techniques cannot process whole blood as it is. As such, whole blood is converted first into either plasma or serum prior to subjecting them to bioanalytical sample preparation techniques. Analytes in whole blood remain partitioned between the aqueous portion and the suspended solid portion of the biological fluids. A significant portion of target analytes/metabolites disappears with the solid matrix components during the transformation of whole blood into plasma or serum followed by extraction of the analytes using conventional sample preparation techniques (e.g.,solid phase extraction (SPE) and Liquid-Liquid Extraction (LLE)). A recent study showed that converting whole blood into plasma resulted in ~30% loss of metabolomic information.[1] Fabric phase sorptive extraction (FPSE), a new generation sample preparation technology, has offered a paradigm shift approach in sample preparation for pre-clinical and clinical research. FPSE innovatively combines the benefits of SPE (exhaustive extraction principle) and solid phase microextraction (equilibrium extraction principle) into a single sample preparation technology platform. FPSE utilizes a flexible fabric substrate, coated with sol-gel sorbents as the extraction membrane. This uniquely designed extraction medium is capable of extracting target analyte(s) directly from whole blood and other biological fluids. Due to the sponge-like porous architecture of sol-gel sorbents, rapid analyte mass transfer occurs between the bulk sample and the extraction medium, resulting in exhaustive extraction within a fraction of time required for other comparable sample preparation techniques.

FPSE is particularly suitable for analyzing target analytes e.g., drug residues, metabolites, biomarkers directly from whole blood without requiring any protein precipitation or other pre-extraction sample cleaning/manipulation.[2]

After extracting the target analyte(s) directly from the whole blood sample, the FPSE membrane is exposed to a small volume of organic solvent (100-500 µL) for eluting the extracted analyte(s). Low viscosity of the organic solvent and the sponge-like porous sol-gel network allows fast diffusion of organic solvent into the FPSE membrane for quick and complete recovery of the extracted analyte(s). As a result, FPSE eliminates the time consuming and error prone solvent evaporation and sample reconstitution step often considered as an integral part of solid phase/liquid-liquid extraction workflow. During the solvent mediated elution, any protein or matrix interferents adhered to the FPSE membrane precipitate out and a final centrifugation of the resulting solution ensures clean particle-free highly concentrated target analyte(s) prior to injecting into the analytical instrument.

FPSE is a green sample preparation technique due to its minimal solvent consumption, simplified workflow, and elimination of solvent evaporation. FPSE has been evaluated using ComplexGAPI and clearly demonstrates its environmentally-friendly characteristics.

FPSE has already developed many sol-gel sorbents specifically suitable for polar drugs/metabolites/biomarkers. These high-efficiency sorbents have been found to be equally effective for analytes with wide range of polarities.

The performance superiority and operational convenience of FPSE have already been presented in many published articles. For example, FPSE was deployed in the determination of synthetic opioids in oral fluid, anti-depressant drugs in biological fluid, non-steroidal anti-inflammatory drug residues in human saliva, seven selected antidepressant drugs in post-mortem samples, and adamantine analogues in human urine.[3-7]

In the current talk, new data on bioanalytical sample preparation using FPSE and a comparison between FPSE and conventional sample preparation techniques will be presented.

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Absorption; Bodily Fluid; Chromatography

L16   A Comparative Analysis of Accuracy, Precision, and LOD in AAS and TMA for the Quantification of Cadmium in the Blood Samples

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Learning Overview: After attending this presentation, attendees will have a better understanding that how trace metal analyser is better than atomic absorption spectrometry in terms of accuracy, precision, and Limit Of Detection (LOD) for the detection of cadmium (Cd) in blood samples.

Impact Statement: This presentation will impact the forensic science community by providing awareness about heavy metals and a brief about various detection techniques used for Cd analysis. Among those techniques, Atomic Absorption Spectroscopy (AAS) flame is the widely used technique that is used for the analysis of Cd in blood, but due to its limitations, it cannot detect trace amounts of Cd; for that, Trace Metal analyser (TMA) can be used for trace amount detection with high accuracy, precision, and a lower LOD.

Heavy metals are a major concern for human health due to their exposure via mining, metallurgy, industrial waste and effluents, automobile emissions and chemical fertilizers. According to the literature, the primary source of toxicity in humans is inhalation of dust or cigarette smoke. Toxic metals especially Cd and lead (Pb) are inhaled through smoke and accumulate in the lungs and tissues [1]. Cd is reported as one of the toxic heavy metals, which is carcinogenic, leading to severe health problems in the body. Cadmium accelerates the synthesis of metallothionein in the liver, which results in osteodynia known as *Itai-Itai*. Cadmium is widely used in the production of lithium-ion batteries and nuclear reactors, as it absorbs neutrons and helps in controlling the rate of the reaction. It is also used in solar cells, paint pigment, and gold manufacturing industries, etc, [2]. There are various techniques that are used for the detection of Cd metal in biological samples. Among these, AAS and TMA (i.e., differential pulse anodic stripping voltammetry) are the majorly used techniques for quantitative analysis [3].

In the present study, techniques are compared based on precision, accuracy, and LOD in the biological samples. Blood samples were collected and spiked at different concentrations ranging from 10ppb to 1000ppb. The samples were digested by using the open digestion method. In the case of AAS, the following set parameters i.e., wavelength= 228.8m, lamp current=5, and slit size= 0.4, were used for analysis. In the case of TMA, parameters used for the analysis were fixed to mode- anodic, drop size-4, electrode-Hanging Mercury Drop Electrode (HMDE), stirrer speed- 2000 rpm, and voltage- 6mV, etc.

The present study was done to compare the accuracy and cost-effectiveness of AAS and TMA. For AAS, the standard deviation for a sample with a 10ppb spiked concentration (lowest limit) of Cd was shown as ±5ppb; whereas, for TMA, the same sample showed a standard deviation of ±0.01 ppb. However, in AAS, the cost of lamps and gases used are also higher when compared with TMA. Hence, as Cd concentration is in trace amounts in blood samples, TMA can be preferred over the AAS flame method to detect this amount of Cd because of its features like accuracy, precision, & reproducibility and most importantly the cost of sample analysis.

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Cadmium; FAAS; TMA

L17   The Determination of Cadmium in Blood by Voltammmetry Using Hanging Mercury Drop Electrode (HMDE)

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Learning Overview: After attending this presentation, attendees will learn about a simple and reliable method for the determination of cadmium (Cd) in blood using hanging mercury drop electrode (HMDE) and its application in toxicology.

Impact Statement: This presentation will impact the forensic science community as the developed voltammetry method is a selective, sensitive, and reproducible technique for the quantitative determination of cadmium in blood using HMDE on the instrument Trace Metal analyser (TMA) and thus can be used for the analysis of Cd in forensic and clinical toxicology laboratories.

Cadmium is a metallic element present in our environment and it is considered a pollutant, as it is derived from anthropogenic sources. Cadmium poisoning isn't very common today, but its carcinogenic nature can cause serious health ailments. Most of the toxicological problems associated with cadmium are the result of occupational and environmental exposure. Inhalation of tobacco smoke is one of the major routes of cadmium exposure. The disease caused by exposure to Cd is known as Itai -Itai (ouch-ouch) disease. As Cd can accumulate in the body, Cd poisoning can occur in chronic smokers or by eating food or crops grown in contaminated soil or water or by working in places where cadmium is used or produced. Various sophisticated analytical techniques like AAS, ICP, ICP-MS etc. are usually used to assess levels of Cd in blood.[1-3] An attempt has been made to develop an alternative method for the determination of Cd in blood with a voltammetric technique using HMDE on the TMA.

Blood samples were spiked with solution of Cd. The samples were processed by the closed digestion method using nitric acid. Microwave digestor (MDS-10) was used for the digestion of samples. Voltammetry determination of Cd in the blood samples was carried out using HMDE. The electrode was washed well with ultrapure water. 10 ml ultrapure water and 1 ml ammonium acetate buffer were taken in the voltammetric vessel and blank voltammograms were recorded under the voltammetric conditions, with peak potential of Cd as -560mV. After completion of blank voltammograms, 0.1 ml of digested sample was added in the voltammetric vessel and voltammograms were recorded. After completion of sample voltammograms, 0.1 ml of standard solution of Cd was added and the voltammogram was recorded. An extrapolation graph gives the amount of Cd in the blood sample.

Cadmium was successfully detected in all the spiked blood samples. The developed method on the TMA is suitable for the determination of Cd in blood samples. The limit of detection of the method was 0.1 µg/L for cadmium.

The developed voltammetry method is a selective, sensitive, and reproducible technique for the quantitative determination of Cd in blood using HMDE.

References:
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Cadmium; Blood; Hanging Mercury Drop Electrode

L18   Identifying Designer Benzodiazepines in Blood Using Gas Chromatography/Mass Spectrometry

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Learning Overview: After attending this presentation, attendees will have a better understanding of a method that utilizes Gas Chromatography/Mass Spectrometry (GC/MS) to quantitate flualprazolam, etizolam, and bromazolam, as well as identify adinazolam and 4'-chloro-deschloroalprazolam.

Impact Statement: This presentation will impact the forensic science community by providing a validated method for identifying designer benzodiazepines (DBZD) using GC/MS. Some toxicology laboratories may not have access to Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) or other similar instrumentation. This method uses an instrument that is universal to most forensic toxicology laboratories; therefore, making testing for select DBZD more accessible.

DBZD have been found increasingly in the illicit drug market, most frequently found with other drugs, such as fentanyl. New DBZD continue to be manufactured and distributed, highlighting the importance of updating analytical methods for their detection. One such method, originally validated for alprazolam using GC-MS, was expanded to quantitate flualprazolam, etizolam, and bromazolam, as well as identify adinazolam and 4'-chloro-deschloroalprazolam.

This expanded method was validated and evaluated for use as a confirmation method with authentic specimens. Blood and urine samples from decedents were received by the Forensic Toxicology Laboratory at the University of Florida as part of routine casework. If initial testing or case history suggested use of synthetic opioids by a decedent, a urine sample was screened using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) for a panel of synthetic opioids (n=16). As part of this process, the data were compared to a personal compound database and library containing the targeted DBZD. Twenty-two (22) samples between February 2022 and April 2023 indicated the presence of one or more of these drugs.

To confirm the presence of the DBZD, 1 mL blood samples were extracted using UCT Clean Screen® Extraction columns after the addition of 100 µL of the internal standard, bromazolam-D5 (1 µg/mL). Extracts were reconstituted in 50 µL of methanol and analyzed by GC-MS in selected ion monitoring mode (flualprazolam: *m/z* 297.2, *222.2, 326.2*; etizolam: *m/z* 342.2, *313.1, 266.2*; bromazolam: *m/z* 325.2, *352.2, 353.2*; adinazolam: *m/z* 308.2, *280.1, 205.1*; 4'-chloro-deschloroalprazolam: *m/z* 279.2, *310.1, 308.2*; and bromazolam-D5: *m/z* 330.0, *357.2*).

Key validation parameters, including Limits Of Detection and Quantitation (LOD and LOQ, respectively), calibration model, accuracy, precision, carryover, and interference studies, met acceptance criteria. LOD for the targeted DBZD was determined to be 1.25 ng/mL with LOQ of 5 ng/mL. Calibration models were assessed for flualprazolam, bromazolam, and etizolam over 3 days with linearity from 5 to 100 ng/mL. Precision and bias were evaluated at 25 ng/mL in triplicate over 3 days and all results were within ±20%. No carryover was observed and no interferences were found when several common drugs of abuse, prescription medications, and over-the-counter medications were tested, including alprazolam.

Blood samples from 22 decedents were analyzed using this method after their urine samples screened positive. Of those, 12 confirmed positive for one or more DBZD. One explanation for the 54% confirmation rate was that the screen was in urine and confirmation in blood. One decedent (white male, age 44) had etizolam (90 ng/mL) and flualprazolam (< 5 ng/mL). The other 11 decedents were positive for bromazolam. Two had concentrations less than 5 ng/mL and nine had bromazolam concentrations ranging from 5.9 to 314 ng/mL (mean, 53 ng/mL; median, 16 ng/mL). The mean age of the decedents positive for bromazolam was 32, ranging from 20 to 49 years. The majority of the decedents were white males (63.6%). Polydrug use was detected in all decedents. The most prevalent drugs found with bromazolam were fentanyl (100%), methamphetamine and/or amphetamine (54.5%), and cannabinoids (45.5%).

The validated method uses GC-MS to quantitate flualprazolam, bromazolam, and etizolam, as well as detect adinazolam and 4'-chloro-deschloroalprazolam with no interference from alprazolam or other common drugs of abuse. Adinazolam and 4'-chloro-deschloroalprazolam are two of the newer emerging drugs in this class, so this method was designed to start monitoring for their presence in casework. The method had acceptable values for precision and accuracy and can be applied to detect select DBZD in casework. While bromazolam was the most prevalent DBZD observed in this set of cases, methods must continually be expanded to account for evolving trends.

Novel Psychoactive Substances; Method Development; Mass Spectrometry

L19 The Impact of Gold Top SST (Gel and Clot) Collection Tubes on Cannabinoid Quantitative Analysis in Blood

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Learning Overview: After attending this presentation, attendees will have a better understanding on the impact of time, temperature, and gold top Serum Separator Tube (SST) (gel and clot) collection tubes on the stability of cannabinoids in whole blood samples.

Impact Statement: This presentation will impact the forensic science community by presenting evidence of how different storage conditions using gold top SST collection tubes can impact the concentration of cannabinoids in blood, as well as detection of the isomers, delta-8-tetrahydrocannabinol (delta-8-THC) and delta-9-tetrahydrocannabinol (delta-9-THC) and their metabolites, using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

The expanded use of recreational and medicinal cannabis products, as well as the appearance of new cannabinoid derivatives, increases the importance of implementing pre-analytical (sample collection) protocols, and the development of recommendations for cannabinoid testing in biological samples. The accurate quantification of cannabinoids in biological samples is critical for providing a correct result interpretation, especially if specific thresholds have been legally set, as in drugs and driving cases in certain states.

This study focused on the impact of different storage conditions using gold top SST (gel and clot) collection tubes in the analysis of delta-9-THC, delta-8-THC, cannabidiol (CBD), and their hydroxy (delta-9-THC-OH, delta-8-THC-OH, 7-OH-CBD) and carboxy (delta-9-THC-COOH, delta-8-THC-COOH, 7-COOH-CBD) metabolites in blood samples stored at Room Temperature (RT) and at 4°C up to one month.

Pooled stability samples were prepared at 3 and 30 ng/mL in human whole blood samples from BioIVT (Westbury, NY) preserved in K_2EDTA. The spiked pooled blood samples were mixed in a rotator for 30 min followed by immediately taking three aliquots of 0.5 mL as the time zero reference. The rest of the spiked blood samples were transferred to the gold top SST (serum separator tube) tubes, which were mixed, but not spun down to mimic the conditions of how some authentic samples were received in the lab, and stored at RT and at 4°C for 4 days, one week and one month. All stability samples were analyzed in triplicate. The blood samples were submitted to anion exchange solid phase extraction after protein precipitation in acetonitrile, and were analyzed by LC/MS/MS, employing gradient mode and ESI+. The method was fully validated following ANSI/ASB 036 guidelines. The method was linear from 1 to 100 ng/mL, bias ranged from -8.6% to 8.6%, and imprecision from 5.3 to 27.9%. All analytes, except for 7-OH-CBD, showed acceptable imprecision (<20%). Extraction efficiency ranged from 19.1 to 53.7%, and ion suppression was observed for all analytes (from -42% to -91%).

Most cannabinoids showed stability issues at room temperature, with the low concentration (3 ng/mL) samples being more affected than the samples at 30 ng/mL. Delta-8 and delta-9-THC showed similar stability behavior. Both compounds showed stability issues at RT at 3 ng/mL (up to total loss after one month) and at 30 ng/mL (39 and 37% loss, respectively), but they were mostly stable at 4°C. Delta-9-THC-COOH was slightly more stable than delta-8-THC-COOH. Both compounds were stable at 4°C, but they showed losses at RT (delta-9-THC-COOH with 39 and 55% loss at 3 and 30 ng/mL, respectively; delta-8-THC-COOH with up to total loss at 3 ng/mL and 63% loss at 30 ng/mL). Delta-9-THC-OH had stability issues at RT at 3 ng/mL (up to total loss after one month) and 30 ng/mL (55% loss), but it was stable at 4°C. Delta-8-THC-OH was stable in all conditions. Regarding CBD and metabolites, all were stable at 4°C (within 20% of starting concentration). CBD-COOH and CBD-OH showed stability issues, with up to total loss, at RT for one month (CBD-COOH) or one week (CBD-OH) at both concentrations, while CBD was stable.

Blood storage at room temperature should be avoided due to the large decrease in cannabinoid concentrations. Overall, delta-8 and delta-9-cannabinoids showed similar stability behavior, except delta-9 and delta-8-THC-OH. As a next step, a comparison of these results with blood samples stored in K_2EDTA tubes could indicate if the observed losses were due to the gel present in SST tubes.

Cannabis; Liquid Chromatography; Validation

L20 Designer Benzodiazepine ELISA Cross-Reactivity

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Learning Overview: After attending this presentation, attendees will understand the utility of the Neogen® Benzodiazepine kit to screen for various Designer Benzodiazepines (DBZ) by Enzyme-Linked Immuno-Sorbent Assay (ELISA).

Impact Statement: This presentation will impact the forensic science community by sharing the results of the cross-reactivity study of several DBZ with the Neogen® Benzodiazepine kit.

Method: Working controls for the target molecule and designer benzodiazepines were prepared at 2 µg/mL. The working control of clonazepam was used to prepare five concentrations, in duplicate, for both blood and urine to create a standard curve. The standard curve spanned the range of the LOD, 5 or 10 ng/mL for blood or urine, respectively, to 100 or 200 ng/mL for blood or urine, respectively. Separate controls for 4'chloro deschloroalprazolam (4-CDA), 8-aminoclonazolam, alpha-hydroxy bromazolam, bromazolam, clonazolam, desalkylgidazepam, and flualprazolam were prepared at various concentrations in each matrix. Whole blood was diluted 1:5 using EIA buffer offline and urine was diluted 1:10 online. The samples were analyzed by a Dynex ^{DSX} Automated ELISA System as described previously ([1]). The cutoffs used for blood and urine were 10 and 25 ng/mL of clonazepam, respectively.

Results: Cross-reactivity was evaluated by comparing instrument responses for the various DBZ to the standard curves of clonazepam in blood and urine. In contrast, cross-reactivity determined by the manufacturer was performed in EIA buffer using oxazepam as the target analyte. The cross-reactivity determined for the seven DBZ evaluated herein as well as three previously evaluated (1) are presented in Table I. Eight out of the ten DBZ listed on the table showed excellent cross-reactivity in both blood and urine. While 8-aminoclonazolam showed lower cross-reactivity than its parent drug, clonazolam, the response was still sufficient to allow detection at relevant concentrations with the low cutoff levels employed. The lack of cross-reactivity for 4-CDA means it will likely go undetected in both urine and blood samples screened by ELISA.

Table I. ELISA Cross-reactivity for various designer benzodiazepines

Drug	Observed Urine % Cross-reactivity	Observed Blood % Cross-reactivity	Manufacturer % Cross-reactivity
4'chloro deschloroalprazolam	1	10	Not reported
8-aminoclonazolam	65	45	Not reported
Alpha-hydroxy Bromazolam	227	193	Not reported
Bromazolam	274	303	Not reported
Clonazolam	126	99	120
Desalkylgidazepam	182	168	Not reported
Etizolam	238	200	87
Flubromazolam	277	114	253
Phenazepam	413	101	190
Flualprazolam	246	163	467

Conclusion: The Neogen® Benzodiazepine kit using clonazepam as the target can detect a wide range of routine, non-routine, and non-FDA approved designer benzodiazepines. Excellent cross-reactivity was observed for most of the DBZ evaluated, which has also been demonstrated in authentic case specimens. However, there may be some novel designer benzodiazepines that may go undetected as demonstrated for 4-CDA.

Reference:

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Benzodiazepine; Cross-Reactivity; ELISA

L21 EC-SERS and DART®-TOF/MS for Toxicological Screening

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Learning Overview: After attending this presentation, attendees will be able to explain the theory behind Electrochemical Surface-Enhanced Raman Spectroscopy (EC-SERS) and Direct Analysis In Real-Time-Time-Of-Flight/Mass Spectrometry (DART®-TOF/MS) as they relate to the analysis of drugs. Attendees will also understand the advantages and disadvantages of these techniques for toxicological screening

Impact Statement: This presentation will impact the forensic science community through the investigation of novel methods for toxicological screening. These methods are focused on improving the speed and reliability of screening in toxicology, as well as investigating the quantitative ability of the methods. This work will provide the first steps toward demonstrating the ability of EC-SERS alone and in orthogonal combination with DART®-TOF/MS for future use in clinical and forensic toxicological settings.

Fast and efficient screening methods have become a focal point in many disciplines of forensic science and research including toxicology. Both clinical and forensic toxicology settings can benefit from fast screening methods capable of identifying potential drugs and toxins within samples. Mass spectrometry is especially powerful in the toxicological field due to the high sensitivity and specificity inherent in these methods. However, applications for screening samples using smaller, cheaper, and portable instrumental techniques may be desirable in a number of settings including drug and compliance monitoring, Driving Under the Influence Of Drugs (DUID) check-point testing, and rural community testing.

EC-SERS demonstrates potential as a novel, sensitive, and selective analytical method capable of rapid drug identification. The benefits of such a method have been shown in the seized drug space with the assessment of authentic casework samples [1,2]. This work demonstrates the proof-of-concept for using EC-SERS methods for toxicological samples and presents the initial stages of combining EC-SERS with Direct Analysis in Real Time – Mass Spectrometry, providing orthogonal data for improved identification with two rapid technologies.

The initial analyte panel included fentanyl, norfentanyl, 4-ANPP (despropionyl fentanyl), cocaine, and benzoylecgonine. Urine was selected as the matrix of choice for this proof-of-concept due to its prevalence in drug testing and monitoring and ease of use as a matrix. Three sample preparation procedures were assessed including Liquid-Liquid Extraction (LLE), Solid-Phase Extraction (SPE), and DPX in-tip extraction using neat and matrix-matched calibration curves between 1 ng/mL and 500 ng/mL. Extraction efficiencies for these procedures were compared using EC-SERS and Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS), where the DPX extraction performed poorly, followed by LLE with an average efficiency of 60% and SPE with 89%.

The combination of orthogonal techniques (EC-SERS and DART-TOF-MS) was especially useful for this panel of analytes as one analyte (benzoylecgonine) did not present discernible SERS signals but DART-TOF-MS detection was achieved. The EC-SERS detection was carried out using silver screen-printed electrodes, where the SERS substrate was electrochemically generated. Excellent selectivity was apparent from the generation of high-resolution mass spectra from the TOF instrument. Several challenges were identified during testing, including the need for supporting electrolyte in EC-SERS experiments, matrix effects/interference, and wide range of analyte concentrations typically found in toxicological samples. Initial identification of the analyte panel was achieved in urine using the EC-SERS and DART-MS approach with current work focused on improving the detection capabilities and assessing quantitative ability.

Future work is focused on the next stage of this approach, which is the development of an in-line EC-SERS + DART-MS platform, allowing for simultaneous collection of spectroscopic and spectrometric orthogonal data from the same sample. The rapid and efficient nature of EC-SERS and DART-TOF-MS suggests that this technology could be effective in workplace drug testing, compliance testing with rehab/treatment centers and parole/prisons, drug checking/needle exchange, and in rural communities. In addition, the quantitative ability of these methods will be assessed in future experiments to provide a confirmatory answer immediately.

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EC-SERS; DART-TOF-MS; Toxicology

L22 **A Validated Method for Quantification of Buprenorphine/Naloxone and Its Primary Metabolites in Clinical Human Breast Milk, Maternal and Infant Plasma Samples Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)**

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Learning Overview: After attending this presentation, attendees will better understand the use of opioid maintenance medication buprenorphine/naloxone in pregnant women and detectable concentrations of buprenorphine, naloxone, and their metabolites in different biological matrices.

Impact Statement: This presentation will impact the forensic science community by increasing awareness about the safety and efficacy of fetal and infant exposure to buprenorphine/naloxone combination therapy. The method presented in this proof-of-concept study could also be applied to monitor drug compliance to a prescribed treatment plan among pregnant and nursing women with Opiod Use Disorder (OUD).

Untreated OUD has been associated with increased risk of fetal growth restriction, preterm delivery, stillbirth, and more severe Neonatal Abstinence Syndrome (NAS) ([1]). The buprenorphine/naloxone combination product, Suboxone®, is not recommended for pregnant women use due to lacking safety information. There are also concerns of active Suboxone® components excreting into breastmilk postpartum. In particular, naloxone could have teratogenic potential that might result in birth defects (2) and could inadvertently reverse morphine treatment in breastfed infants with NAS. This research aimed to quantify concentrations of buprenorphine (BUP), Buprenorphine-Glucuronide (BUP-Gluc), norbuprenorphine (NBUP), norbuprenorphine-glucuronide (NBUP-Gluc), naloxone (NAL), and Naloxone-Glucuronide (NAL-Gluc) in clinical breastmilk, maternal and infant plasma samples.

Samples were assayed using an analytical scheme adapted from Swortwood et. al (3) with modifications. Sample clean-up was performed with protein precipitation using acetonitrile and solid-phase extraction. Separation was achieved using a Restek Raptor Biphenyl (2.7 μm, 100 x 2.1 mm) column with matching guard (5 μm, 5 x 2.1 mm). Samples were analyzed via an Agilent 1290 Infinity liquid chromatograph coupled to an Agilent 6470 triple quadrupole mass spectrometer. Electrospray ionization was in positive mode. The mass analyzer was operated in multiple reaction monitoring mode with one quantifier and two qualifiers for analyte identification. Method validation was performed per SWGTOX guidelines.

The mean gestational age of maternal participants was 25.9 ± 2.8 weeks at the time of Suboxone® treatment entry. Paired breastmilk and maternal plasma were collected 2-3 hours post-sublingual dosing on days 2, 3, 4, 14 and 30 postpartum. Infants were born at full term. Two infants required morphine treatment for NAS. Infant plasma (n=3) was collected via a heelstick on day 14. All mothers and infants tested negative during the perinatal period for substances other than BUP.

All validation parameters met SWGTOX acceptance criteria. The linear range was 0.5-50 ng/mL for BUP, NBUP, and NBUP-Gluc, 0.5-25 ng/mL for BUP-Gluc, 1.25-50 ng/mL for NAL, and 2.5-50 ng/mL for NAL-Gluc ($R^2 > 0.99$ for 5 runs). The LOD was 0.5 ng/mL for BUP, BUP-Gluc, NBUP, NBUP-Gluc, NAL, and 1.25 ng/mL for NAL-Gluc. The LOQ was 0.5 ng/mL for BUP, BUP-Gluc, NBUP, NBUP-Gluc, 1.25 ng/mL for NAL, and 2.5 ng/mL for NAL-Gluc. Bias, intra- and inter-day precision, and dilution integrity were acceptable. Positive and negative controls were free from carryover and endogenous/exogenous interferents. Plasma extracts were stable beyond 60 hours when stored at 4°C or at room temperature.

While BUP-Gluc and NAL-Gluc were variably detectable, BUP (0.8-16.5 ng/mL), NBUP (1.1-8.4 ng/mL), and NBUP-Gluc (0.7-19.7 ng/mL) were consistently quantified across all breastmilk samples (n=18). NAL (1.3 ng/mL) was found in one subject's breastmilk on day 30. Similarly, BUP (1.6-17.7 ng/mL), BUP-Gluc (0.7-13.1 ng/mL), NBUP (2-15.8 ng/mL), NBUP-Gluc (20.7-86.3 ng/mL), and NAL-Gluc (9.6-39.2 ng/mL) were quantified in all maternal plasma samples, with NAL undetected or < LOQ. Infant plasma (n=3) was negative for BUP, BUP-Gluc, NBUP-Gluc, NAL, and NAL-Gluc, with NBUP concentrations of 0.9 and 5.4 ng/mL found in two infants respectively.

Despite a low NAL concentration detected in one breastmilk sample, the poor oral bioavailability of BUP and NAL renders any ingestion by breastmilk likely to be inconsequential for the neonate. NBUP concentrations found in infant plasma may support previous findings concluding minimal *in utero* and breast-milk exposure of BUP for the developing fetus and infant. The absence of detectable NAL in infant plasma is reassuring. While the NAL component may contribute to decreased abuse potential for the mother, clinically insignificant NAL concentrations found in breastmilk are unlikely to interfere with NAS treatment in breastfed infants of Suboxone®-maintained mothers. These results demonstrate Suboxone® as an effective and safe medication for neonate and pregnant/nursing women with OUD.

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Validation; Tandem Mass Spectrometry; Opioids

L23 An Evaluation and Comparison of the Evidence MultiSTAT to GC/MS for the Detection of Morphine, Codeine, and Thebaine in Urine and Oral Fluid

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Learning Overview: After attending this presentation, attendees will learn about the ability of new immunoassay instrumentation and Gas Chromatography/Mass Spectrometry (GC/MS) to detect thebaine as a biomarker for poppy seed detection.

Impact Statement: This presentation will impact the forensic community by informing attendees on the use of different instrumentation for the screening of opiate samples and how authentic samples after poppy seed ingestion present using each instrument.

The ongoing opioid epidemic has increased the "poppy seed defense" to explain opiate-positive samples coming from the ingestion of opium poppy seeds before drug testing. To support or refute this, laboratories may test for thebaine as a biomarker for poppy seed use versus pharmaceutical or illicit opiate use. The Randox Evidence MultiSTAT is a new immunoassay tool that conveniently uses cartridges which house all required reagents for testing many drug classes for one sample. The opiate panel demonstrates a low cross-reactivity to thebaine, but offers low detection limits for other opiates to identify opiate-positive samples, which could be beneficial for poppy seed confirmation.

To evaluate the utility of the Evidence MultiSTAT for opiate detection in comparison to traditional screening methods, a qualitative GC/MS (Agilent 7890A GC/Agilent 7683B MS) method was developed and validated according to ASB 036 qualitative method validation guidelines for the detection of morphine, codeine, and thebaine in Oral Fluid (OF) and urine. Authentic urine and OF samples were collected from 12 participants (7 female, 5 male) after the consumption of Betty Crocker™ poppy seed muffins. OF was collected at specified time points until 24 hours, while urine samples were collected at every void until 24 hours. Samples were analyzed using the Evidence MultiSTAT and GC/MS.

GC/MS samples were extracted using Solid-Phase Extraction (SPE). Samples (500 µL) were fortified with internal standard (morphine-D_3) at 50 and 20 ng/mL for urine and OF, respectively. After elution, samples were dried and reconstituted with 25 µL ethyl acetate and 25 µL BSTFA with 1%TMCS. Following derivatization, 2 µL of sample was injected onto the GC/MS using split mode (10:1) on a DB-5ms GC column (30 m x 250 µm x 0.25 µm). Temperature programming was used for analysis beginning at 150°C until reaching 300°C. The MS was operated in selected ion monitoring mode. The target ions for each analyte were *m/z* 432, 429, 371, and 311 for morphine-D_3, morphine, codeine, and thebaine, respectively.

The GC method resulted in Limits Of Detection (LOD) of 7.5 ng/mL for OF and 100 ng/mL for urine, which represent 50% of the Evidence MultiSTAT cutoff. Matrix interferences and carryover were below the administrative cutoff point for both matrices. Morphine and codeine were stable at low (matrix LOD) and high (30 ng/mL in OF and 400 ng/mL in urine) concentrations in both matrices for up to 48 hours. Thebaine was stable for at least 24 hours in both matrices at high concentrations but was variable at low concentrations. Of the MultiSTAT-screened OF samples, all but 3 participants were negative before the 3-hour time point, but all participants were negative by 24 hours. Urine sample testing was limited by the number of testing cartridges available, so samples collected earlier in the day and at the 24-hour time point were prioritized. By the 9-hour mark, all participants had tested positive at least once. By 24 hours, two participants still screened positive for opiates. Upon GC/MS testing, only 1 OF sample and 2 urine samples had responses above the Evidence MultiSTAT cutoff. No samples demonstrated a response greater than the recommended SAMHSA cutoff for either matrix.

In conclusion, samples that screened positive using both instruments showed similar detection limits. However, the gap in time between screening and GC/MS analysis and the possibility of thebaine instability could account for detection discrepancies. For laboratories looking to test for thebaine as a poppy seed marker, the Evidence MultiSTAT would be a sufficient screening method. Based on these studies, a separate thebaine-only confirmatory method should be utilized using GC-MS, or other methods such as LC/MS/MS could be explored as a viable alternative.

Opioids; Immunoassay; Oral Fluid

L24   An Investigation of Phthalate Levels in Teething Devices for Children in the United Arab Emirates

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Learning Overview: Phthalates, a class of synthetic chemicals used as plasticizers in teethers to enhance their physical properties, raise concerns regarding potential risks to children's health. This presentation investigates the prevalence of phthalates in children's teethers sourced from various suppliers across the United Arab Emirates (UAE), prioritizing product quality and safety.

Impact Statement: The examination of phthalate concentrations within children's toys in the UAE not only contributes to the enforcement of safety rules and the protection of public health, but also increases the forensic science community's skill in evaluating chemical compounds in consumer items. Forensic scientists improve their skills in researching possible dangers in numerous products, not only toys, by inventing and implementing breakthrough analytical techniques to identify and quantify phthalates precisely, which is useful in addressing larger consumer safety issues. Furthermore, their participation in this vital study fosters collaboration among forensic scientists, regulatory agencies, and companies, supporting a multidisciplinary approach to goods safety and advocating for the welfare of children and the community.

To promote flexibility in delicate PVC items, phthalates including DEHP, DINP, and DIDP are incorporated into PVC toys. These phthalates are not strongly bonded to the plastics, but rather are prevalent as movable elements of the polymer backbone. Children may come into physical contact with such substances when using soft PVC items, particularly those meant for maximum touch and mainly when manual force is used (for example, when a child chews off a PVC teether). Considering its elevated consumption per unit of body mass, undeveloped digestive systems capacity, and growing hormonal and reproductive systems, children are more susceptible to the possible harmful repercussions of phthalates. Hence, it was deemed essential to conduct research on the presence of phthalates in kids' toys and other toy products. There are no particular phthalate labelling regulations. Typically, phthalates are not listed on teether labels. In truth, most toys do not specify if they are formed from PVC on the packaging. Other producers are starting to mark their toys and childcare articles as "phthalate-free," presumably providing consumers with the knowledge they require to render informed selections. There are no regulations or guidelines to control the "phthalate-free" label or guarantee that goods with this label genuinely don't possess phthalates. Children between the ages of 0.5 and 4 are projected to consume the most. It is challenging to instantly measure the non-dietary consumption of phthalates that kids may experience when they are mouthing, sucking, or chewing on toys or similar things that possess phthalates. Predictions are created by integrating information on the length of duration children mouth objects with information on the quantity of DINP and DEHP that leaches from items that contain phthalates in research on adult mouthing behavior. There are suggestions that DINP (and DEHP) be taken out of children's teether as consumption levels could be too severe to be safe.

The practical employment of PVC plastics is meant to be prohibited by the 0.1% limit for toys, presumably as a whole or for each of the specified distinct phthalates. For PVC to be soft, phthalates must be present in amounts of at least 10% of its weight. Applying phthalates for their designated mechanical purpose is practically prohibited by the regulation limit of 0.1%, or even 1%. As a result, these restrictions are not predicated on potential health risks or hazards. The UAE's ESMA (Emirates Authorities for Standardization and Metrology) has set a 0.1% limit on four different phthalates (DEHP, BBP, DBP, and DIBP). It is anticipated that this has not resulted in a decline in the usage of plasticizers overall, but rather a change in the sort of phthalates that are most frequently employed.

This research investigates the prevalence of phthalates in children's teethers sourced from various suppliers across the United Arab Emirates, prioritizing product quality and safety. Gas chromatography with mass spectroscopy was employed to analyze six specific phthalates: DMP, DEP, DBP, BBP, DEHP, and DnOP. The analysis unveiled diverse levels of phthalates in several teethers, with the majority of samples meeting the requirement of being below 0.1%. However, certain samples exceeded the permissible limit for specific phthalate compounds according to the law of UAE. The practical employment of PVC plastics is meant to be prohibited by the 0.1% limit for toys, presumably as a whole or for each of the specified distinct phthalates. For PVC to be soft, phthalates must be present in amounts of at least 10% of its weight. Applying phthalates for their designated mechanical purpose is practically prohibited by the regulation limit of 0.1%, or even 1%. As a result, these restrictions are not predicated on potential health risks or hazards. The UAE's ESMA (Emirates Authorities for Standardization and Metrology) has set a 0.1% limit on four different phthalates (DEHP, BBP, DBP, and DIBP). It is anticipated that this has not resulted in a decline in the usage of plasticizers overall, but rather a change in the sort of phthalates that are most frequently employed. These findings emphasize the necessity for rigorous quality control measures to minimize potential health risks associated with phthalate exposure in children's teethers. Continuous monitoring and adherence to safety regulations are vital to safeguard the well-being of young consumers. Furthermore, as the study progresses, it is predicted to impart awareness into the prevalent types of phthalates in teethers, potentially fostering a shift in their usage patterns without compromising overall polyvinyl chloride (PVC) consumption.

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Toxicity; Phthalates; GCMS

L25   The Validation of an LC/QqQ/MS Method for the Detection of Single Doses of Drugs in Hair

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Learning Overview: The goal of this work is to present a validation of a Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) method for detecting single doses of drugs in hair.

Impact Statement: This presentation will impact the forensic science community by presenting a validated analytical method for the detection of 40 different drugs in hair.

Forensic toxicology benefits from drug testing in hair in many different ways. Hair analysis can be effective in identifying exposures for drugs of abuse beyond the typical detection window of other specimens. This makes hair a valuable matrix for long term exposure, as well as chronic use overall. Numerous analytical methods exist for the analysis of drugs of abuse in hair. Cumulative drug exposure over time can be assessed by analysis of the full hair shaft, while episodic exposures can be determined by segmental hair analysis. A major challenge in forensic hair analysis is the detection of single doses of drugs, which can be important in identifying cases of Drug Facilitated Crime (DFC). To date, limited work has been published on single dose hair analysis. The expected concentration range following a single dose exposure of drug in hair is 1-20 pg/mg. This project focuses on the validation of a method for the detection of single doses of drugs in hair using a previously optimized LC-QqQ-MS method.

The optimized sample preparation includes a decontamination procedure consisting of one 30 minute wash with water followed by three 30 minute washes with dichloromethane. The hair is then dried overnight, weighed out, placed into a steel milling jar, and pulverized in a Mini-Bead Beater 24 ball mill (Biospec; Bartlesville, OK, USA) in 10 second intervals for 30 seconds at 3200 rpm. The drug extraction is performed by adding 12.5 μL/mg of hair swelling solution consisting of methanol, acetonitrile, and 2 mM ammonium formate (25:25:50, v/v, pH 5.3). The samples are then placed in a 37°C shaking incubator for 2 hours.

The final LC-QqQ-MS method includes 40 compounds potentially relevant to DFC cases, with individual deuterated internal standards for each analyte. The three MRM transitions with the highest values, one quantitative and two qualitative, for each of the 40 compounds are included in the analytical method. An Agilent 1290 UHPLC coupled to a 6470 LC-QqQ-MS/MS with electrospray ionization in positive and negative mode was utilized for this work. A Zorbax Eclipse Plus C18 column 3.0 x 100 mm, 1.8 mm with guard column was used with a gradient that starts at 5% B for 5 min then increases to 95% until 8 min and is held for 2 min at a flow rate of 0.3 mL/min. Aqueous mobile phase (A) is 5 mM ammonium formate in 0.1% formic acid in water and organic phase (B) is 0.1% formic acid in methanol. The LC-QqQ-MS method demonstrates good selectivity and sensitivity for the selected 40 drug compounds.

The method was validated based on the ANSI/ASB 036 validation standards. The validation consisted of assessment of calibration models, carryover, bias, precision, interferences, matrix effects (ionization suppression and enhancement), Limit Of Detection (LOD), Limit Of Quantitation (LOQ), and sample (extract) stability. The calibration curves were matrix matched using blank hair at 0.0025, 0.005, 0.025, 0.05, 0.25, 0.5, 1.25, 2.5, 5.0, and 10.0 pg/mg concentrations. Calibration curves were initially made in methanol with majority of the LOD and LOQ values at ranges of 0.007-0.2 ppb and 0.02-0.8 ppb respectfully. Matrix effects were evaluated using at least 10 samples of blank hair obtained from non-drug users. The blank hair was decontaminated and extracted according to the optimized method. The method validation is currently ongoing.

This research presents a validated method for quantifying drugs in hair at low concentrations that can accurately detect single doses in hair. Future work will include the analysis of authentic single dose hair samples.

Toxicology; Method Validation; Hair

L26   The Development of a Reference Material for Chemical Hair Analysis

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Learning Overview: This presentation will demonstrate the importance of laser-based sampling for chemical analysis of hair. After attending this presentation, attendees will better understand the importance of the development of a matrix-matched reference material for human hair for forensic toxicology. Hair is a viable source of biological evidence as it can maintain a temporal record of exposure in an individual.

Impact Statement: This presentation will impact the forensic science community by showing the importance of biomonitoring of hair by laser ablation for forensic toxicology. There is a need for a matrix-matched reference material that emulates the chemical and physical properties of human hair. These results can pave the way for a new set of standard reference materials for chemical analysis of hair.

Beyond glass, there is an interest in laser ablation analysis within the forensic science community. In forensic toxicology, chemical analysis of hair has become an established analytical procedure for drugs of abuse. In comparison to other biological matrices, human hair can be useful as a source of biological evidence as it provides a minimally invasive sample collection and is highly temperature stable. Hair can also provide a more sensitive approach as it holds a higher concentration of analytes, especially when evaluating past and present exposure to metals and metalloids.[1] Thus, this makes hair useful to keep a biological temporal record of organic and inorganic components in the body.

Laser ablation of hair is a powerful approach for analysis at a micron scale, eliminating the need for sample modification prior to analysis. Laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been established as a sensitive analytical technique for determining the concentration and distribution of trace metals within hair samples. However, chemical analysis of human hair by laser ablation still encounters the challenge of a lack of standards that are matrix-matched.[2] It is imperative for a calibration material to show similarity to the chemical, physical, and optical properties of its matrix. We have developed a method for a new reference material using keratin film as a matrix-matched standard for laser-ablation based analysis. A new reference material for human hair will provide opportunity for single hair analysis to obtain a quantitative chronological record of potential exposure. For this method, keratin was extracted from human hair then purified, spiked, and cross-linked to obtain a thin film. The films were doped with metals of interest to use as a calibration material for laser-ablation analysis. Characterization of the reference material's chemical and physical properties were studied. These results provide a high-quality standard reference material for forensic analysis of hair.

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Hair; Laser; Spectroscopy

L27   Streamlining Blood LC/MS/MS Workflows to Meet ASB/OSAC Recommendations for Medicolegal Death and Impaired Driving Cases

Jason Truskowski*, PinPoint Testing, Little Rock, AR

Learning Overview: After attending this presentation, attendees will have learned lean strategies in processing blood samples for toxicological investigations that meet newly published Academy Standards Board/Organization of Scientific Area Committees (ASB/OSAC) recommendations for medicolegal death and impaired driving Cases. Concepts and methods presented eliminate time-consuming steps in sample preparation, optimize analytical steps to support concordance testing, and improve data quality and defensibility.

Impact Statement: These methods and concepts will impact the forensic science community by giving forensic toxicologists the ability to become ASB/OSAC compliant for medicolegal death and impaired driving Cases.

In March 2023, the Academy Standards Board (ASB) released updated requirements for blood toxicology testing in medicolegal death and impaired driving cases.[1,2] Unfortunately, decreased discretionary funding makes it difficult to meet this challenge. To sustain ASB complaint testing, forensic laboratories require technologies that provide high data quality and reduces turnaround times.

This study created a simplified Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) workflow to meet ASB/OSAC recommendations. The study stress-tested analytical standard test kits and Supported Liquid Extraction-Diatomaceous Earth (SLE-DE) with optimized LC-MS/MS mobile phases and gradients on C18 or phenyl hexyl stationary phase using LC-MS/MS.

ToxBox® is a premanufactured kit that incorporates calibrators, quality controls, and internal standards using NIST traceable certified reference materials in a 96-well plate. Three analytical plates were designed and manufactured for this study - TOX2001, TOX2002, and TOX2003 – which included 123 analytes. Analytes covered drug classes including opioids, benzodiazepines, antihistamines, antitussives, cocaine, sympathomimetic amines, antidepressants, anticonvulsants and antipsychotics, as well as several other illicit and prescribed drug to cover and go beyond ASB/OSAC guidelines. Targets were chosen to limit interference while maximizing the range of detection and sensitivity of methods and instrumentation. Several manufacturers of SLE-DE plates were performance tested on C18 and phenyl hexyl stationary phases.

Mock casework specimens were added to the three analytical plates. A single extraction procedure using SLE-DE plates and a single mobile phase gradient was tested. This method was validated and optimized on all 123 analytes. Performance was tested between C18 and phenyl hexyl stationary phases showing equivalence. Bias and precision measurements ranted from 0.01 to 9.3% and 0.53 to 10.5% for C18 and phenyl hexyl respectively. Sensitivity issues were reported for analytes in samples with low sample volumes for buprenorphine and norbuprenorphine. Buprenorphine meets ASB/OSAC cut off recommendations, whereas norbuprenorphine is not listed in the guideline, but is required in driving impairment cases. Other limitations include the recovery of benzoylecgonine from the SLE-DE products tested. Further optimization of extraction procedures and/or use of more sensitive equipment is believed to improve recovery observed.

This study provides validation that high-throughput technology can be used to quickly adopt new guidelines suggested by ASB/OSAC for medicolegal death and impaired driving cases. With optimized analytical plate layouts, toxicologists can run parallel processing of two separate stationary phases in as little as one hour.

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Blood; Toxicology; ASB/OSAC

L28 Streamlining Urine LC/MS/MS Workflows to Meet ASB/OSAC Recommendations for Drug-Facilitated Crime Investigations

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Learning Overview: Attendees of this presentation will learn lean strategies in processing urine samples for toxicological investigations that meet newly published Academy Standards Board/Organization of Scientific Area Committees (ASB/OSAC) recommendations for Drug-Facilitated Crime Investigations (DFCI). Concepts and methods presented eliminate time-consuming steps in sample preparation, optimize analytical steps to support concordance testing, and improve data quality and defensibility.

Impact Statement: This presentation will impact the forensic science community by giving forensic toxicologists the ability to become ASB/OSAC compliant for DFCI.

Introduction: In March 2023, the Academy Standards Board (ASB) released updated requirements for urine toxicology testing in DFCI.[1,2] Unfortunately, decreased discretionary funding makes it difficult to meet this challenge. To sustain ASB complaint testing, forensic laboratories require technologies that provide high data quality and reduced turnaround times. This study followed ASB method validation protocols and ISO17025 accreditation to meet DFCI recommendations for LC-MS/MS.[1]

ToxBox® is a test kit that incorporates calibrators, quality controls, and internal standards using NIST traceable certified reference materials in a 96-well plate. The analytical plate included 60 analytes representing drug classes; opioids, benzodiazepines, cannabinoids, antidepressants, analgesics, sedatives, as well as several other illicit and prescribed drugs and their metabolites. Some analytical plates included a newly developed hands-free Suspended-State™ technology. This technology stabilized analytical standards aliquoted at precise concentrations in the presence of sample preparation reagents, including sample matrix, β-D-glucuronidase and enzymatic buffers. ToxBox® kits with Suspended State™ were removed from storage, incubated with samples at either ambient temperature or 60°C, and assayed using the validated LC-MS/MS method.

Drug residue in the traditional ToxBox® was matrix-matched by reconstituting with 100 µL of blank urine. Calibration (0.1 ng/mL to 100,000 ng/mL) and QC (0.3 ng/mL to 90,000 ng/mL) concentrations met sensitivity requirements established for DFCI ([1]). Samples were processed with the addition of β-glucuronidase and concentrated by Supported Liquid Extraction (SLE) using the ToxBox® kit protocol. Chromatographic separations were validated for concordance testing using two orthogonal C18 and phenyl-hexyl stationary phases. Each phase was validated separately using identical mobile phases and gradients. MS/MS parameters maximized analytical measurement ranges on an Agilent 6420 LC-MS/MS. Two m/z transitions (quantitative and qualitative) that minimized potential interferences for each analyte were chosen for confirmational analysis. Interlaboratory comparison studies were executed to assess suitability of the validated procedure in fortified human urine samples.

Results and Discussion: The LC-MS/MS method was validated for 60 analytes within their validated reportable range, recovery (% bias), within and between run precision (%CV), and measurement uncertainty was < 20%. Except for lorazepam, no difference was observed between the two stationary phases. All other analytes met performance specifications and ASB reporting requirements. The clonazepam internal standard interfered with the confirmation ion of lorazepam when the phenyl-hexyl column was used. The C18 column provided adequate resolution to confirm lorazepam above 10 ng/mL (ASB defined cut-off is 5 ng/mL). More sensitive LC-MS/MS instrumentation or a larger sample is required to meet DFCI reporting requirements for lorazepam. All analytes tested were within 0.1 to to 1.6 standard deviation of the reported mean.

Next, the validated procedure was used to evaluate suspended-state technology kits for urine toxicology methods. No differences were observed when results were compared. The %bias and %CV were < 20% for all analytes.

This study validates a streamlined LC-MS/MS testing procedure for two test kits designed for DFCI. This study met accreditation requirements for all analytes tested; however, lorazepam could only be validated to 10 ng/mL using 100 µL sample and entry level LC-MS/MS equipment. The LC-MS/MS testing procedure is suitable for DFCI as described in ASB/OSAC 2023 recommendations.

References:

1. *ANSI/ASB Standard 121*; Colorado Springs, CO, 2021.
2. *ANSI/ASB Standard 036*; Colorado Springs, CO, 2021.

Toxicology; Urine; ASB/OSAC

L29   **"Hang on a Minute": Developing Improved Separation Methodologies for Weakly Retained Polar Molecules in LC/MS/MS**

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Learning Overview: This goal of this presentation is to explore chromatographic parameters to overcome separation difficulties and sample sensitivities by optimizing High-Performance Liquid Chromatography (HPLC) column chemistry, particle technology and column dimensions. The complexity of HPLC and the possible method enhancements are often misunderstood in laboratory settings. This work is intended to demonstrate the power of chromatographic parameters for difficult analyses.

Impact Statement: This presentation will impact the forensic science community by expanding the arsenal of strategies that toxicologists can look to for enhancement of sample signal and for optimization of compound retention and separation for identification and quantitation. With the ever-expanding list of illicit drugs on the open market, toxicologists need to expand their options for analysis. Further, this work demonstrates the power of Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) and argues for transition of methodology in forensics labs to LC/MS/MS methods.

Background: Polar analytes present challenges for optimizing HPLC and LC/MS/MS methods since they are often not well retained using reversed phase methods. This is particularly true for a broad panel of analytes that combines both polar and non-polar analytes of forensic interest. The current study is for the evaluation of poorly retained street drug samples. The goal of this research is to optimize a method for a representative set of polar compounds. Column parameters were studied including silica type, (fully porous at 1.9 μm with surface areas 300 m^2/g and 200 m^2/g and superficially porous at 2.7 μm with surface area of 120 m^2/g), column phase chemistry (C18, amide and perfluorophenyl (PFP)), chromatographic conditions (see below). Biphenyl phases were not evaluated since they have a reputation of high column bleed.

Methods: Columns were either 100 X 2.1 mm or 100 X 1.5 mm. Column length was fixed for the sake of comparison. Evaluation consisted of comparing compound resolution, run time, signal sensitivity, and peak shape. Seven cathinones, three glucuronide metabolites, and six opiates were chosen as an initial evaluation set of compounds. The cathinones were cathinone, methcathinone, mephedrone, butylone, α-PVP, pentylone, and 3,4-MDPV. The glucuronides included were morphine glucuronide, lorazepam glucuronide, and amitriptyline glucuronide. The opiates involved were morphine, hydromorphone, hydrocodone, oxymorphone, codeine, and heroin. Multiple methods were tried, but the following was settled on: flow rate: 0.40 mL/min, mobile phase A: 2.5 mM ammonium acetate with 0.1% acetic acid (pH: 4.15), mobile phase B: acetonitrile. The gradient went from 0 to 60% acetonitrile in 15 minutes.

Results: Of the C18 columns, the 1.9 μm column with a surface area of 200 m^2/g and a pore diameter of 200Å, and the 2.7 μm superficially porous column performed the best. The large 200Å pore diameter allows for better surface wetting at low acetonitrile conditions. For both columns, the peak shapes were excellent (asymmetry < 1.2 on all compounds) and baseline resolution was obtained with good sensitivity. The 300 m^2/g (120 Å) did not provide baseline resolution and no further work was done on this column. The superficially porous C18 phase predictably gave better efficiency and better sensitivity (an average of 1.3 times the peak intensities). Both columns gave excellent intensities down to 125 ng/mL with minimum area counts of greater than 500,000.

The polar embedded amide C18 phase (300 m^2/g, 3 micron and 120 m^2/g, 2.7 micron) was chosen since the amide function enhances retention and alternate chromatographic selectivity. Both amide phases failed to give complete baseline resolution, but they did provide good peak shape (asymmetry < 1.2 on all compounds). Efficiency and peak intensity were predicably higher on the 2.7 micron column.

Lastly the column geometry was investigated using the superficially porous, 2.7 micron C18. Column internal diameter of 2.1 mm is common for LC/MS/MS applications. However, 1.5 mm column diameter was investigated. Reducing the flow rate to 0.20 mL/min gave retention times similar to the 2.1mm columns. Baseline resolution and good peak shape was obtained on all compounds, but the 1.5 mm diameter column demonstrated peak intensities of more than double on all compounds.

Standard curves were prepared for the two C18 columns, and all analytes had values at 0.98 or better.

Conclusion: In closing, this work is intended to explore chromatographic parameters that drug analysts can use for optimization of compound retention and sensitivity. Sensitivity in LC/MS/MS was optimized by bringing analytes to a higher percent acetonitrile, which enhances ionization. Full resolution of all compounds was accomplished by the proper choice of silica particles. The 200Å particles performed very well when compared to the superficially porous particles.

Liquid Chromatography; Metabolite; Tandem Mass Spectrometry

L30   The Separation of Fentanyl Analogs and Fentanyl Geometric Isomers by LC/MS/MS

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Learning Overview: This work investigated the use of chromatographic modes for solving separatory challenges. Attendees will learn about which chromatographic methods are ideal for resolution of fentanyl analog and fentanyl analog isomers. Reversed phase chromatography was investigated with the aim of developing a general method which can be expanded to other drug classes in ultimately a larger panel. The use of Hydrophilic Interaction Liquid Chromatography (HILIC) mode provided methodology for the resolution of geometric and positional isomers. Evaluation of column geometry was instructional for demonstrating the power of column length and column internal diameter to increase resolution and sensitivity of analysis.

Impact Statement: Geometric isomers in general are difficult to analyze for any class of drugs. This presentation will impact the forensic science community by providing methodology that can be used to accomplish those separations. The evaluation of geometric isomers can be important in profiling for connection of street samples to manufacturing sources. Further, this work begins the task of developing larger panels of fentanyl analogs by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis. LC/MS/MS is versatile and provides high sensitivity. As testing laboratories transition to LC/MS/MS, methodologies need to be developed for all drug classes.

Background: Fentanyl and fentanyl analogs are powerful synthetic opioids, which are increasingly found as adulterants in seized samples of cocaine and heroin. Modifications by illicit manufacturers have caused a rapid proliferation of new fentanyl derivatives. As the number of fentanyl seizures and overdoses increases, and new fentanyl analogs are introduced to the illicit market, it is essential for forensic toxicologists to be able to reliably separate and characterize these drugs. This goal is further complicated by the presence of both positional and geometric isomeric fentanyl analogs, which are difficult to separate and cannot be resolved using mass spectrometry alone, since the monoisotopic mass and the fragmentation patterns are the same for geometric isomers and very similar for positional isomers. Therefore, chromatographic separation with unique retentions are mandatory for identification and quantitation of geometric and positional isomers.

Method and Results: This study includes LC/MS/MS analysis of fentanyl analogs using a HILIC mode. Particle types were superficially porous products (Surface area: $135m^2/g$). For the specific resolution of geometric fentanyl isomers (ortho, para, meta) of fluorofentanyl, despropionylfluorofentanyl, and fluoroisobutyryl fentanyl were investigated and for the resolution of positional isomers methylfentanyl analogues (alpha, beta, +/- trans-3-methyl) were investigated.

Separation of the geometric isomers in HILIC mode was attempted using 100 X 2.1mm columns of PFP and HILIC (SiO_2) phases. Mobile phases were varied at 85%, 90% and 95% acetonitrile with 5mM ammonium acetate and 0.1% acetic acid. The PFP column gave long retention (greater than 25min) and poor peak shape. The superficially porous SiO_2 column worked very well, but resolution of the fluorofentanyl isomers was barely baseline. Increasing column length to 150mm gave good resolution, efficiency, and asymmetry at 95% acetonitrile and 5% 5mM ammonium acetate / 0.1% acetic acid with a flow rate of 0.4mL/min. Complete resolution of all geometric isomers in HILIC except for despropionylfluorofentanyl was achieved using a HILIC gradient on the 150 X 2.1mm HILIC column, with an A mobile phase of 5mM ammonium acetate / 0.1% acetic acid and a B mobile phase of acetonitrile. After a 20-void volume equilibration, the mobile phase was held isocratically at 98% B for 1-minute and then ramped down from 98 – 87% B with a gradient time of 17-minutes, utilizing a flow rate of 0.4 mL/min throughout. The order of elution of the fluorofentanyl isomers was ortho (Rt: 9.31min / k': 5.21), meta (Rt:10.43min / k': 5.95) and para (Rt: 10.99min / k': 6.33). For the fluoroisobutyrylfentanyl isomers the results were ortho (Rt: 8.24min / k': 4.49), meta (Rt: 9.08min / k': 5.05) and para (Rt: 9.54min / k': 5.36). The results for the methylfentanyls were alpha (Rt: 14.08min / k': 8.39), beta (Rt: 8.70min / k': 4.80), and trans-3-methyl (Rt: 10.04min / k': 5.69). Following separation on the 150 X 2.1mm columns, the method was performed on a 150 X 1.5mm column with a flow rate of 0.2mL/min, showing approximately a twofold increase in signal intensity.

The despropionylfluorofentanyl set was resolved using a standard 100 X 2.1mm C18 column using an A mobile phase of water with 0.1% formic acid and a B mobile phase of acetonitrile with 0.09% formic acid. The separation was performed isocratically at 36% B with a flowrate of 0.4 mL/min. The results for the despropionylfluorofentanyls were para (Rt: 2.44 / k': 1.80), ortho (Rt: 3.00 min / k': 2.45), and meta (Rt: 3.33 min / k': 2.83).

We successfully resolved the geometric and positional isomers of fluorofentanyl, methylfentanyl, and fluoroisobutyrylfentanyl using HILIC mode LC, and the positional isomers of despropionylfluorofentanyl using reverse phase LC. Chromatographic data and standard curves will be presented.

Fentanyl; Liquid Chromatography; Tandem Mass Spectrometry

L31   What Are You Smoking? A Derivatization to Improve the Analysis of Tetrahydrocannabinol (THC) Isomers Via LC/MS/MS

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Learning Overview: After attending this presentation attendees will be aware that derivatives are frequently used in Gas Chromatography/Mass Spectrometry (GC/MS) to enhance chromatographic or mass spectrometric properties of analytes. In this study, this concept was extended to compounds that aren't amenable to Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis. A derivative methodology was learned. Additionally, parameters for High-Performance Liquid Chromatography (HPLC) analysis were explored, including phase chemistry, media type, and column geometry. These parameters influence the selectivity and sensitivity of the analytes in question.

Impact Statement: THC positional isomers and other analogs from the cannabis family are becoming more and more prevalent in both the illicit and commercial markets. Forensic laboratories often have difficulty distinguishing the isomer as phenols are poorly ionized. This presentation will impact the forensic science community by providing an analytical methodology for the identification and quantitation of cannabis-related products by LC-MS/MS.

Over the past decade, many states have been debating and approving laws regarding cannabinoids originating from *Cannabis sativa*[1]. Cannabinoid is a term that refers to, but is not limited to, both tetrahydrocannabinol (THC) and cannabidiol (CBD). Many states have approved THC for both recreational and medical use. However, many states have approved only the Delta-8-tetrahydrocannabinol isomer, commonly referred to as Delta-8.

Of the several known isomers of THC, the most popular two being debated are Delta-8 and Delta-9. The only difference between Delta-8 and Delta-9 is the position of the olefinic double bond. Although this is a minor difference, the position of the alkene produces different toxicological effects. Of the two, Delta-9 is more potent, which is why it is illegal in many states. With the legalization of Delta-8, many clinical and forensic laboratories have difficulty distinguishing legal Delta-8 from illegal Delta-9 via normal analytical methods. Mass spectrometry (MS) does not provide absolute differentiation between the two since these compounds will have identical mass-to-charge ratios and fragmentation patterns.

Further, the naturally neutral structures of the THCs are difficult to ionize using the soft ionization methodology used in LC-MS/MS. In this study, derivatization of THC analogues including Delta-8, Delta-9, Delta-6, and Delta-10 has been carried out with the aim of producing products that provide for positive ion mode and hence greatly enhanced sensitivity. Derivatization was accomplished using 4-N,N,-dimethylaminobenzoylchloride. This technique was used to derivatize cannabinoids in the form of CRMs, but also plant and oil materials. The analytes were extracted using chloroform and then filtered through a fine glass frit. Derivatization was accomplished in chloroform using the derivatization agent and pyridine as an acid scavenger for the reaction. As an example, 100 mg of oil was suspended in chloroform and 100 mg of derivatizing agent (an excess) along with 50 mg of pyridine were mixed and heated at 50ºC for 1 hour. The chloroform was then evaporated using a nitrogen flow and then reconstituted to 1 mL with acetonitrile. The incorporation of the dimethylamino functionality allows LC-MS/MS analysis in positive ion mode, providing enhanced sensitivity. The non-derivatized samples require negative ion mode, which is less sensitive.

Chromatographic optimization was performed using superficially porous C_{18} and amide columns at 100 X 2.1 mm and 100 X 1.5 mm. The amide-embedded C_{18} phase chemistry is particularly useful since it has high selectivity for compounds with polar moieties. Comparing the amide column to the straight C_{18} the amide column produced the best baseline separation. All further work was conducted on the amide columns. The mobile phase conditions were 2.5 mM ammonium acetate/0.1% acetic acid and acetonitrile as the organic. A gradient was used with a 1-minute hold at 70% acetonitrile then ramped to 84% acetonitrile over 6.5 minutes for a total 7.5-minute run time. A flow rate of 0.40 mL/min was used for the 2.1 mm column while 0.20 mL/min was used for the 1.5 mm column. The carboxy metabolites of Delta-8 and Delta-9 had retention times of 2.29 and 2.59 minutes respectively on the 2.1 mm column and 2.12 and 2.52 minutes respectively for the 1.5 mm column.

As of this writing, we are still developing methods for the derivatized samples, but that data will be available at the time of the presentation.

The derivatization of the samples went according to plan, and once we have chromatographic/MS data, we will determine percent recovery data and standard curves for analysis of street samples. We will also demonstrate the enhanced sensitivity provided by the derivatizations.

Reference:
1.   Meng, Q., Buchanan, B., Zuccolo, J., Poulin, M. M., Gabriele, J., & Baranowski, D. C. (2018). A reliable and validated LC-MS/MS method for the simultaneous quantification of 4 cannabinoids in 40 consumer products. *PloS one*, *13*(5), e0196396. https://doi.org/10.1371/journal.pone.0196396.

Liquid Chromatography; Tandem Mass Spectrometry; THC

L32 **The Quantitation of 106 Drugs in Urine Using a High-Throughput, Seven-Minute Method by High Resolution Mass Spectrometry and Triple Quadrupole**

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Learning Overview: After attending this presentation, attendees will: (1) understand the power of productivity as it relates to a high throughput and robust Liquid Chromatography/Mass Spectrometry (LC/MS) method and how this can be applied to a forensic laboratory; (2) learn about the advantages of a Orbitrap™ technology in terms of screening and quantitative method and the high-resolution instrument and the heightened sensitivity of the triple quadrupole technology; and (3) observe the wide dynamic range of this fast, quantitative method used to detect 106 of the highest-frequency drugs tested by forensic and clinical labs nationwide.

Impact Statement: This method was built around the highest-frequency tested drugs in the United States along with the most common isomers from many different drug classes. This presentation will impact the forensic science community by informing attendees that whether you are using an Orbitrap™ or triple quad, the method shows the power of productivity in its ability to analyze 106 drugs of abuse analytes at once and allows labs to easily customize this list.

Drugs of abuse testing in urine is an essential and routine requirement for forensic toxicology labs. With the ever-growing number of abused drugs and increase in overdoses, it is of great importance to develop a fast, high-throughput liquid chromatography mass spectrometry (LC-MS/MS) method to accommodate drugs of different hydrophilicities, chemical structures, and produce baseline separation of isomers. Moreover, standardization guidelines like ANSI/ASB 113 "Standard for Identification Criteria in Forensic Toxicology" must be followed to ensure the accuracy of the results. Here we present a method for quantitative analysis of 106 drugs of abuse with complete sample preparation workflow and a fast, 7-minute LC-MS/MS method by Orbitrap technology and triple quadrupole.[1]

Methods: 106 drugs, chosen based on high frequency testing in forensic labs, were spiked into negative urine spanning a concentration range of 0.05 to 5,000 ng/mL. 200 μL of each sample were extracted using SOLAμ™ SPE plates. Analytes were separated by Ultra-High Performance Liquid Chromatography (UHPLC) using a 7-min gradient and C18+ column. An Orbitrap mass spectrometer was used for targeted screening and quantitation with full scan and targeted data dependent MS2 scanning used with an inclusion list for targeted compounds. The samples were also run by triple quadrupole using optimized SRM transitions with the same sample preparation and chromatography. Each sample was injected in triplicate.

Results: Limits of detection (LOD) and quantitation (LOQ) were determined for the 106 drugs of over ten different drug classes. LOQ was defined as the back calculated concentration where % difference and % RSD were less than 20%. Lower limits of quantitation ranged from 0.1 to 100 ng/mL meeting ANSI and industry concentration standards. The data shows a wide dynamic range for compounds such as norbuprenorphine, which produced a linear calibration curve between 0.5 and 5,000 ng/mL. Finally, this method adheres to the ANSI/ASB 113 guidelines as both the high resolution Orbitrap and triple quadrupole methods allow you to achieve greater than the minimum required points for analyte identification.

Conclusion: This fast, quantitative method on both Orbitrap and triple quadrupole platforms was used to detect, identify, and confirm 106 high frequency drugs. Due to the robustness of the chromatography and the sensitivity of the mass spectrometers, extremely low LOQs and wide dynamic ranges of calibration curves were produced.

Reference:
1. Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA, USA 95134.

Mass Spectrometry; Drugs; Isomer

L33   Urine Immunoassay Cross-Reactivity of Various OTC and Prescription Drugs

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Learning Overview: This presentation will demonstrate the importance of evaluating urine test cups for the potential of cross reactivity with common Over-The-Counter (OTC) medications. After attending this presentation, attendees will better understand cross-reactivity of structural similar drugs tested.

Impact Statement: This presentation will impact the forensic community by adding to the body of knowledge about the lack of cross reactivity of guaifenesin, orphenadrine, fluoxetine, norfluoxetine, doxylamine, and clomipramine in standard 5-panel immunoassay-based urine test cups. This presentation also highlights the need to consider the potential for common OTC or prescription medications to be evaluated for cross reactivity in these rapid tests.

Immunoassay-based urine cups are a commonly used rapid drug analysis methods and are used in medical examinations, legal applications, and often in various hiring processes.[1,2] These devices detect the presence of pre-specified drugs or drug classes, their metabolites, or both if present at a concentration above the cup's limit of detection.[3] These devices are often preferred due to their non-invasive nature and do not require the collection of blood, sweat, saliva, or hair.[4] This type of testing remains popular due to the rapid results produced in minutes without the use of laboratory instruments.[4] These devices identify pre-specified drugs or drug classes, their metabolites, or often both; while testing for one or multiple compounds. The compounds of interest are amphetamines, opiates, phencyclidine (PCP), cocaine and metabolites, and marijuana and metabolites.[5]

Immunoassay-based testing methods are often sensitive but not specific, meaning that there is the chance for false positive results to occur due to the presence of a cross-reacting, non-target compound; for many common OTC drugs, this cross reactivity is often overlooked or is unknown.[2,3] This study was performed in order to determine whether guaifenesin, orphenadrine, fluoxetine, norfluoxetine, and doxylamine cross-react with the antibodies of the target compounds in 3 commonly used, immunoassay-based 5-panel urine testing devices (Abbott iCup Drug Screen, Wondfo T-cup, and Noble). 30 mL of known negative, untreated urine was spiked with the drug of interest at concentrations of 500 ng/mL, 1,000 ng/mL, and 100,000 ng/mL. Positive control urine was produced by spiking 30 mL of known negative, untreated urine with cocaine, heroin, or methamphetamine; urine samples positive for amphetamine and THC were obtained from volunteers. No cross reactivity (in the form of false positives or false negatives) was observed among guaifenesin, orphenadrine, doxylamine, fluoxetine, or norfluoxetine; with the target analytes in the Abbott iCup Drug Screen, Wondfo T-cup, or Noble test cups.

References:
1. Brahm, N. C., Yeager, L. L., Fox, M. D., Farmer, K. C., & Palmer, T. A. (2010). Commonly prescribed medications and potential false-positive urine drug screens. *American Journal of Health-System Pharmacy*, 67(16), 1344–1350. https://doi.org/10.2146/ajhp090477
2. Lin, C.-N., Nelson, G. J., & McMillin, G. A. (2012). Evaluation of the NexScreen and DrugCheck waive RT Urine Drug Detection Cups. *Journal of Analytical Toxicology*, 37(1), 30–36. https://doi.org/10.1093/jat/bks087
3. Saitman, A., Park, H.-D., & Fitzgerald, R. L. (2014). False-positive interferences of common urine drug screen immunoassays: A Review. *Journal of Analytical Toxicology*, 38(7), 387–396. https://doi.org/10.1093/jat/bku075
4. Skaugen, J. M., Scoccimarro, A., Pizon, A. F., Rymer, J. A., Giannoutsos, S., Ekins, S., Krasowski, M. D., & Tamama, K. (2019). Novel ketamine analogues cause a false positive phencyclidine immunoassay. *Annals of Clinical Biochemistry: International Journal of Laboratory Medicine*, 56(5), 598–607. https://doi.org/10.1177/0004563219858125
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Drug Analysis; Immunoassay; Forensic Science

L34   Don't Huff Before You Blow!

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Learning Overview: The goal of this presentation is to provide an overview on the intoxicating effects of inhaling 1,1-Difluoroethane (DFE) and the subsequent impact on the evidentiary breath alcohol instrument DataMaster DMT-C when a sample is provided. Attendees will learn the different possible responses of an infra-red instrument to the interfering presence of DFE in the breath of an individual providing a sample and the cues that should prompt an officer to suspect the presence of DFE in an individual.

Impact Statement: This presentation will impact the forensic science community by providing an overview on the intoxicating effects of inhaling DFE and the subsequent impact on the evidentiary breath alcohol instrument DataMaster DMT-C when a sample is provided. Attendees will learn the different possible responses of an infra-red instrument to the interfering presence of DFE in the breath of an individual providing a sample and the cues that should prompt an officer to suspect the presence of DFE in an individual.

Drivers stopped for impaired driving following the use of DFE may be asked to provide a breath sample for alcohol quantification by an officer. In the province of Quebec (Canada) the evidentiary instrument used is the DataMaster DMT-C (similar to the Intox DMT). The absorption of infrared (IR) light by a volatile is used by the instrument to quantify ethanol. The sample chamber is monitored every 0.25 sec during expiration with a 3.44 μm wavelength filter. Following the acceptance of the sample, the identity of ethanol is confirmed using the absorbance ratio of 3 wavelength filters (3.44, 3.37 and 3.50 μm). From the concentration of ethanol in the breath sample, the DataMaster DMT-C then calculates an ethanol concentration in blood, based on a distribution factor of 2100:1 (blood:breath ratio) and the output is a value of ethanol in mg/100 mL of blood (80 mg/100 mL is equivalent to 0.080 g/210L of air).

1,1-Difluoroethane (DFE) gas can be inhaled recreationally. This gas is found in various household items such as compressed air dusters, refrigerants, and propellants. DFE, also known as Freon 152a or refrigerant-152a, is a central nervous system depressant causing euphoria, disinhibition, confusion and in some cases reduced consciousness ([1]). DFE is known to absorb IR light in the range used by the DMT-C. Therefore, if an individual provides a breath sample after DFE use, the instrument should react to it. Indeed, when DFE was added to a wet-bath simulator containing an ethanol solution, the resulting vapor caused a "Detector overflow" message. This type of message is obtained when the measurement capacity of the instrument of 700 mg/100 mL (0.700 g/210L of air) is exceeded. This situation can arise from variations in both the absorption coefficient and the concentration in breath of the substance. The later being affected by the amount in blood but also, it's volatility. Since DFE is highly volatile, the latter explanation is the more likely.

In a real case, the presence of DFE in blood (and therefore the presence in breath attributable to its volatile nature), also led to a response from the instrument. The message "Invalid sample" was obtained on two consecutive breath samples. This message is displayed when the level of IR absorption diminishes during breath sampling. On the result printout the estimated ethanol concentration from the continuous IR absorption monitoring was above 0.600 g/210L of air for the first breath sample and around 0.550 g/210L for the second. The presence of DFE was confirmed (qualitative method) in the apprehended driver's blood sample taken 2 hours later.

The presence of DFE could also lead to an "Interference" message (lack of agreement between the IR absorption ratios of the three wavelengths) although a complete sample (meeting the instrument acceptance criteria for volume, air flow and slope) has yet to be obtained.

In conclusion, the combination of ethanol-like impairment symptoms in combination with overflow or breath sample variability messages, especially if the presence of aerosol computer cleaners or other DFE-containing products were noted on the scene, should direct the investigation towards inhalant drug impairment. The vigilance of the arresting officer and the instrument operator are key in properly identifying unusual situations. The detection window for DFE in blood following abuse ranges from 6.5 to 12.8 hours (2) providing plenty of time for the collection of a blood sample following driving.

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Alcohol; Blood Alcohol Concentration; Impaired Driving

L35   Evaluating Drug Positivity Trends in Fatally Injured Drivers, Passengers, and Pedestrians in Connecticut

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Learning Overview: After attending this presentation, attendees will be able to evaluate the prevalence of drugs detected in fatally injured drivers, passengers, and pedestrians who were comprehensively tested for drugs using the scope recommendations developed by the National Safety Council's Alcohol, Drugs and Impairment Division Committee (NSC ADID).

Impact Statement: This presentation will impact the forensic science community by providing an evaluation of current drug positivity rates and recent trends in traffic fatalities. This information will benefit the criminal justice system by providing a detailed account of the drugs contributing to traffic fatalities in drivers, passengers, and pedestrians.

The National Highway Traffic Safety Administration (NHTSA) reported traffic fatalities reached a 16-year high in 2021. Moreover, beginning in 2019, data acquired through the Fatality Analysis Report System (FARS) showed an upward trend with an increase in motor vehicle fatalities, despite several years where numbers had been steadily declining. Trends related to increases in motor vehicle fatalities are likely attributed to numerous factors including returning to pre-COVID roadway traffic and the ongoing opioid/polydrug epidemic among other factors.

Deaths of drivers, passengers, and/or pedestrians from Connecticut were used for the data set. Toxicology testing was performed by NMS Labs and included the basic postmortem toxicology panel, which tests for all Tier I drugs recommended by the NSC-ADID. The manner and Cause Of Death (COD) along with basic demographic information were provided by the Connecticut Medical Examiner's Office.

A total of 907 cases were sent for toxicology testing between 2019-2022. At the time of composing this abstract, 81 cases had been reviewed. With respect to the drivers, fifteen (28%) of the 53 cases were negative for the presence of drugs or alcohol. The most frequently detected substance was ethanol confirmed in 22 (41.5%) of the 53 cases. Postmortem blood (identified as femoral or peripheral) ethanol concentrations (n=14) ranged between 71 mg/dL and 342 mg/dL with a mean and median of 184 and 179 mg/dL, respectively. Twelve cases were above the per se threshold of 80 mg/dL. In five cases in which antemortem blood was provided, the ethanol concentrations ranged from 73 mg/dL to 283 mg/dL with a mean and median of 159 mg/dL and 166 mg/dL, respectively. Two cases had vitreous only, and one case only had muscle tissue.

Other than ethanol, n=19 (35%) of cases were positive for one or more drugs. The most frequently detected drug was delta-9-THC, which was identified in 11 (20.7%) of cases. Postmortem blood concentrations ranged between 0.56 ng/mL to 40 ng/mL with an average of 10 (±11) ng/mL. Three of the cases submitted had antemortem blood and concentrations were 1.8, 3.8 and 18 ng/mL, respectively. The interpretability of postmortem delta-9-THC concentrations with respect to impairment have recently been called into question. Other Tier I drugs identified in drivers more than once included: alprazolam (n=3, 6%), cocaine and metabolites (n=3, 6%), methadone (n=3, 6%), amphetamine (n=2, 4%) and morphine (n=2, 4%). Of interest, phencyclidine, a Tier II drug, was identified in three drivers (6%). With respect to the number of drugs identified in a single driver, there was a 40-year-old man with 11 findings including ethanol, fentanyl, methadone, clonazepam, alprazolam, cocaine, and flubromazolam along with their respective metabolites. The COD in this case was smoke inhalation and thermal injuries.

With respect to passengers (n=12), the most frequently detected drug was delta-9-THC identified in four cases (33%). Four of the passengers were negative for any of the tested for drugs. Of the 16 pedestrians included in the data set, six (37.5%) were positive for delta-9-THC, four (25%) were positive for fentanyl, and four (25%) had none detected. Of interest, a 28-year-old pedestrian whose COD was from blunt head neck and torso trauma was positive in antemortem blood for fentanyl (19 ng/mL), norfentanyl (11 ng/mL), xylazine (19 ng/mL), methadone (150 ng/mL), caffeine, and cotinine. The presence of xylazine suggests that this was illicit fentanyl use.

As traffic-related fatalities continue to increase nationally, the likelihood of drug detection in these cases also increases. In addition, recreational marijuana became legal in Connecticut on July 1, 2021. Continued vigilance and comprehensive testing aligned with testing recommendations is necessary to assess the impact of drugs in fatalities among drivers, passengers, and pedestrians.

Traffic Fatality; Postmortem Toxicology; Medical Examiner

L36 Drivers Under the Influence of Alcohol and Drugs: A Six-Year (2017–2022) Retrospective Analysis in a Southern Italian Region

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Learning Overview: After attending this presentation, attendees will gain an understanding about the Italian trend in alcohol and drug use among motor vehicle drivers involved in road traffic accidents.

Impact Statement: This presentation will impact the forensic science community by sharing toxicological findings derived from extensive analysis conducted by one of southern Italy's leading forensic toxicological laboratories on biological samples (blood and urine) from 3.252 drivers suspected at roadside screening of Driving Under the Influence of alcohol (DUI) or Drugs (DUID) from 2017 to 2022.

Driving a motor vehicle under the influence of alcohol (DUI) or drugs (DUID) is worldwide considered a crime. An Italian Road Traffic Law (L. 41/2016) has updated the crimes related to DUI and DUID and the penal sanctions have been raised.

Based on the 2021 report of the National Institute for Statistics (ISTAT), 151.875 road accidents with 204.728 injured victims occurred in Italy. 9.014 out of 151.875 road accidents were reported in Campania with 12.883 injured victims.[1-3]

Aims of this retrospective study are: a) to assess the trend of consumption of alcohol and illicit/licit drugs by the drivers involved in fatal and non-fatal road accidents and admitted to the emergency departments located in Campania from 2017 to 2022. b) to collect data useful for the improvement of toxicological analyses and preventive policies. Confirmation tests of positive toxicological screening analyses were performed on biological samples (blood/urine) collected from 3.252 hospitalized drivers. Therefore, 3.252 blood samples and 2.178 urine samples were analyzed in total. BAC on whole blood was analyzed by GC/HS-FID and quali/quantitative analyses for drugs were accomplished by GC/MS or LC-MS/MS.

Results: 2.914 drivers (89.5%) tested positive for alcohol and/or drugs: among them, 1.056 (36.2%) were positive only for alcohol; 1.423 (48,8%) took licit/illicit drugs, individually or in combination, while 435 drivers (15%) were positive for alcohol and drugs in combination. The number of drivers suspected of DUI increased from 499 drivers (in the first three years of the study, 2017-2019) to 557 (in the late three years of the study, 2020-2022). A similar increasing trend has been observed for drivers suspected for DUID raised from 216 (in the first three years of the study 2017-2019) to 219 (in the last three years of the study 2020-2022).

Toxicological analyses were negative for alcohol and/or drugs in 338 drivers out of 3.252 suspected cases of DUI and DUID previously recognized positive at screening tests. It is worth of mentioning that these negative results (10.5% of the sample study) were obtained in 219 blood samples and 171 urine samples.

BAC >1.5g/L was found in 474 out of 1.056 (44.9%) suspected cases of DUI and in 183 out of 435 (42.1%) suspected cases of DUID. Of all the subjects tested positive for drugs, 426 out of 1.858 (22.9%) had taken multiple drugs and 1432 out of 1858 (77.1%) had taken one single drug. Among cases of single-drug use, D9-THC (641 out of 1432 cases - 44.8%), cocaine (438 out of 1432 cases - 30.6%) followed by benzodiazepines (10.1%) and morphine (9.3%) were the most frequent substances. Cocaine and D9-THC was the most common combination in polydrug users (152 out of 426 cases – 35,7%).

In conclusion, suspected cases of DUI or DUID related road traffic accidents were confirmed in 89.5% of drivers; this is a better performance compared with the previous one (86.4%) reported in our 8-year retrospective study over 1.797 suspected drivers.

False positive results of the preliminary screening tests also fell to 10.5% from 15.4% reported in the previous study. Such achievement can be related to an improvement of our laboratory protocols, more sensitive instruments of quantitative analyses or better reliability of screening methods by immunoassay than confirmation procedures.

However, false positive results of the preliminary screening tests could be also caused by late blood sampling that should be done in 3 hours from the road crashes, to prevent modification due to pharmacokinetic. Late blood samples can lead to negative results in confirmation test. Therefore, to improve the accuracy of toxicological analysis performed on subjects suspected of DUID, time-factor should be also taken in consideration and implemented into government laws.

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Driving Under the Influence of Alcohol (DUI); Driving Under the Influence of Drugs (DUID); Road Traffic Accidents

L37   Pediatric Poisoning: A Plan of Action for Early Diagnosis and Treatment

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Learning Overview: This presentation aims to highlight the lack of an unequivocal diagnostic protocol in the case of suspected pediatric intoxication in structures not provided with a forensic toxicological service/unit.

Impact Statement: This presentation will impact the forensic science community by proposing an algorithm to be used in the Emergency Room (ER) showing the paramount role of collecting biological samples according to a strict forensic protocol.

The pediatric population represents the most vulnerable and at risk for unintentional poisoning, with children younger than 6 years old accounting for nearly half of poison exposures.[1,2]

The main risk factors for children are improper storage of substances, lack of supervision and distraction from caregivers. Other known risk factors are single parent households, parental illness or disability, accessibility to toxic agents, grandparent caretaking and a desire of the child to imitate adult behavior.[3]

Poisoning is a time-dependent emergency and the need to reach a scientific agreement on a diagnostic protocol and treatment seems to be crucial.

Starting with the experience of a pediatric hospital located in the South of Italy regarding the case of a 4-year-old boy (hospitalized for drowsiness) limits and pitfalls of a traditional clinical diagnostic approach are highlighted.

In the presented case, the mother reported that the little boy was playing in the garden and suddenly became drowsy. No family history of similar episodes was reported. All his psychomotor developmental stages were normal. Diagnosis of acute buprenorphine intoxication was only reached after three days when an in-depth diagnostic investigation became necessary and complete forensic toxicological analyses were performed. The case evidenced an alarming lack of an unequivocal diagnostic protocol in suspected intoxication in structures not provided with a forensic toxicological service/unit. Collection of biological specimens according to forensic protocols at hospital admission plays a paramount role in the definitive diagnosis of intoxication.

Accidental intoxication in pediatric population exposes healthcare professionals to risks possibly related to judicial consequences. In fact, the availability of biological samples suitable for forensic analyses is mandatory. A diagnostic algorithm that focuses on medical history and biological specimen collection timing is herein proposed, in order to unify emergency approach to the suspected poisoned child.

Finally, the paucity of data on buprenorphine pediatric intoxication sets a need to improve the knowledge of the phenomenon, promoting a virtuous collaboration between emergency departments and forensic toxicological units.

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Poisoning; Buprenorphine; Emergency

L38   The Effects of Ethanol on GABA Catabolism Via GABA-Transaminase

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Learning Overview: This research presents one potential aspect of the mechanism ethanol may utilize to affect the Central Nervous System via its influence on the concentrations of the inhibitory neurotransmitter, GABA. After attending this presentation, attendees will learn how ethanol impacts the GABA catabolic pathway and its effect on the kinetics of the enzymes involved.

Impact Statement: This research may provide insight to the mechanism by which ethanol causes inhibitory effects in the central nervous system. This presentation will impact the forensic science community by being useful to forensic practitioners in their efforts to understand the effects of ethanol alone in the body, as well as in tandem with other toxic agents.

Ethanol is the primary active ingredient in alcoholic beverages, and functions in the body as a central nervous system (CNS) depressant.[1] The overt and recognizable effects ethanol has on the body are well documented, such as diminished cognitive ability, loss of motor control, slurred speech, impaired balance, etc. The similarity between the effects of ethanol and those of drugs mimicking the inhibitory neurotransmitter GABA (e.g., benzodiazepines, barbiturates) has led to investigations regarding a potential agonist effect of ethanol on GABA receptors.[2] However, despite extensive efforts, no definitive binding site or agonistic mechanism has been identified.[3]

The structural homology between ethanol and the C3, C4 carbons of GABA lead us to hypothesize that ethanol may acts as a CNS depressant in part via the inhibition of GABA catabolic enzymes, thereby increasing the functional concentration of GABA in the inhibitory neuron and the inter-neuronal synaptic space following an inhibitory depolarization event. The two primary enzymes involved are GABA-transaminase (GABA-T) and Succinyl Semialdehyde Dehydrogenase (SSADH). GABA-T exchanges an amine group from GABA with a keto group from a-ketoglutarate to produce Succinyl Semialdehyde (SSA) and glutamate.[4] SSADH then oxidizes SSA to succinic acid, using either NAD+ or NADP+ as the oxidizing agent. Inhibition of either, or both, enzymes would slow the catabolism of GABA, presumably thereby increasing the concentration of GABA in both the pre-synaptic neurotransmitter vesicles and synapse as a consequence of depolarization.

We have previously investigated the effect of ethanol on SSADH and determined that there was a competitive inhibitory effect at physiologically relevant concentrations of ethanol (0.08 – 0.32 g/dL). In this study, we have investigated the effect of ethanol on GABA-T, using the commercially available enzyme GABase, containing both GABA-T and SSADH.

The enzyme assay protocol is a modified version of that outlined in Tsukatani, Higuchi & Matsumoto (2005).[5] Activity of the enzyme system was measured indirectly by the amount of NADH (or NADPH) produced using a UV-Vis spectrophotometer @ 340 nm. A series of substrate-velocity experiments were conducted with varying concentrations of GABA in the presence and absence of ethanol (0.4 g/dL) to evaluate the potential for ethanol-based inhibition of the enzyme system. To isolate the effect of ethanol on GABA-T, we utilized a pre-incubation period where the necessary cofactor for the oxidation by SSADH, NAD+, was initially withheld. Formation of the SSADH reaction substrate, SSA, would then be expected to be constant unless affected by ethanol-based inhibition. After the pre-incubation, NAD+ was added to the reaction and the absorbance was monitored. As such, if and to the extent that ethanol inhibited the GABA-T reaction, the concentration of SSA available as a substrate for the subsequent NAD+ (NADP+) – dependent reaction would be reduced. These results were compared to various controls, including those without the pre-incubation, in order to determine the effect of ethanol on GABA-T alone.

Michaelis-Menten coefficients (K_M; reflecting substrate affinity for enzyme binding site) and maximal reaction rates (V_{max}) were then determined. Noncompetitive inhibition was present at physiologically relevant levels (i.e., ~0.4g/dL). K_M of GABA-T was unaffected by the addition of ethanol, indicating it does not alter the enzymes affinity for the substrate. However, V_{max} was reduced in response to ethanol demonstrating that it functions as noncompetitive inhibitor of the enzyme.

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Ethanol; GABA; Kinetics

L39   *Crotalus* Snake Venoms of North and South America: Their Symptoms, Treatments, and Potential Medical Applications

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Learning Overview: This presentation will discuss the compositions of *Crotalus* genus snake venoms and their respective symptomologies, then compare the differences in physiological effect between the species of the Americas. Attendees will learn about the challenges of antivenom production and how inter- and intra-species venom variation complicates the treatment process. Finally, the attendees will hear of the therapeutic potentials of these rich and varied sources of biologically active molecules.

Impact Statement: This presentation will impact the forensic science community by collating available knowledge on *Crotalus* snakebite envenoming, thereby highlighting the best practices for treatment and prevention so available public information and care can be improved. This presentation hopes to draw attention to currently under-researched fields surrounding the topic, such as analgesic usage of venom components and antivenom supply/storage.

The *Crotalus* genus of vipers is widespread across the Americas and is known for containing several species of rattlesnake. Between 1989 and 2018, 93 of 101 snakebite fatalities in the USA were associated with envenoming from this genus.[1] They are normally reclusive, and only bite in self-defence and are even capable of 'warning strikes' containing no venom to dissuade a would-be predator. Despite this, envenomation is common, with 5 to 10 thousand general envenomations per year in the US alone, though the true number is suspected to be higher as less serious bites or bites in isolated communities go unreported. Although fatalities are rare, bites can cause death if untreated, and a longer interval between envenomation and care typically worsens sequelae, such as thrombocytopenia, tissue loss, and amputation.

Snake venoms are complex mixtures of various enzymatic and non-enzymatic toxins. For the *Crotalus* genus, the composition of this venom will vary based on several factors, including species, age and location, but generally follow a rule of containing metalloproteases to aid digestion, as well as a balance between neurotoxic components and myotoxic components. Phospholipase A_2 activity is common among crotaline toxins, but the most prevalent is crotoxin, which is made of two subunits and has a wide range of effects.[2] Generally, *Crotalus* snakes of North America have more myotoxic venoms, while South American species have more neurotoxic venoms, particularly for inducing respiratory paralysis.

As these venoms can cause various combinations of symptoms based on the balance of toxins, such as swelling, limb weakness and necrosis, identification of the biting snake is important for planning a patient's care . Furthermore, the correct antivenom must be administered, as while polyvalent antivenoms exist, they are typically limited to only a few species.[3] Fortunately, most care centres in the USA carry antivenoms for any local venomous species, though cost and supply are important factors, as well as underestimated expiry dates resulting from a lack of research. Snake envenomation is a disease of poverty, disproportionately affecting lower income, rural groups, especially agrarian workers, who are more likely to be bitten occupationally. Countries like Brazil, where this demographic is large are affected much worse than the USA and Canada, especially as these rural communities are often isolated and far from urban health centres.[4]

Venoms are rich sources of biologically active molecules, with crotaline toxins, such as crotoxin and crotalphine being studied in animals for their use as analgesics, particularly when administered with carrier molecules. Secondly, crotoxin acts preferentially towards actively proliferating cells, thus is being investigated for use as a cancer treatment. Venom toxins can also act with anti-plasmodial and antibacterial properties, which in response to increasing drug resistances are of particular value to research.[5]

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Toxicity; Histological; Species

L40 A   Review of Popular Illegal Performance-Enhancing Drugs in Sports and Gyms

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Learning Overview: The goals of this presentation are to provide a comprehensive compendium of the effects and side effects, both physical and mental, of some of the most popular Illegal Performance-Enhancing Drugs (IPEDs). The side effects are extremely numerous and in additional to mental side effects, the general systems that are affected by the side effects are the cardiovascular, endocrine, hepatic, liver, renal, gastrointestinal, musculoskeletal, skin, and central nervous systems. Anabolic steroids affect all of the listed systems, while other IPEDs present lead to side effects only in some systems. The main systems usually affected by side effects are the cardiovascular system, the musculoskeletal system, and the endocrine system in some cases. Further goals intend to highlight the prevalence among the general and athlete populations, provide information on some aspects of usage, and evaluate the research present on each topic.

Impact Statement: This presentation will impact the forensic science community, by providing an easier and faster way to find general information regarding IPEDs. This can potentially make future access to the information simpler and reduce the number of papers needing to be read, thus reducing the time and energy needed to comb through research for relevant information.

PEDs (Performance-Enhancing Drugs) are present throughout society, with illegal performance-enhancing drugs (IPEDs) having a prevailing presence in gym environments and among athletes. Androgenic Anabolic Steroids (AASs) have an estimated prevalence of between 1 to 3 million people in the United States (Statpearls Middlebrook and Schoener 2022) The popular IPEDs discussed in this paper will be AASs, specifically testosterone, Growth Hormone (GH), ephedra/ephedrine, and 1,3-dimethylamylamine (DMAA). The studies and information used for the paper were obtained by using the website PubChem and utilizing the search engine Google and Google Scholar to find articles using keywords, such as prevalence, AASs, IPEDs, and performance enhancement. The research papers and studies were only included as potential information if they were written after the year 1980. However, most studies utilized were written after the year 1990 and were used to provide more up-to-date information about IPED use and its effects. Most studies used were full papers, however, from some studies only abstracts were used. Additionally, one book on doping in sports, published by Springer and accessed online was used.

The side effects of AAS and ephedrine/ephedra mostly focused on the severe side effects from usage affecting the cardiovascular system, however, unlike ephedrine/ephedra, AAS also significantly affected the endocrine system. Growth hormone (GH) negatively affects cardiac functions and pulmonary functions, with some evidence pointing to GH being a potential carcinogen.[1] research, while cardiovascular side effects were observed on the supposed side effects of DMAA, the side effects however only seem to be present at very high dosages, above 100 mg.[2] However, the observed severe side effects were usually observed at dosages 19 to 37 times the recommended dosage of 25-50 mg.[3] The research regarding androgenic anabolic steroids (AASs) is extensive and well documented, with deep analysis into the effects, motivations, and prevalence among a variety of populations. hGH is often used in conjunction with AAS. Among AAS users, the lifetime prevalence of hGH use is 27%.[4] Among both steroid and non-steroid users, Nelson et al. report that over 50% of athletes considered using PEDs, and 25% have used GH daily.[5] All aspects of hGH are well documented, possibly due to the prion diseases that can be contracted with hGH use. The use of ephedrine in men was reported to be 25%, while the prevalence percentage was around 13% among women.[6] Information regarding ephedra/ephedrine, especially the history, is well documented, with documentation that can be traced to traditional Chinese medicine use in a plant named Ma Huang. DMAA has a severe lack of information in all aspects. Prevalence and use statistics are mostly missing and there is inconsistent information regarding the effects of use. Overall, DMAA is lacking in concrete information regarding the drug. The studies and information regarding the prevalence have been mostly from North America, Europe, and Arabic countries while missing other continents.

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Doping; IPEDs; Sport

L41 An Evaluation of Long-Term Health Effects From Acute Exposure to Toxic Chemicals

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Learning Overview: The goal of this presentation is to review the work done by the Department of Homeland Security's Chemical Security Analysis Center to develop a modeling tool to determine long-term health effects after an acute exposure to toxic chemicals. This work has been an on-going effort to define bin chemicals into toxidromes and use the toxidrome-based data to develop these long-term effects.

Impact Statement: This presentation will impact the forensic science community by relating that the hope for this data and model is to help the communities plan for longer-term needs and support from acute exposures. There is also a military aspect to maintain protective military exposure guidelines to these chemicals.

Since 2016, the Chemical Security Analysis Center of the Department of Homeland Security Science and Technology Directorate and the Army Public Health Center (APHC) have partnered to develop guidelines for acute exposure to chemicals of concern resulting in chronic effects. The Acute Exposure/Chronic Effect (AECE) project utilizes a toxic syndrome (i.e., toxidrome) based approach where the likelihood of long-term health outcomes are evaluated as a function of the acute exposure level (mild, moderate, severe, life-threatening). Six toxidromes (cholinergics, blood, opioid, irritant/corrosive – upper pulmonary, irritant/corrosive – lower pulmonary, and vesicants) have been completed and three toxidromes are planned for FY22-23 (convulsant, hemolytics, metabolic). Collecting data based on a toxidrome rather than a single chemical addresses the issue of sparse data, as findings made for a toxidrome can be leveraged for any chemical in that toxidrome. The supporting assumption for this generalization is that chemicals in the same toxidrome elicit similar acute effects, and that long-term health effects can be estimated based on the extent of the injury demonstrated by acute effects.

This project utilizes Subject Matter Experts (SMEs) and peer reviewed journal articles to identify the acute exposure symptoms, which are categorized by health effect severity. SMEs then identify potential long-term symptoms which are separately categorized by health effect. Then the probability of a long-term health effect based on each level of acute exposure can be elucidated by the SMEs and combined into a single probability for each acute exposure level. These effect probabilities are combined with acute effect dose-response estimates to yield long-term health effect curves for each chemical in the toxidrome. APHC will utilize these calculations to expand on the Military Exposure Guidelines (MEGs). Currently, MEGs only consider the acute effects of acute exposures despite documented evidence that acute exposures can lead to long-term effects. Such evidence includes incidences with military relevance, such as exposure to mustard agents during World War I and sarin attacks in Syria from 2012 until present day. Better knowledge of how an acute exposure can lead to long-term effects allows for better military planning, as long-term effects could manifest during the course of a deployment in ways that would impact operational readiness. The CSAC will apply these values to modeling potential harm to civilian populations to inform the Homeland Security Enterprise (HSE).

Toxicity; Modeling; Public Health

L42 Future Strategies in Drug Trend Monitoring and Harm Reduction

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Learning Overview: This presentation will describe future strategies in drug trend monitoring and harm reduction. After attending this presentation, attendees will better understand the importance of harm reduction.

Impact Statement: This presentation will impact the forensic science community by showing that harm reduction is to mitigate the adverse effects connected with different actions or pursuits, particularly those linked to substance consumption and hazardous behaviors. Rather than concentrating solely on complete refraining, harm reduction recognizes that certain actions may continue and endeavors to diminish the potential detriment to individuals and communities. This approach is based on empathy, practicality, and evidence-supported methodologies. Harm reduction techniques can be employed in diverse domains, although they are most frequently linked with substance utilization, such as the usage of drugs and alcohol.

Objectives: By minimizing the adverse effects of some risky behaviours, particularly those related to substance abuse and high-risk activities, harm reduction is a pragmatic and evidence-based approach to public health. Harm reduction strategies focus on minimizing the harmful effects of such behaviours on individuals, communities and society.

Methods: A review of instrumental techniques such as Gas-Chromatography Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Fourier Transform Infrared (FTIR) and nuclear magnetic resonance in harm reduction strategies in various settings.

Results: This study recognizes the dynamic nature of drug markets, the emergence of new user groups, the prevalence of polydrug use, the increasing potency of substances, and the rise of emerging stimulants within the nightlife economy. It identifies barriers to the effective implementation of harm reduction and education programs and suggests potential approaches to address these challenges. Moreover, the study emphasizes the value of online surveys as a versatile tool in harm reduction strategies, education programs, and the monitoring of future drug trends. Ultimately, this research contributes to our understanding of effective approaches to harm reduction on a global scale, recognizing the evolving nature of substance-related issues and the importance of evidence-based strategies. This research highlights the importance of staying updated on evolving drug trends to adapt testing methodologies accordingly.

Discussion: Education programs are essential in harm reduction and future drug trend monitoring. This review will consider recommendations on a number of key areas on the topic of emerging drug trends, nightlife drug use and novel responses to drug checking provisions. A focus on the considered use of tailored web surveys to obtain data on the needs of all the parties (and consider the advantages/disadvantages) involved will be outlined. The value of these surveys for selected parties (intervention planning, environmental planning, scientific testing planning, identification of staff, identification of peer volunteers, a training program and robust mechanism for evaluation) will be discussed where applicable.

Conclusions: Due to changing drug markets, new user groups, poly drug use, higher potency and emerging stimulants in the nightlife economy this work reviews the barriers to implementation of harm reduction and education programs and suggests potential approaches to address these and the value of online surveys in harm reduction approaches worldwide, education programs and future drug trends. This research contributes to the forensic toxicology community by offering insights into the application of advanced analytical techniques, the identification of emerging substances, data collection methods, and the broader context of harm reduction and prevention efforts. It encourages a holistic approach to addressing substance-related issues, which can ultimately benefit the forensic toxicology field in its mission to provide accurate and meaningful information in legal and public health contexts.

Education; FTIR; Mass Spectrometry

L43   A Review of Factors Affecting the Interpretability of Postmortem Cannabinoid Concentrations and Their Limitations

Sherri L. Kacinko, NMS Labs, Horsham, PA; Daniel S. Isenschmid, NMS Labs, Horsham, PA; Barry K. Logan*, Center for Forensic Science Research and Education, Horshan, PA

Learning Overview: After attending this presentation, attendees will be able to describe some of the known limitations on the interpretability of postmortem (PM) cannabinoid concentrations from both an analytical and interpretive perspective.

Impact Statement: This presentation will impact the forensic science community by drawing attention to the increasing knowledge base that suggests that cannabinoid concentrations in PM blood samples are subject to changes in magnitude and direction, likely due to a combination of factors that are unpredictable from case to case, and that they should be interpreted with a high degree of caution.

Circumstances, including cases where recent cannabis use, or the sobriety of the decedent may be an issue in either criminal or civil litigation. Interpretation of these results is dependent upon multiple factors, including the assumption that the postmortem (PM) concentration of drugs including cannabinoids and their metabolites accurately reflects the amount of drug circulating proximate to the time of death, and that there is a relationship between the measured concentration of the drug and the dose/effects of the drugs.

In living subjects, delta-9-tetrahydrocannabinol (delta-9-THC) is known to persist in the blood of chronic users for days or weeks at measurable concentrations following time of last use, making interpretation of degree of impairment, or time of last use based solely on blood concentrations, a dubious practice.

In postmortem scenarios there are many additional variables that complicate measurement and interpretation. In general, femoral blood is considered the specimen of choice for postmortem toxicology because it is considered most likely to represent the perimortem drug concentrations and is least likely to be impacted by Postmortem Redistribution (PMR). During the postmortem period drugs may move from areas of higher concentrations (the organs) to areas of lower concentration (the blood), complicating interpretation.

PMR can be studied by comparing the Central Blood to Peripheral Blood (CB:PB) ratio of drugs. In addition to PMR, the stability of analytes between the time of collection and the time of testing can also be a factor in the degree of redistribution. PMR studies of cannabinoids have been performed using animal models and have demonstrated that neither concentrations from blood samples collected 2 hours or 24 hours after death reflected the perimortem concentrations.

Studies on PMR evaluating human heart:iliac blood delta-9-THC ratio demonstrated marked site dependence of concentrations, and a trend towards greater PMR with increasing postmortem interval. Another study evaluating delta-9-THC concentrations in samples collected antemortem (AM), at mortuary admission (AD) and at autopsy and showed a 7.2 fold increase in median delta-9-THC concentrations over a median time period of 33 hours between the AM and the AD specimens.

Analyte stability is another factor that must be considered for accurate interpretation of PM drug concentrations. Delta-9-THC concentrations are known to decrease over time when stored in untreated glassware. In one study, at refrigerated temperatures less than 10% of the initial delta-9-THC concentrations remained after a period of 196 days.

Finally, when evaluating the usefulness of quantitative toxicology, the interpretive value of a concentration must be considered. There is no established lethal concentration of delta-9-THC, and there is no evidence-based threshold for impairment based on delta-9-THC measurements in postmortem blood.

Based on the documented site specific differences in concentration, and the magnitude of the changes that can occur in delta-9-THC concentrations during the PMI and during storage, combined with the limited interpretive value of cannabinoid concentrations even in living persons, quantitative determination of postmortem cannabinoid blood concentrations with respect to establishing the concentration at the time of death, or using the concentrations to infer impairment, are not warranted. At best, the detection of delta-9-THC in postmortem blood is evidence of ingestion of or exposure to the drug at some indeterminate time prior to death. Similarly, low or none-detected concentrations of delta-9-THC do not rule out the possibility of impairment at some earlier time [1-8].

Given these considerations, interpretation of postmortem blood delta-9-THC concentrations with respect to impairment or time of last use should not be considered a forensically reliable practice.

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Marijuana; Impaired Driving; Postmortem

L46   An Update on Standards Development Activities in Forensic Toxicology

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Learning Overview: After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of forensic toxicology.

Impact Statement: This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to forensic toxicology. This presentation will also increase awareness regarding training, tools and resources that support implementation, compliance monitoring, and broader understanding.

Standards continue to impact daily work in the field of forensic toxicology. Through the efforts of both the Organization of Scientific Area Committees (OSAC) for Forensic Science and the Academy Standards Board (ASB), there have been over a dozen forensic toxicology standards published over the last six years. This presentation will provide an update on the current published standards, those nearing completion, as well as standards you should expect to see in the coming years. During this presentation, updates related to standards development in forensic toxicology will be presented. These include:

ASB-published documents that have been added to the OSAC Registry:

- ANSI/ASB 017: Standard Practices for Measurement Traceability in Forensic Toxicology;

- ANSI/ASB 036: Standard Practices for Method Validation in Forensic Toxicology;

- ANSI/ASB 037: Guidelines for Opinions and Testimony in Forensic Toxicology;

- ANSI/ASB 053: Standard for Report Content in Forensic Toxicology;

- ANSI/ASB 054: Standard for Quality Control Programs in Forensic Toxicology Laboratories;

- ANSI/ASB 119: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations;

- ANSI/ASB 120: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations;

- ANSI/ASB 121: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigation; and ANSI/ASB 152: Standard for the Minimum Content Requirements of Forensic Toxicology Procedures

ASB-published documents that have yet to go through the OSAC Registry approval process:

- ANSI/ASB 098: Standard for Mass Spectral Data Acceptance in Forensic Toxicology;

- ANSI/ASB 113: Standard for Identification Criteria in Forensic Toxicology;

- ANSI/ASB 153: Standard Practices for Proficiency Testing for Forensic Toxicology Laboratories; and

- ANSI/ASB 156: Guidelines for Specimen Collection and Preservation in Forensic Toxicology

Documents currently in development by the ASB:

- ASB 055: Standard for Breath Alcohol Measuring Instrument Calibration;

- ASB 056: Standard for Evaluation of Measurement Uncertainty in Forensic Toxicology Laboratories and Breath Alcohol Programs;

- ASB 118: Standard for Breath Alcohol Instrument Specifications;

- ASB 122: Best Practice Recommendation for Performing Alcohol Calculations in Forensic Toxicology; and

- ASB 173: Standard for Education, Training, Continuing Education, and Certification of Forensic Toxicology Laboratory Personnel

- ASB 017: Standard for Metrological Traceability in Forensic Toxicology, Second Edition

- ASB 036: Standard for Test Method Selection, Development, Validation, and Verification in Forensic Toxicology, Second Edition

Documents currently being drafted at the OSAC:

- Quality Management Systems in Forensic Toxicology Laboratories;

- Standard Method for Blood Ethanol Identifications and Quantitations; and

- Carbon Monoxide Analysis in Forensic Toxicology

Priorities for revisions to existing standards, new documents or work products, and other highlights.

Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

Standards; Quality Assurance; Court

L47   Quantitation of Hexahydrocannabinol and Metabolites in Blood From Recreational Users

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Learning Overview: After attending this presentation, attendees will better understand the complexity of hexahydroxycannabinol analysis and the evaluation of blood concentrations in the living. Attendees will be able to describe the metabolism of hexahydrocannabinol and compare it to that of tetrahydrocannabinol.

Impact Statement: This presentation will impact the forensic science community by presenting human data from a new cannabinoid, hexahydrocannabinol, and provide information about immunochemical cross reactivity and the blood concentrations from recreational users. This presentation will also provide some insight into hexahydrocannabinol's metabolism and propose major human metabolites.

Hexahydrocannabinol (HHC) emerged on the drug market as an ingredient of infused hemp flowers, vape products, and edibles around mid-2021 first in the USA and then elsewhere. It was first reported in the EU in May 2022. HHC has three chiral carbon atoms, but only (6aR,9R,10aR)-HHC (9R-HHC) and (6aR,9S,10aR)-HHC (9S-HHC) have been encountered in HHC products. The human pharmacokinetics and metabolism of HHC has not been published. Studies on the *in vitro* metabolism and *in vivo* animal species indicate that the initial oxidative steps are similar to those observed for tetrahydrocannabinol (THC), namely hydroxylation and carboxylation in the C11 position. Receptor activation studies have indicated that 9R-HHC is significantly more potent than 9S-HHC. No blood concentrations of HHC or its proposed metabolites have been reported in the scientific literature.

To fill this knowledge gap, the goal of this study was to develop and validate a method for the quantitative analysis of 9R-HHC, 9S-HHC, 11-OH-9R-HHC, 11-carboxy-9R-HHC, 11-carboxy-9S-HHC, and 8-OH-9R-HHC. In addition, an objective was to investigate the immunochemical cross reactivity. Consecutive blood samples from cases screened positive for cannabis using ELISA and then confirmed negative for THC, 11-OH-THC, and 11-carboxy-THC were reanalyzed with a newly validated HHC method to investigate the presence of HHC and metabolites. Cross reactivity on an ELISA method was investigated separately for 11-carboxy-9R-HHC and 11-carboxy-9S-HHC at a concentration range between 5-200 ng/mL and found to be 111% for 11-carboxy-9R-HHC and 44% for 11-carboxy-9S-HHC.

The LC-MS/MS method was validated for matrix effects, Lower Limit Of Quantification (LLOQ), calibration model, precision, bias, and autosampler stability. Matrix effects were evaluated in three sources at two concentrations. 11-carboxy-9R-HHC, 11-carboxy-9S-HHC, and 11-OH-9R-HHC showed matrix effects less than 25% at both concentrations while 8-OH-9R-HHC, 9R-HHC, and 9S-HHC matrix effects exceeded 25% at both concentrations but showed good precision and low bias in the further validation. The LLOQ was investigated using three matrices fortified and analyzed over three days and established at 0.2 ng/mL for all analytes except the carboxylated metabolites that had an LLOQ of 2.0 ng/mL. Calibration functions were evaluated with 6-point curves and found to be quadratic over the working ranges 0.2-10 ng/mL and 2.0-100 ng/mL. Precision and bias were evaluated during five runs using triplicate Quality Controls (QC) at 0.4 and 4.0 ng/mL for the low QC, and at 8.0 and 80 ng/mL at the high QC level. Precision was between 4.3 and 9.0% at both concentrations with bias < 5% for all analytes at both concentrations. During 72h autosampler stability, the analyte and internal standard areas slightly increased, probably due to solvent evaporation.

All cases between January and May 2023 (N=146) that were positive for cannabis in blood by ELISA screening but were confirmed negative were analyzed for HHC and metabolites. In 32 cases (22%) the presence of HHC and/or metabolites were confirmed. In Table 1 the quantitative results are summarized.

Table 1. Analyte concentrations in recreational users (ng/mL whole blood).

	9R-HHC	9S-HHC	11-carboxy-9R-HHC	11-OH-9R-HHC
Number	24	15	32	12
Min	0.21	0.28	2.20	0.26
Max	7.7	3.8	96	1.3
Median	0.85	0.66	11	0.49
Mean	1.48	0.97	20	0.65

11-carboxy-9R-HHC was always found and to the contrary 11-carboxy-9S-HHC was not found above 2.0 ng/mL in any case. Neither was 8-OH-9R-HHC found in any case above 0.2 ng/mL. 9R-HHC was always higher than 9S-HHC with an average ratio of 2.3 (N=15). An observation is that even though both parent isomers were found in several cases, only 11-carboxy-9R-HHC was present in the samples.

We conclude that the major metabolite in blood after administration of HHC was 11-carboxy-9R-HHC followed by 11-OH-9R-HHC and that presumptive positive cases are caught by the routine ELISA cannabis screening.

Hexahydrocannabinol; New Psychoactive Substances; LC-MS/MS

L48   Determining Delta 9-THC Acetate Analog Metabolites Using Human Liver Microsomes to Determine the Structure and Rate of Metabolism

Natalie Ortiz*, Virginia Commonwealth University, Richmond, VA; Justin L. Poklis, Virginia Commonwealth University, Richmond, VA; Kimberly Karin, Virginia Commonwealth University, Germantown, WI; Grace R. R. Williams, Virginia Commonwealth University Health, Richmond, VA; Carl E. E. Wolf, Virginia Commonwealth University, Richmond VA, VA

Learning Overview: Currently, there is no presented research on how delta 9-tetrahydrocannabinol (delta 9-THC) acetate metabolizes. After attending this presentation, attendees will have the ability to understand how rapidly human liver microsomes metabolize the analog and discover what the analog is metabolized into.

Impact Statement: This presentation will impact the forensic science community by helping attendees understand the anticipated similar chemical synthesis between delta 9-THC-O-acetate (delta 9-THC-O-A) and heroin, resulting in subsequent potentially similar rapid metabolic products. It's important to confirm which biomarkers will be useful in laboratory analysis when trying to detect and confirm the presence of delta 9-THC-O-A.

Introduction: The legality of delta 9-tetrahydrocannabinol (delta 9-THC) and other cannabinoid analogs (e.g., delta 8-THC, cannabidiol (CBD), acetates, etc.) is complex and varies between federal and state statutes. This is more complex since the Agricultural Improvement Act of 2018 (2018 Farm Bill) defined 'hemp.'[1] Delta 9-THC-O-acetate (delta 9-THC-O-A) is classified as a Schedule I controlled substance because it does not occur naturally in the cannabis plant and can only be obtained synthetically.[2]

Similar to the synthesis of heroin from morphine, delta 9-THC-O-A can be synthesized from delta 8-THC, delta 9-THC, and delta 10-THC by an acetic anhydride reaction.[3] Although little is known about the effects of delta 9-THC-O-A, reports indicate that this analog is being called a "spiritual cannabinoid" due to its psychedelic properties of producing vivid hallucinations.[4]

Delta 9-THC-O-A has caused reason for concern due to its increasing prevalence in gummies and vapes. Currently, there is no published information on how delta 9-THC-O-A metabolizes. Due to its structural similarity to heroin, it's suspected to be metabolized similarly to heroin by the liver.

Methods: For the metabolism studies, human liver microsomes maintained at body temperature (37°C) were fortified with either a negative control (drug-free), positive control (delta-9-THC), or delta-9-THC-O-A, in triplicate. Analytes were prepared in a solution containing 1μm delta 9-THC-O-A or delta 9-THC, 5μL of DMSO, 417.5μL of DI water, 50μL of 1M phosphate buffer pH 7.4, and 15μL of 5mM NADPH. Following a 5 min equilibration of the solutions at 37°C, 12.5μL of human microsomes (20mg protein/mL) was added. Addition of 200μL of acetonitrile was used to terminate the metabolic reactions at 2, 4, 8, 16, and 32 mins after the microsomes were added to the positive control and delta-9-THC-O-A solutions. Similarly, the metabolic reactions were stopped at 2 and 32 mins after application of the microsomes to the negative control. A previously validated high-performance liquid chromatography tandem mass spectrometry method was used to analyze the acetonitrile layer for the following analytes and metabolites: delta 9-THC-O-A, delta 9-THC, 11-hydroxy-delta 9-THC (11-OH-delta 9-THC), and 11-nor-9-carboxy-delta 9-THC (11-COOH-delta 9-THC).

Results: In the acetate solutions, delta 9-THC-O-A was < 50ng/mL after 8 minutes, delta 9-THC was >50ng/mL at 4 minutes, the 11-OH-delta 9-THC was >50ng/mL at 2 minutes, and the 11-COOH-delta 9-THC was < 50ng/mL at all time points. In the positive control, delta 9-THC was >50ng/mL at all time points, the 11-OH-delta 9-THC was >50ng/mL at 2 minutes, and the 11-COOH-delta 9-THC was < 50ng/mL at all time points. In the negative control, no cannabinoids were detected at either time point.

Discussion: Delta 9-THC-O-A was metabolized at a much faster rate than delta 9-THC. This is consistent with heroin, where heroin has a half-life of 2-6 minutes and delta 9-THC-O-A is observed to have a similar half-life. This increased metabolism may affect the ability to detect acetate metabolites in urine.

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Delta 9-THC-acetate; Microsomes; Cannabinoids

L49   The Metabolism of THC-O-Acetate (THCO)—An Emerging Drug Threat

Ya-Chih (Jessica) Cheng*, Sam Houston State University, Huntsville, TX; Charles R. Perkins, Houston Forensic Science Center, Houston, TX; Sarah Kerrigan, Sam Houston State University, Huntsville, TX

Learning Overview: After attending this presentation, attendees will have gained insight into the specific enzymes involved in the metabolism of Δ9-THC-O-acetate (Δ9-THCO) and Δ8-THC-O-acetate (Δ8-THCO). Biotransformation was investigated using pooled human plasma, Human Liver Microsomes (HLMs), recombinant cytochrome P450 enzymes (rCYPs), and human carboxylesterases (hCES).

Impact Statement: This presentation will impact the forensic community by providing new information regarding the biotransformation of two emerging drug threats (Δ9-THCO and Δ8-THCO).

The Agriculture Improvement Act of 2018 (Farm Bill) legalized cannabis plants containing 0.3% or less Δ9-THC as hemp (no longer a controlled substance). Recreational use of THC isomers and synthetic cannabinoids has increased significantly following the passage of the bill. The Centers for Disease Control and Prevention (CDC) issued a report in response to the increased availability of potentially dangerous cannabis-derived products, including THCO. Synthetic acetate derivatives of Δ8- and Δ9-THC are widely available in liquids, edibles, oils, and waxes. Reports suggest that THCO might be twice as potent as their corresponding THC counterparts, possibly due to the increased lipophilicity of the drug. Δ9-THCO and Δ8-THCO have emerged as notable drug threats and have been classified as schedule I controlled substances. Despite their potency and widespread availability, the enzymes responsible for the conversion of THCO to THC have not yet been reported. Identification of the specific enzymes involved in their metabolism is important from the standpoint of adverse drug reactions, drug-drug interactions, and potential toxicity.

Despite the presence of an ester moiety, THCO did not undergo hydrolysis in aqueous buffer during enzymatic incubations. However, notable loss of the analyte was observed due to adsorption of THCO isomers onto plastic surfaces. To address this issue, enzymatic incubations were carried out using glass tubes to minimize sample loss. Drugs and metabolites were analyzed using positive Electrospray Ionization (ESI+) using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and Liquid Chromatography-Quadrupole/Time Of Flight-Mass Spectrometry (LC-Q/TOF-MS). The metabolic profiles of Δ9-THCO and Δ8-THCO were similar. Pooled human liver microsomes (HLMs) demonstrated stepwise metabolism of Δ9- and Δ8-THCO to Δ9- and Δ8-THC and their respective hydroxylated species (11-hydroxy-THC). None of the eleven rCYP enzymes were responsible for the conversion of Δ9- and Δ8-THCO to Δ9- and Δ8-THC (n=2 for each rCYP enzymes). Transformation of THCO to THC was observed using pooled human plasma, hCES1, and hCES2. Activity of hCES1 and hCES2 was confirmed using simvastatin and loperamide as inhibitors. All experiments were conducted in triplicate (n=3), unless specified otherwise.

These results highlight the critical role of carboxylesterases (CES1, CES2b and CES2c) for the biotransformation of THCO derivatives to pharmacologically active THC. In humans, CES1 is expressed largely in the liver and lung, whereas CES2 is predominantly expressed in the gastrointestinal (GI) tract and liver. These finding have implications for the pharmacological and toxicological profiles of Δ8-THCO and Δ9-THCO since these drugs can be administered orally and by smoking. The findings from this study significantly expand our understanding of these novel synthetic cannabinoids and their metabolic pathways, presenting valuable insights for future research and forensic applications.

Metabolism; THCO; Carboxylesterases

L50   A Quantitative Confirmation Assay of New and Emerging Synthetic Cannabinoids by LC/QqQ/MS

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Learning Overview: After attending this presentation, attendees will have an understanding of the development and validation process for a new Liquid Chromatography/triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) method for the quantitative confirmation of seven novel synthetic cannabinoids. Attendees will have the opportunity to examine data from the implementation of the validated method to authentic human toxicological samples.

Impact Statement: This presentation will impact the forensic science community by providing a validated LC/QqQ/MS method to forensic scientists for detecting and quantifying novel synthetic cannabinoids in their forensic casework.

Novel Psychoactive Substances (NPS) are chemical compounds that are designed to imitate the desired effects of traditional recreational drugs. NPS are often synthesized by clandestine chemists or in legitimate laboratories outside of the United States with the end goal of circumventing current national and international drug scheduling laws. Synthetic cannabinoids represent one of the five main drug categories of NPS and present with a wide array of adverse effects that can lead to death. The synthetic cannabinoid drug market remains highly dynamic as new drugs are appearing monthly, which in turn, requires forensic scientists to develop and validate new methods for their detection and quantitation. One additional challenge is that some new synthetic cannabinoids do not always circulate in the drug market for extended periods, meaning the utility of methods may be of high timely importance but also short-lived. These challenges affect multiple professions including law enforcement, health care providers, forensic chemists, toxicologists, and scientific researchers.

Laboratory analysis for synthetic cannabinoids can be difficult and complex due to several factors. First, like other NPS classes, synthetic cannabinoids can exist in isomeric forms, including those known to the drug market and others yet to emerge. Second, unlike other NPS classes, synthetic cannabinoids and their metabolites are often neutral and acidic in chemical nature, requiring specialized sample preparation procedures. Third, toxicologically significant concentrations of synthetic cannabinoids are typically low, meaning less than 5 ng/mL or even sub-1 ng/mL. This all necessitates the development of an analytical method that is sensitive and specific, while also remaining robust and resource-conscious. A wide variety of methods have been previously published for the identification, detection, and quantitation of synthetic cannabinoids; however, none have included the most recent synthetic cannabinoids found on the drug market today.

This study includes the development and validation of a confirmatory quantitative method to identify and detect new and emerging novel synthetic cannabinoids using liquid chromatography-tandem quadrupole mass spectrometry (LC-QQQ-MS). The assay includes seven synthetic cannabinoids of toxicological interest: MDMB-4en-PINACA and its metabolite, MDMB-4en-PINACA-3,3-dimethylbutanoic acid, ADB-BUTINACA and its metabolites, ADB-BUTINACA-N-butanoic acid and ADB-BUTINACA-N-4-hydroxybutyl metabolite, ADB-5'Br-BUTINACA, and ADB-5'Br-PINACA.

The instrument used was an Agilent 6495 LC-QQQ-MS. Chromatographic separation was achieved using an Agilent InfinityLab Poroshell EC-C18 (3.0 x 100 mm x 2.7 μm) analytical column. The Aqueous Mobile Phase (MPA) was 0.1% formic acid in water and the organic Mobile Phase (MPB) was 0.1% formic acid in acetonitrile. A reverse-phase gradient was used with initial conditions at 70:30 (MPA:MPB), gradually reaching 5:95 (MPA:MPB) at 5 minutes and transitioning back to 70:30 (MPA:MPB) until 6.50 minutes. Additional parameters include: flow rate 0.5 mL/min, column temperature 50°C, and injection volume 10 μL.

Validation studies are being performed in accordance with the AAFS Standards Board (ASB) standard for Method Validation in Forensic Toxicology. Validation experiments include calibration model, accuracy, precision, interferences, recovery, matrix effects, process efficiency, dilution integrity, carryover, limit of detection/quantitation, processed sample stability, and matrix matching. The validated assay will be applied to authentic human biological specimens, including blood and urine, from forensic casework.

Novel Psychoactive Substances; Synthetic Cannabinoids; LC-QQQ-MS

L51 A Case Series Involving the Synthetic Cathinone Alpha-PiHP in Postmortem Forensic Investigations

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Learning Overview: After attending this presentation, attendees will be able to assess the impacts of novel synthetic stimulant use on public health and safety as well as recognize the importance of testing for novel psychoactive substances.

Impact Statement: This presentation will impact the forensic science community by emphasizing the importance of qualitative differentiation and quantitative testing of novel psychoactive substances to establish reference concentrations.

Synthetic stimulants, often referred to as bath salts or research chemicals, are novel psychoactive substances increasingly found in medicolegal death investigations and postmortem forensic toxicology casework. Several synthetic stimulants, including alpha-PVP (referred to as Flakka in south Florida) and methylenedioxy-alpha-PHP (referred to as Monkey Dust in the UK), have garnered high media attention due to strange and sometimes violent adverse effects. The primary challenge with many of the novel synthetic stimulants is their inherent structural isomers, making identification of the true intoxicant difficult for forensic laboratories without dedicated methods for their characterization. alpha-PiHP is the latest novel stimulant of the pyrrolidinohexiophenone cathinone subclass to emerge and proliferate, and is a structural isomer of alpha-PHP, an internationally controlled drug. alpha-PiHP was first detected in 2016 and has similar psychostimulant effects to methamphetamine, such as tachycardia and vasoconstriction. Synthetic cathinones have become more prevalent over the last decade, mixed in or substituted for MDMA commonly sold as party drugs 'Molly' or 'Ecstasy'.

A laboratory method was developed and validated for the quantitation of alpha-PiHP, alpha-PHP, methylenedioxy-alpha-PiHP, and methylenedioxy-alpha-PHP. Fentanyl-D5 was used as the internal standard. Quantitation was performed by standard addition and the range was assessed from 0.2 to 50 ng/mL. Up-spike concentrations of 0.2, 2, and 20 ng/mL were used. Samples (0.5 mL) were prepared using a basic liquid-liquid extraction with borax buffer (10 mM, pH 10.4) and an extraction solvent of *N*-butyl chloride/ethyl acetate (70:30 *v:v*). Analysis was performed using a Waters Xevo TQ-S Micro tandem quadrupole mass spectrometer coupled to a Waters Acquity I-Class ultra-performance liquid chromatograph. Chromatographic separation was achieved using an Agilent InfinityLab Poroshell 120 EC-C18 (3.0 x 150 mm, 2.7 μm) under gradient elution. Mobile phase compositions were 0.1% formic acid in water and 0.1% formic acid in methanol. The flow rate was 0.4 mL/min. The I njection volume was 5 μL. The column temperature was 60 ℃. A modified method validation was performed based on experiments outlined in the ASB 036 and 054 standards. Authentic forensic specimens were collected as part of medicolegal death investigations and subjected to the method. Basic demographic and case history information were available.

The method was successfully validated and applied to cases screening positive for alpha-PHP/alpha-PiHP and methylenedioxy-alpha-PHP/methylenedioxy-alpha-PiHP (the isomer pairs were not distinguishable via LC-QTOF-MS screening alone). Nine postmortem specimens from seven individuals (blood n=6, urine n=1, liver n=1, vitreous fluid n=1) collected between May 2022 and March 2023 were quantified. Decedents were primarily male (71%) ranging from 25 to 61 years in age. Cases originated from Florida (57%), New York (14%), and the United Kingdom (28%). Case histories included vehicular accident, homicide, suspected overdose, and cardiac arrest. Blood concentrations of alpha-PiHP ranged from 0.3 to 840 ng/mL (n=6, mean: 259±320 ng/mL, median: 83 ng/mL). The alternative matrices in this case series had quantitative values for alpha-PiHP of 88 ng/g (liver), 2600 ng/mL (urine), and 350 ng/mL (vitreous fluid). Blood concentrations of methylenedioxy-alpha-PHP of 3.9 and 97 ng/mL (n=2) were found. Alpha-PHP was qualitatively identified in one blood sample and methylenedioxy-alpha-PiHP was not detected in any authentic specimens. Alpha-PiHP was often detected alongside traditional opioids (44%), such as fentanyl and tramadol, and other NPS (33%), such as dimethylpentylone and designer benzodiazepines (e.g., bromazolam and desalkylgidazepam).

Our assay described was successful in accurately identifying and quantifying two synthetic cathinone isomer pairs in medicolegal death investigations. Based on this case series, alpha-PiHP was most commonly identified and was the primary pyrrolidinohexiophenone in the United States and the United Kingdom during this time period. Similar to previous investigations involving synthetic cathinones, laboratories should be prepared to accommodate large concentration ranges for these drugs.

Stimulant; Synthetic; NPS

L52   2C or Not Tusi? The Emergence of a New Drug Combination in Miami

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Learning Overview: This presentation will present attendees with information regarding an emerging substance, "Tusi," and its role in a death investigation in Miami-Dade County, FL.

Impact Statement: This presentation will impact the forensic science community by emphasizing the importance of toxicological investigation in overdose fatalities as well as by highlighting the impact of a new deadly drug combination

A 24-year-old female was found unresponsive by hotel staff at a Miami Beach hotel. The decedent was last been seen the previous night entering a hotel room with an unknown male. When hotel staff discovered that no one checked out of the room, a welfare check was conducted at which time the decedent was found unresponsive in the bathtub with a towel wrapped around her hair. Investigation revealed the presence of an unknown pink powdery substance located on the coffee table along with a clear baggie and cut straw containing a similar substance. Search of the decedent's purse revealed another unopened baggie containing more of the pink powdery substance. Further investigations revealed a call to room service from the room by a female around 8:00 AM requesting breakfast for one. The hotel staff did not realize the decedent was unresponsive in the tub when the food was delivered to the room. She was pronounced deceased at 1:22 PM.

The decedent was brought to the Miami-Dade Medical Examiner department for further examination. The paraphernalia found on the scene was also submitted to the laboratory for testing. Medical records indicated that the decedent suffered from seizures that were controlled by lamotrigine. Toxicology testing of aorta and iliac vein blood revealed multiple substances in her blood (Table 1). Testing of the pink powder revealed ketamine, 3,4-methylenedioxymethamphetamine (MDMA), and oxycodone. Based on the toxicology findings, the case was ruled as an accidental overdose, and the cause of death was determined to be "Acute Combined Toxic Effects of Multiple Drugs."

The pink powdery substance was determined to be "Tusi" or "Pink Cocaine." Contrary to its most popular names, the drug combination does not contain psychedelic 2C-B nor cocaine. Originally from Colombia, "Tusi" is a combination of ketamine and MDMA plus other varying drugs as fillers; in this case oxycodone. The name Tusi is a phonetic translation of the number "2" and the letter "C" (2C), because of its similar psychoactive effects as synthetic 2C-B.[1]

Drug	Source	Concentration
Alprazolam	Aorta	Not quantified
Methamphetamine	Iliac	< 0.010 mg/L
Amphetamine	Iliac	< 0.010 mg/L
Methylenedioxymethamphetamine	Iliac	1.8 mg/L
N,N-Dimethylpentylone	Aorta	Not quantified
Ketamine	Iliac	1.8 mg/L
Norketamine	Iliac	1.0 mg/L
Oxycodone	Iliac	< 0.010 mg/L
Noroxycodone	Aorta	Not quantified
Methylenedioxyamphetamine	Iliac	0.208 mg/L

Table 1: Drugs present in postmortem blood.

Reference:
1.   Palamar JJ. Tusi: a new ketamine concoction complicating the drug landscape. *Am J Drug Alcohol Abuse*. 2023 May 10:1-5. doi: 10.1080/00952990.2023.2207716

Postmortem; Overdose; New Psychoactive Substances

L53   The Frequency and Analysis of Kratom in Postmortem Cases

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Learning Overview: The goal of this presentation is to explore kratom exposure in postmortem cases where mitragynine was the main toxicological finding of significance in blood.

Impact Statement: Kratom's popularity is on the rise. The supplement is readily available in a variety of forms and touted as being as "safe as a cup of coffee" due to it being a natural substance. Yet, the toxicological significance of mitragynine, the most notable alkaloid of kratom, is not well understood. This presentation will impact the forensic science community by discussing mitragynine concentrations and trends in postmortem toxicology casework. Cases where mitragynine is the main toxicological finding, with a case history indicative of kratom exposure, will be discussed in further detail.

Kratom, derived from the leaves of the tropical tree *Mitragyna Speciosa*, has become a popular dietary and herbal supplement over the last several years due to its stimulant and opioid-like effects. Kratom contains many active alkaloids, with the most prevalent being the compound mitragynine. Although the complete pharmacology of mitragynine is not fully understood, there is mounting evidence indicating its role in serious adverse effects, up to and including death. Mitragynine has been listed as a significant toxicological finding in postmortem casework, particularly when present in elevated blood concentrations (>1000 ng/mL).

This presentation will analyze the trends in positivity and concentration of mitragynine in postmortem forensic toxicology casework performed at NMS Labs from 2018 through 2022. Positivity rates of mitragynine in routine postmortem toxicology casework have increased from 1.24% to 1.86% during that time, reflecting the increase in popularity of the supplement. Of the 7,300+ cases positive for mitragynine, 14% had concentrations greater than 1,000 ng/mL, with the highest blood concentration reported at 11,000 ng/mL.

	2018	2019	2020	2021	2022
n	788	1131	1708	1874	1878
Mean (ng/mL)	352	328	411	403	466
Median (ng/mL)	120	110	130	120	150
Range (ng/mL)	5.9 - 7000	5.0 - 8900	5.0 - 11000	5.5 - 8000	5.5 – 9900
Positivity Rate	1.24%	1.67%	1.88%	1.75%	1.86%

	2018	2019	2020	2021	2022
N (>1000 ng/mL)	102	112	249	262	309
% of Total	13%	10%	15%	14%	16%

Toxic blood concentration ranges have not been well established for mitragynine due to a number of confounding factors such as polypharmacy and underlying pathology. Further, mitragynine exhibits limited stability, so pre-analytical factors may also play a role when interpreting blood concentration.

Case studies will be presented in further detail, with a focus on cases where mitragynine was found at highly elevated concentrations with limited co-positivity. Case history and autopsy findings will be discussed to help evaluate the significance of mitragynine in cause of death. Some examples include:
- 27-year-old body builder who died at home with an autopsy finding of hemorrhagic pulmonary edema. Toxicology testing revealed 3,500 ng/mL of mitragynine in his blood.
- 25-year-old who suffered a fatal heart attack while driving to work. Their immediate family was aware of his kratom use and had seen an increase in usage prior to death. Toxicology testing revealed 2,700 ng/mL of mitragynine in his blood.

Mitragynine; Kratom; Postmortem

L54 Postmortem Diagnosis of Ketoacidosis: The Importance of Beta-Hydroxybutyrate Quantification as Part of Routine Toxicological Screening at Autopsy

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Learning Overview: After attending this presentation, attendees will understand the added value of including Beta-Hydroxybutyrate (BHB) quantification into their routine toxicological screening at autopsy.

Impact Statement: This presentation will impact the forensic science community by providing toxicological data on pathologically significant concentrations of BHB identified during routine toxicological screening and its relation to the cause of death in forensic autopsy cases in The Netherlands between January 2016 and January 2023.

Ketoacidosis is a biochemical disturbance in the body. If no glucose is available, the body will utilize fatty acids as an alternative fuel pathway and ketone bodies will be produced. The increase of ketone bodies (acetoacetate, acetone and beta-hydroxybutyrate (BHB) in the blood will lower the blood pH. In the literature, BHB concentrations are considered normal below 0.5 mmol/L, elevated up to 2.5 mmol/L, and pathologically significant over 2.5 mmol/L.[1,2] Different forms of ketoacidosis exist, of which alcoholic ketoacidosis as a result of chronic alcohol abuse, diabetic ketoacidosis as a result of a reduction in insulin and starvation ketoacidosis as a result of poor nutrition are best known. The symptoms of the different forms are very similar and include nausea, vomiting, abdominal pains, loss of appetite, lethargy, weakness, unconsciousness and ultimately death.

At the Netherlands Forensic Institute (NFI), routine toxicological screening at autopsy is a standard part of the cause of death investigation. As of 2016, BHB quantification is part of this screening procedure. Before 2016, BHB was usually only quantified in cases with known risk factors for ketoacidosis (e.g., history of diabetes or alcohol abuse), or in cases where no anatomical/toxicological cause of death could be concluded. Cases of fatal ketoacidosis could then be missed if BHB quantification was not performed at all or could lead to a prolonged investigation process if BHB was quantified during a later stage. Therefore, the aim of this research was to evaluate the added value of early stage BHB quantification as part of the routine toxicological screening procedure at autopsy.

To achieve this, the results of BHB quantification in forensic autopsy cases at the NFI from January 2016 to January 2023 were examined. All cases with BHB levels in blood, quantified during routine toxicological screening, of ≥2.5 mmol/L were included.

Between January 2016 and January 2023, 27 cases were included; 18 male and 9 female; age ranged between 20 and 87 years. BHB concentrations in blood ranged between 2.5 to 15.4 mmol/L. Of these 27 cases, six had a known history of diabetes, four had a known history of alcohol abuse, and two had a known history of both diabetes and alcohol abuse.

For 20 cases, ketoacidosis was suggested as a possible cause of death by the pathologist in the final autopsy report (diabetic (n=6), alcoholic (n=1), other/unspecified (n=13)). Of these 20 cases, nine cases had no known history of diabetes and/or alcohol abuse. In those cases, BHB quantification would, in the past, most likely not have been performed at the outset of the investigation, based on the absence of known risk factors prior to autopsy.

For 10 of the 20 cases, ketoacidosis was suggested as the only possible cause of death. For the other 10 cases, ketoacidosis was suggested as a possible cause of death next to one (n=5), two (n=2) or three (n=3) other possible causes of death. For those cases with other possible causes of death and no background of diabetes and/or alcohol abuse (n=5), no indication for BHB quantification would have arisen during the investigation. Hence a fatal ketoacidosis would have been missed.

In conclusion, routine quantification of BHB at autopsy results in valuable information in an early stage in the cause of death investigation on ketoacidosis as a possible cause of death. BHB quantification as part of routine toxicological screening is especially relevant in cases where a fatal ketoacidosis is not directly considered. For example, in cases with no known prior risk factors for ketoacidosis and in cases for which other possible causes of death are already identified.

References:
[1] Iten, P.X. Meier, M. Beta-Hydroxybutyric acid – an indicator for an alcoholic ketoacidosis as cause of death in deceased alcohol abusers, *J Forensic Sci* 45: 624-632 (2000).
[2] C. Palmiere, Augsburger, M. The postmortem diagnosis of alcoholic ketoacidosis. *Alcohol Alcohol.* 49(3): 271–281 (2014).

Ketoacidosis; BHB; Postmortem

L55   A Case Series of Suicides by Sodium Azide Ingestion: An Update

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Learning Overview: After attending this presentation, attendees will understand the importance of matrix selection, analytical strategy, and interpretation of toxicological results in cases in which an intentional overdose with sodium azide was suspected.

Impact Statement: This presentation will impact the forensic science community by providing toxicological data on the presence and concentrations of azide in various biological samples, including (femoral) blood and vitreous humor in 26 apparent suicides.

Sodium azide is a chemical used as preservative for diagnostic reagents and propellant in automobile airbags. In the 1950s, it was investigated as a potential antihypertensive agent in oral doses of 0.01 to 0.03mg/kg. Nowadays, it is easily available for online purchase. Azide is believed to cause its toxic effects through the inhibition of cytochrome oxidase resulting in cellular hypoxia. Toxic effects that may occur include hypotension, cardiac arrhythmia, respiratory depression, unconsciousness, coma, and death.

The objective of this study was to investigate the involvement of sodium azide in 26 apparent suicides and provide toxicological data on the presence and concentrations of azide in various biological samples including (femoral) blood and vitreous humor. In 2022, the results of 13 cases in which an intentional overdose with sodium azide was suspected were presented.[1] In the past year, another 13 cases were included.

As azide cannot be detected by routine screening, only cases with suspicion of an intentional sodium azide ingestion confirmed by analysis of azide in blood or vitreous humor were included. The cases were screened for the presence of alcohol, drugs of abuse and medicines in blood. Azide in blood was identified and quantified after derivatization with pentafluorobenzylbromide and extraction with tetradecyldimethylammonium as liquid phase-transfer catalyst using Gas Chromatography/Mass Spectrometry (GC/MS) and isotopically labeled azide as internal standard.[2] In some cases, other biological matrices were available for analysis as well.

Twenty-six cases were included (male/female: 9/17; age range 27–88 years). Most bodies were found in their homes. Two victims were found in the garden needing resuscitation and eventually died in the hospital. In 16 cases, a small container labeled with sodium azide was found at the scene and the total dose in the container, if classified, varied from 2 to 3gram. Toxicological analysis for the presence of alcohol, drugs of abuse and medicines resulted in no indications for a toxicological cause of death. In all cases, medicines were found, especially analgesics (21 cases), opioids (4 cases), anti-emetics (17 cases), and benzodiazepines (11 cases). Alcohol was detected in four cases. The median azide blood concentration was 16mg/l (n=25, range 130mg/l). Median concentrations in vitreous humor, heart blood, and urine were 24mg/l (n=5, range 9–29mg/l), 7mg/l (n=5, range 0–6mg/l) and 5mg/l (n=5, range 2–11mg/l), respectively. The higher azide concentrations measured in vitreous humor suggest good permeation and postmortem stability. Even more interesting was one case in which azide tested negative in heart blood, but positive in vitreous humor with an azide concentration of 8.6mg/l.

In 22 cases, concentrations of azide were found in the blood (ranging from 3.6 to 30mg/l), corresponding with concentrations that were measured in persons who died after apparent intentional ingestion of sodium azide.[3]

In two cases, no toxicologically relevant substances were found except for azide. However, the measured azide concentrations were lower (about 1mg/l), possibly due to degradation. These victims were found together, and a container labeled with sodium azide was found near them. In a third case, the measured azide concentration in blood was also lower (1.0mg/l), but no clear explanation was found.

It was concluded that the measured azide concentrations in blood could explain the death in 25 of our cases. In one case, the death was explained by the measured azide concentration in vitreous humor. Although femoral blood is the specimen of choice in forensic toxicology, the results of azide in vitreous humor show that it is a valuable additional matrix, and it is advised to collect femoral blood and vitreous humor when suspecting an azide overdose.

References:
1. I.J. Bosman et al. A case series of suicides by sodium azide ingestion. *Abstract in Toxac* 3 (34): S45-46, 2022.
2. S. Kage et al. Determination of azide in blood and urine by gas chromatography -mass spectrometry. *J. Anal. Toxicol.* 24: 429-432, 2000.
3. R.C. Baselt. *Disposition of toxic drugs and chemicals in man*. Twelfth edition (2020), monograph of azide, pp. 180-181.

Azide; Postmortem; Vitreous Humor

L56 Ketamine in Driving Under the Influence of Drugs (DUID) Cases: 2015–2023

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Learning Overview: After attending this presentation, attendees will understand the prevalence of ketamine detected in DUID cases.

Impact Statement: This presentation will impact the forensic science community by presenting the prevalence and concentrations detected of ketamine in DUID cases.

Ketamine (R,S-ketamine) is a dissociative anesthetic that is chemically related to Phencyclidine (PCP). It was initially developed in 1970 as a safer alternative to PCP; however, it was found to have significant adverse effects and potential for abuse. Between 2017 and 2022, the High Intensity Drug Trafficking Areas (HIDTA) program analyzed data on the total number of ketamine seizures and reported a 349% increase, suggesting increased availability may be leading to increased illicit use.[1] Despite its potential for abuse, ketamine also has shown promise in the medical field. In 2019, intranasal S-ketamine was approved by the Food and Drug Administration (FDA) for use in the treatment of depression. This research investigates the prevalence and concentrations of ketamine in DUID casework and its potential significance in the context of FDA approval.

Blood samples were submitted to the laboratory for analysis under the DUID/**Drug Recognition Expert (**DRE) panel. The samples were screened by high performance Liquid Chromatography/Time Of Flight/Mass Spectrometry (LC/TOF/MS) with a Reporting Limit (RL) of 10ng/mL for ketamine and 20ng/mL for norketamine. Positive screens were confirmed via Gas Chromatography/Mass Spectrometry (GC/MS) (RL = 40ng/mL). Positive ketamine and/or norketamine samples submitted to the laboratory between January 2015 and April 2023 were reviewed.

Of approximately 148,000 DUID cases submitted, 78 submissions confirmed positive for ketamine and/or norketamine. Drivers were comprised of 53 males, 11 females, and 14 of unknown sex. The age range was 19–60 years (mean ±standard deviation [±SD] = 32 ±10 years, median = 30 years). The concentration range of ketamine was 40-8,200ng/mL (mean ±SD = 653 ±1,228ng/mL, median = 280ng/mL) while the range for norketamine was 41–2,100ng/mL (mean ±SD = 330 ±350ng/mL, median = 250ng/mL). Submitting agencies were contacted to verify whether ketamine positivity was due to medical intervention; however, 50 cases could not be distinguished.

A total of 13 submissions (16.6%) were identified as illicit ketamine use. Ten males and 3 females with an age range of 20-40 years (mean ±SD = 28 ±6.8 years, median = 28 years) comprised this subgroup. The concentration range for ketamine was 86–400ng/mL (mean ±SD = 251 ±127 ng/mL, median = 245ng/mL) while the range for norketamine was 47–810ng/mL (mean ±SD = 276 ±262ng/mL, median = 145ng/mL). Other findings included detection of benzodiazepines, cannabinoids, hallucinogens, opioids, and stimulants, where cannabinoids (69%) were most prevalent among cases. Of six cases where norketamine was reported without ketamine (46%), one case had a concentration of 48ng/mL with no other findings. Positivity over the 9-year period reached as high as 0.02% in 2018 and 2022, with 4 and 3 positive cases, respectively.

Fifteen submissions were individuals confirmed to have received ketamine due to medical intervention. The age range was 19–48 years (mean ±SD = 30 ±7.3 years, median = 30 years). The concentration range for ketamine was 40–2,200ng/mL (mean ±SD = 667 ±676ng/mL, median = 430ng/mL), and norketamine was 42–580ng/mL (mean ±SD = 290 ±165ng/mL, median = 325ng/mL). Other findings included anticonvulsants, antidepressants, antihistamines, benzodiazepines, cannabinoids, muscle relaxants, opioids, and stimulants.

Ketamine showed low positivity in DUID cases throughout the years and remained consistent despite increased seizures and its potential significance in the context of FDA approval; however, positivity is based on a small population size. When comparing concentrations of ketamine between illicit and medical use, the concentrations overlapped despite lower mean and median concentrations in illicit use. Therefore, if ketamine is detected, more information regarding the totality of the case is needed to draw conclusions about type of use and its potential contribution to an individual's impairment.

Reference:

1. Palamar JJ, Wilkinson ST, Carr TH, Rutherford C, Cottler LB. Trends in Illicit Ketamine Seizures in the US From 2017 to 2022. *JAMA Psychiatry. 2023;* 80(7):750–751.

Ketamine; Driving Under the Influence of Drugs; Impairment

L57 DUID Cannabinoid Investigations: Blood, Oral Fluid, or Urine—Which Is the Superior Specimen?

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Learning Overview: After attending this presentation, attendees will understand the benefits of using oral fluid for testing in Driving Under the Influence of Drugs (DUID) cannabinoid investigations.

Impact Statement: This presentation will impact the forensic science community by demonstrating the relevancy of oral fluid drug testing in DUID investigations and the implementation of an oral fluid drug testing program.

In 2018, the Alabama Department of Forensic Science (ADFS) became the first state crime laboratory to conduct oral fluid evidentiary confirmation testing, in addition to traditional blood testing, for DUID cases.[1] ADFS is in a unique position to present cannabinoid prevalence and concentrations in blood, oral fluid, and urine from drivers charged with DUID. The primary psychoactive cannabinoid, delta-9-THC, can be detected in blood and oral fluid, whereas urine contains predominately metabolites (e.g., carboxy-THC). The primary objective was to evaluate the presence of delta-9-THC in the blood, oral fluid, and urine. The timeliness of specimen collection may affect the ability to detect cannabinoids.

A review of test results from 1,585 cases was performed over a five-year period (2018–2022). Median (average) concentrations, positivity rates, and the prevalence of cannabinoids and their metabolites (i.e., delta-9-THC, hydroxy-THC, and carboxy-THC) in blood, oral fluid, and urine were determined. Analytical methods complied with Academy Standards Board (ASB) Standard 036(2) and the National Safety Council's recommendations for cannabinoid scope, cutoffs, and limit of detections.[3] Biological specimens were drug screened by Enzyme-Linked Immunosorbent Assay (ELISA) using a Randox® Evidence Analyzer or Tecan Evo 75 with Immunalysis reagents. Quantitation of cannabinoids was performed by liquid-liquid extraction followed by analysis using an Agilent® 6460 or 6470 Triple Quadrupole mass spectrometer. The Limit Of Detection (LOD) for delta-9-THC was 1ng/mL in all specimen types. The LOD for hydroxy-THC was 0.5ng/mL (blood, urine) and 4ng/mL (oral fluid). The LOD for carboxy-THC was 2.5ng/mL (blood, urine) and 1ng/mL (oral fluid).

In 2022, delta-9-THC or THC metabolites were the most common drug in DUID cases, with a 41% prevalence. From 2018–2022, approximately 85% of the 1,585 cases included both oral fluid and blood specimens. Urine was collected in less than 5% of submissions. When initial presumptive screening indicated cannabinoids, delta-9-THC and carboxy-THC positivity rates were 90% and 5.1% in oral fluid compared to 75% and 98% in blood, respectively. Delta-9-THC oral fluid concentrations greatly exceeded blood concentrations with median oral fluid: blood ratios equal to 14:1. However, the inactive metabolite, carboxy-THC, had median oral fluid: blood ratios equal to 0.02:1. Delta-9-THC median (average) concentrations in blood and oral fluid were 3.5 (5.1) and 31 (293) ng/mL, respectively. Delta-9-THC was not detected in urine specimens. Carboxy-THC median (average) concentrations in blood, oral fluid, and urine were 36 (67), 3.2 (4.6), and 94 (234) ng/mL, respectively. Blood and oral fluid are superior specimens for DUID investigations when compared to urine due to the detectability of the primary psychoactive cannabinoid, delta-9-THC. Carboxy-THC, an inactive metabolite, has no correlation to recent use or impairment. Accordingly, hydrolyzed delta-9-THC-glucuronide in urine is not indicative of recent use contrary to Illinois' holding.[4] Hydrolyzing delta-9-THC-glucuronide in urine to report delta-9-THC as an indication of impairment in lieu of testing blood or oral fluid is strongly discouraged.

Based on the five-year review, blood or oral fluid, but not urine, can provide important information on the potential source of impairment and recency of cannabinoid use. The ability to collect oral fluid proximate to the time of driving (e.g., at the roadside) presents a distinct advantage. Laboratories should consider the use of oral fluid as an additional or alternative specimen in DUID cases. The use of oral fluid may particularly appeal to states that currently use urine only in DUID testing.

References:
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Cannabinoids; DUID; Oral Fluid

L58   The Detection of Cannabinoids in Exhaled Breath Condensate After Cannabis Use

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Learning Overview: After attending this presentation, attendees will understand how Exhaled Breath Condensate (EBC) is collected from participants after cannabis use, how breath samples like these are processed and analyzed with liquid chromatography/mass spectrometry, and what cannabinoids are detected in breath after cannabis use.

Impact Statement: This presentation will impact the forensic scientific community by describing how to investigate a breath matrix that has never before been collected from cannabis users. The study design with multiple time points and use groups allows for comparisons over time and between groups. Liquid chromatography/tandem mass spectrometry allows for the identification of 11 different cannabinoids, if present, which led to the detection of multiple cannabinoids in breath samples from cannabis users.

The legalization and decriminalization of recreational cannabis use across the United States has increased the need for roadside detection of cannabis use during the window of impairment. Uncertainty remains about the relationship of Δ^9-tetrahydrocannabinol (THC) levels in biological matrices to impairment, so current research has focused on determining recent use. THC is theorized to be carried in exhaled breath aerosols, and several published studies have investigated offline analysis of aerosols captured with filters. Multiple breaths are required to produce picogram to nanogram device concentrations. EBC is a rich matrix that contains both aerosols and volatile organic compounds and has yet to be explored in the context of cannabis use.

In this pilot study, the primary objective was to analyze EBC collected with a commercially purchased, non-invasive hand-held device (Respiratory Research, not affiliated with study) and identify compounds associated with recent cannabis use. EBC was collected by chilling exhaled breath to condense water vapor and volatile organic compounds. Aerosols were captured by sedimentation. The collection tube was cooled with a metal collar stored at -80°C. The participants were asked to breathe deeply through the device, with a breathing maneuver to increase breath aerosols, for 5min while their breaths are counted. The three cannabis use groups included participants who smoke cannabis flower daily, participants who vape concentrates daily, and participants who smoke cannabis flower less than daily. Participants provided their own legal-market products (collected but not discussed here) and there was also a non-use control group. EBC was collected before cannabis use (baseline) and at two time points (~1h and ~2h) after monitored cannabis use (the control group relaxed during this time). EBC was stored in the collection tube at -80°C until it was extracted and processed for analyses. EBC was concentrated to dryness by lyophilization and reconstituted in solvent with deuterated cannabinoid internal standards. Analytes were then separated on an Ultrahigh Performance Liquid Chromatography System with Triple Quadrupole Tandem/Mass Spectrometry (UHPLC-QqQ/MS/MS) to detect and quantify THC and ten other cannabinoids. Multiple reaction monitoring of two transitions from each of the 11 cannabinoids and 10 internal standards allowed for stringent identification criteria for all analytes.

While this is an ongoing study, the first batch of samples that includes 16 total participants with four participants from each group has been analyzed. In all samples from participants who used cannabis (12 of 12 participants), THC was detected in the first (1h) post-use sample above the limit of detection. THC decreased (8 of 12 participants) or was not detected (4 of 12 participants) in the second (2h) post-use sample. THC was detected in some baseline EBC samples from the participants in each use group (5 of 12 participants), despite a requested 8h abstinence before the study session. Cannabinol, cannabigerol, tetrahydrocannabinolic-acid, and tetrahydrocannabivarian were detected in some (6 of 12 participants) post-use samples. THC was not detected in any of the samples from the control group, demonstrating that handling protocols effectively prevent cross-contamination. In post-use samples, four different cannabinoids were detected in addition to THC; detection of one or more of these compounds could potentially improve the reliability of breath-based determinations of recent use by having impairment related to the detection of multiple cannabinoids instead of only THC. The results described here are preliminary and represent a small portion of the larger study (90 total participants anticipated in the cannabis use groups) with an intent to understand the viability of the EBC matrix as a means to capture cannabinoids in breath following cannabis use. Future work will compare breath results to blood and impairment data.

Cannabis; Exhaled Breath Condensate; Cannabinoids

L59 Cocaine and Benzoylecgonine in Blood and Oral Fluid of DUID Cases (2018–2022)

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Learning Overview: After attending this presentation, attendees will better understand the prevalence of cocaine and Benzoylecgonine (BE) in blood and Oral Fluid (OF) in Driving Under the Influence of Drugs (DUID) and traffic crash cases between 2018 and 2022 analyzed by the Alabama Department of Forensic Sciences, which is the first state laboratory with an OF testing program.

Impact Statement: This presentation will impact the forensic science community by providing regional data on cocaine and BE blood and OF concentrations in DUID cases.

Introduction: Cocaine is a central nervous system stimulant that is commonly used as a recreational drug. This powerfully addictive substance is made from the leaves of a coca plant, native to South America. The recreational use of cocaine has resulted in the Drug Enforcement Agency classifying it as a Schedule II drug. Studies have shown that cocaine has a negative effect on complex, divided attention tasks, such as driving. In Alabama, cocaine has been a common drug found in DUID cases. Alabama was first state to offer OF confirmation testing in 2018.[1]

Methods: Cases were screened by enzyme immunoassay using either a Randox® Evidence Analyzer or a Tecan Freedom Evo75 with Immunalysis® reagents. Confirmation and quantification of cocaine and BE were performed by either liquid-liquid extraction with Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis or solid-phase extraction with Gas Chromatograph/Mass Spectrometry (GC/MS).[2] Positivity rates were compared at 10ng/mL for cocaine and BE for blood and OF. Positivity rate = n (positive)/n (analyzed) from cases that screened positive for cocaine in blood and/or OF. Demographics, blood and oral fluid concentrations, case type, sample collection times, and year-to-year comparison were looked at to investigate trends. The oral fluid method is validated qualitatively at this time for cocaine/BE. However, the Alabama Department of Forensic Sciences (ADFS) performs a 7-point calibration to collect semi-quantitative for research purposes to better understand OF drug concentrations in the driving populations.

Results: The combined "analyte" of cocaine/BE was the seventh and sixth most prevalent drug in 2021 and 2022, respectively. Cocaine and BE comprised 9% of the drugs detected in 2022, which was an increase of 42% over 2021. Between 2018 and 2022, 544 blood and/or OF cases from DUID and traffic crashes in Alabama were positive for cocaine and/or BE. Seventy-two percent of the subjects were male and 28% were female (ages 17–72). For cocaine cases, the time between incident and time of collection for blood and OF specimens had a median of 135 and 123 minutes, respectively. For BE cases, the time between incident and time of collection for blood and OF specimens had a median of 135 and 124 minutes, respectively. Since 2018, in cases with both blood and OF samples, cocaine had a median concentration of 45 and 213ng/mL, respectively. Cocaine had a 97% prevalence in OF compared to 44% in blood results. Also, in cases with blood and OF samples, benzoylecgonine had a median concentration of 370 and 283ng/mL, respectively. OF-to-blood median ratio was 11:1 for cocaine and 1:1 for BE.

Discussion: In Alabama, cocaine use has recently increased and ranked sixth in DUID and traffic crash cases. Since implementing the OF testing program for DUIDs, data has shown that cocaine has better detection in OF and at higher concentrations. OF is a specimen containing active parent drugs and cocaine is often present in OF when not found in the blood due to its rapid metabolism and short half-life.

References:

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Cocaine; Oral Fluid; DUID

L60 Mile High: A Five-Year Review of Fentanyl Trends in Colorado Impaired Driving Cases

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Learning Overview: After attending this presentation, attendees will be informed about various fentanyl trends from Colorado and case studies will highlight the challenges of interpretation in polydrug cases.

Impact Statement: Jurisdictions could compare their data to the Colorado data that will be presented. This presentation will impact the forensic science community by showing the importance of comprehensive analysis being performed on each case.

While fentanyl has medicinal uses, recreational use has increased dramatically in recent years.[1] This study looks at all Driving Under the Influence (DUI) blood samples submitted to the Colorado Bureau of Investigation (CBI) that reported a positive fentanyl result between January 2019 and April 2023. In all, 1,024 fentanyl positive DUI cases were assessed over the five-year period for trends in polydrug use and fentanyl concentration. Attendees will learn about the trends in Colorado fentanyl-positive casework, including polydrug trends. Case studies will highlight the challenges of interpretation in polydrug cases.

CBI began comprehensive drug and ethanol testing for all DUI cases submitted after July 1, 2019. Prior to July 1, 2019, the submitting agency was able to select testing for ethanol, drugs of abuse, or both. Ethanol is reported at concentrations greater than 0.010g/100 mL. A 14-panel Enzyme-Linked Immunosorbent Assay (ELISA) screen targets the required minimum scope for blood testing in impaired driving investigations,) and confirmations are reflexed.[2] The ELISA screening cutoff for fentanyl is 1.25ng/mL and the confirmation limit of quantitation is 1.0ng/mL. The CBI performs comprehensive testing on all DUI samples and does not have a stop-testing limit program. Limitations to this data evaluation include a shift in testing protocol as well as limited knowledge of incident type and medical aid that may have been given.

The percentage of fentanyl-positive cases has grown each year and has increased from 1.0% in 2019 to 3.8% in 2023. Reported fentanyl concentrations have increased steeply over the study period with the greatest increase seen in cases with more than 10ng/mL. In 2019, the mean and median reported fentanyl concentrations were 6.8ng/mL and 3.0ng/mL and thus far in 2023, they have jumped to 11.3ng/mL and 5.4ng/mL.

Ethanol and delta-9-Tetrahydrocannabinol (THC) are the CBI's most commonly reported drugs in impaired driving cases. Over this time period, approximately 70% of all DUI casework reported ethanol and 45% of all DUI casework reported THC. Fentanyl-positive cases reporting ethanol peaked in 2019 with 35% cases reporting both and have dropped to less than 12% in 2023. Fentanyl and THC co-consumption cases peaked in 2019 with 47% and have dropped to 25% in 2023. The percentage of fentanyl/THC cases reported with a THC concentration greater than the permissible inference of 5.0ng/mL is consistent with all other casework, around 15%

The greatest increase in polydrug use is with fentanyl and methamphetamine. In 2019, 19% of fentanyl cases reported methamphetamine. By 2023, this number has ballooned to 58%. Polydrug use of fentanyl and benzodiazepines and/or other pain management drugs has slightly decreased over the study time period. The most commonly reported benzodiazepines with fentanyl are alprazolam, clonazepam, and designer benzodiazepines. Other drugs in the pain management assay have stayed widely distributed; however, over the past two years, there has been an increased number of cases reporting both fentanyl and methadone.

This presentation will explore the changing trends observed in the CBI's fentanyl-positive case work over a five-year period. By providing comprehensive testing on all DUI cases, polydrug trends can be tracked and reasons for those trends can be explored. Highlighted case studies will demonstrate interpretation challenges in fentanyl-positive casework when drugs from multiple drug classes are present.

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Fentanyl; Impaired Driving; DUID

L61   Impaired Driving Cases With Bromazolam

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Learning Overview: After attending this presentation, attendees will have increased their knowledge of impaired driving investigations involving bromazolam.

Impact Statement: This presentation will impact the forensic science community by outlining the increasing incidence, polypharmacy, and case reports of bromazolam in impaired driving investigations.

Introduction: Bromazolam has become one of the most frequently detected Designer Benzodiazepine (DZB) since the middle to end of 2022.[1] Bromazolam is structurally similar to alprazolam, with the chlorine in alprazolam replaced by bromine in bromazolam. Similar concentration ranges to alprazolam were observed in impaired driving and postmortem cases.[1] Bromazolam is active at gamma-aminobutyric acid A (GABA$_A$) receptors. This affinity is similar to other benzodiazepines, resulting in the increase of the efficacy of the inhibitory neurotransmitter GABA. Therefore, Central Nervous System (CNS) depressant effects similar to other benzodiazepines would be expected, including memory impairment, sedation, anxiolysis, and muscle-relaxation.[2] In the beginning of 2023, a blood Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) confirmation method was updated to include bromazolam. Bromazolam was identified in four impaired driving cases, including one in which bromazolam was the only drug identified.

Methods: For all blood driving under the influence of drugs cases, a volatile analysis was followed by an 11-panel Enzyme-Linked Immuno-Sorbent Assay (ELISA) (Dynex® DSX with kits from Neogen®) and basic drug extraction with full scan Gas Chromatograph/Mass Spectrometry (GC/MS) (Agilent® 7890A/5975C). All positive results were confirmed with GC/MS and/or LC/MS/MS (SCIEX™ 3200 QTrap or SCIEX™ 5500+), and/or LC with high resolution MS/MS (Thermo Scientific™ Q Exactive™). The ELISA cutoff for benzodiazepines using clonazepam as the target was 10ng/mL for blood. Bromazolam cross-reactivity determined in whole blood was 303%. The limit of quantitation and limit of detection for bromazolam was 5.0ng/mL by LC/MS/MS. A Certified Reference Materials (CRM) was not available at the time of validation; therefore, results were reported qualitatively. Approximate concentrations for bromazolam will be discussed.

Results: In five months since updating the blood confirmation method, bromazolam was identified in four impaired driving cases. Other drugs were identified in 75%. The most common combinations of other active drugs identified were opioids (3/4) and cannabinoids (2/4). In one blood case where bromazolam was the only compound identified, officers observed impairment consistent with a Central Nervous System (CNS) depressant. The driver did not maintain a single lane on a multilane interstate and almost collided with two tractor trailers. The driver was observed to be confused and disoriented with droopy eyelids, lethargic behavior, and slow speech. He also had trouble with short-term memory.

Discussion/Conclusion: Bromazolam was identified in both seized drug and impaired driving investigations in 2023 in this jurisdiction in the Southeastern United States. Bromazolam incidence in seized drug exhibits increased at least 100% from 2022 to 2023 (as of September 8, 2023). In one blood case where bromazolam was the only compound identified, driving ability and normal faculties impairment consistent with a benzodiazepine was observed. Other drugs were identified in the majority of the bromazolam cases, consistent with the polypharmacy observed for other benzodiazepines.

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2. Merette S.A.M., Theriault S, Piramide L.E.C, Davis M.D., Shapiro A.M. (2023) Bromazolam Blood Concentrations in Postmortem Cases—A British Columbia Perspective, *J. Anal. Tox*. 47,4, 385–392, https://doi.org/10.1093/jat/bkad005.

Bromazolam; DUID; Impaired Driving

L62 An Outlook on Fentanyl Adulteration: Polydrug Circumstances, Trends, and Tomorrow's Forecast

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Learning Overview: After attending this presentation, attendees will be able to assess the forensic and toxicological impacts of fentanyl adulteration with xylazine, novel benzodiazepines, and other drugs.

Impact Statement: This presentation will impact the forensic science community by expanding knowledge of fentanyl adulteration trends and providing insights into future drug combinations.

In the United States, the Centers for Disease Control and Prevention (CDC) utilize death certificate data to examine changes in drug overdose death statistics. Public health officials have begun attributing waves (or notable rises in the data) of causality beginning with prescription opioids (first wave), transitioning to heroin (second wave), and now synthetic opioids, primarily fentanyl (third wave). Fentanyl remains the primary driver of fatal drug overdose deaths; however, fentanyl is often combined with other drugs and/or adulterants. Polydrug scenarios have increased, leaving scientists wondering whether the drug overdose crisis has entered a fourth wave or is experiencing "nested waves"—a phenomenon coined to signify waves under the fentanyl third wave. In either scenario, the case remains true that adulteration of fentanyl and its co-use with other drugs is dynamic, on the rise, and significant from public health and safety perspectives.

In 2018, the Center for Forensic Science Research and Education (CFSRE) launched a drug early warning system called NPS Discovery with the primary goal of tracking novel drugs and surveilling drug markets. The CFSRE laboratory employs a battery of analytical testing workflows to characterize drug species in a variety of sample types, including toxicology specimens and drug materials. Central to this workflow, a Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) assay is used for comprehensive drug detection and identification. The LC/qTOF/MS method employs generic gradient elution and non-targeted mass acquisition to allow for detection of all drug species present, regardless of known origins or structure. Datafiles acquired are subjected to targeted data processing using a continually updated in-house library database containing more than 1,100 targets, including traditional drugs, Novel Psychoactive Substances (NPS), adulterants, therapeutic agents, and metabolites. All results are reviewed by trained analysts, complied, and exported to Excel® spreadsheets for data analysis.

Between 2018 and 2023, fentanyl was the most frequently detected drug found during our surveillance, accounting for approximately 30% of case positivity in 2018 and rising to nearly 70% in 2023. These data are consistent with the continued increase in fentanyl prevalence associated with the third wave of the overdose crisis. Fentanyl was commonly found alongside other drugs, including NPS benzodiazepines, psychostimulants (e.g., methamphetamine, cocaine), fentanyl analogs (e.g., cyclopropylfentanyl, fluorofentanyl), and xylazine. In 2018, co-positivity of fentanyl and NPS benzodiazepines was less than 5% and by mid-2020 had increased to more than 45%. Over time, xylazine co-positivity with fentanyl has increased to more than 10% in 2023. Fentanyl analog co-positivity varied—in 2018, the positivity was moderately high (up to 20%), but positivity fell in the wake of Drug Enforcement Administration (DEA) core-structure scheduling to a low (less than 5%) in 2020 before increasing again leading up to 2023 due to the proliferation of fluorofentanyl (more than 20%). Psychostimulant co-positivity with fentanyl has steadily increased since 2018 to greater than 40% in 2023.

Fentanyl adulteration and co-positivity with other substances are now common; however, there are nuances to consider when evaluating the data. As xylazine positivity and prevalence have increased, so too have the media reports accounting its "resistance to naloxone" and associated skin wounds. This has led to federal and state government actions to combat xylazine presence alongside fentanyl, including state scheduling and plans for supply eradication. The future of xylazine adulterated fentanyl is unknown, but it is important to understand the origins and purpose of this adulteration, as well as other drugs used to adulterate fentanyl. The exponential rise of NPS benzodiazepine adulterated fentanyl (i.e., benzo-dope) remains a cause for concern as this drug combination has withstood the effects of scheduling (e.g., transition from etizolam to bromazolam) and is susceptible to additive CNS depressant effects.

Fentanyl; Xylazine; Benzodiazepine

L63 Controlled Oral Kratom Leaf Administration to Healthy Humans: Abuse Potential- and Withdrawal-Related Effects, Adverse Events, and Mitragynine and 7-Hydroxy-Mitragynine Plasma Pharmacokinetics

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Learning Overview: After attending this presentation, attendees will be able to describe the abuse potential of kratom and mitragynine in humans, the treatment-emergent adverse events, and withdrawal following controlled administration of kratom or mitragynine. Attendees will be able to describe the pharmacokinetics of mitragynine and 7-hydroxymitragynine after single and oral dosing with raw leaf kratom powder.

Impact Statement: This presentation will impact the forensic science community by presenting these data from the first large oral kratom (mitragynine) administration study ever conducted. This study was approved by the ethical committee and Health Canada. The abuse potential and withdrawal of four doses of kratom or mitragynine are presented for the first time after single and 15 multiple doses. Furthermore, the pharmacokinetics of mitragynine and 7-hydroxymitragynine and their ratio are presented to improve the interpretation of these concentrations.

Introduction: Approximately 70% of kratom consumers self-report using kratom to support energy and wellness and for chronic pain relief and 30% self-report using kratom to self-manage opioid use disorders. Surveys suggest kratom-related withdrawal and dependence occur in some consumers, but rates are low, and reports may involve polysubstance use. An evaluation of abuse potential and withdrawal from kratom or mitragynine alone in humans was needed. The National Institute on Drug Abuse (NIDA) and the National Institutes of Health (NIH) Helping to End Addiction Long-term® Initiative (HEAL) are evaluating kratom as potential treatment for opioid withdrawal and opioid use disorder.

Objectives: To evaluate abuse potential- and withdrawal-related effects and Treatment-Emergent Adverse Events (TEAE) following Single (SD) and Multiple (MD) kratom doses.

Methods: KAPTURE is a randomized, double-blind, placebo-controlled, dose-escalation study with SD and 15-day MD encapsulated oral dried kratom powder doses of 500, 1,000, 2,000, and 4,000mg Mitra-Leaf containing 6.65, 13.3, 26.6, and 53.2mg mitragynine. Participants (114) were kratom-naive or had not taken kratom in the past year and received active (48) or placebo (66) kratom during 31 in-person visits over 47 days. A Visual Analog Scale (VAS) for "Do you feel a drug effect right now?" and abuse potential-related TEAE evaluated kratom's abuse potential. Withdrawal-related TEAE and the Clinical Opioid Withdrawal Scale (COWS) and Subjective Opioid Withdrawal Scale (SOWS) assessed kratom withdrawal for 72h after the last MD. Mitragynine and 7-hydroxymitragynine plasma concentrations were quantified prior to and 0.25, 0.5, 0.75, 1, 1.33, 1.67, 2, 2.33, 2.67, 3, 3.5, 4, 5, 6, 9, 12, 24, 48, 72h and up to 23 days after single and 15 mitragynine MD.

Results: VAS scores for feeling drug effect and the number of participants reporting abuse potential-related TEAE generally increased as the dose increased in the SD and MD phases. Maximum scores and Area Under the Curve (AUC) were not statistically significantly different from pooled placebo except at the highest 53.2mg dose (p=0.002 and 0.005, respectively). There were no statistically significant differences from pooled placebo for Visual Analog Scale (VAS) score during and after MD. The most common preferred terms for abuse potential, euphoric mood, and feeling drunk were reported by only 6.1% and 4.1% of participants after SD, respectively, and 0% and 9.1%, respectively, after MD. Less specific terms, (i.e., dizziness) was the most commonly reported term following SD and feeling abnormal after MD. Abuse potential-related TEAE after any dose in the SD and MD phases were reported in a comparable or greater percentage of participants than placebo. Mean Clinical Opiate Withdrawal Scale (COWS) and Subjective Opiate Withdrawal Scale (SOWS) scores following the MD were similar to pre-dose scores and did not differ from placebo. There were no reported COWS withdrawal symptoms. Several participants receiving active Mitra-Leaf reported withdrawal symptoms on the SOWS, but none exceeded the mild withdrawal threshold (≤10), and there were no withdrawal-related TEAE. After the highest 53.2mg SD, median (range) mitragynine Cmax was 130ng/mL (34.2-204) and Tmax 1.3h (0.75, 2.0), and for 7-OH-MTG 21.7ng/mL (12.5-38.6), and 1.7h (1.0, 2.3), respectively. After 53.2mg MD, median mitragynine Cmax was 155ng/mL (64.3-215) and Tmax 1.7h (1.0, 3.0), and for 7-hydroxymitragynine 20.9ng/mL (13.3-31.7) and 2.0h (1.3, 3.5), respectively. There was slightly less than dose-proportional pharmacokinetics, with almost no accumulation following MD. Mean maximum 7-hydroxymitragynine to mitragynine plasma concentration ratios were 0.31–0.51 across all doses, with higher ratios after SD compared to MD and highest at the lowest doses.

Discussion: Abuse potential drug effects decreased from SD to MD, suggesting desensitization or tolerance despite comparable exposure, and there were generally no withdrawal symptoms. Abuse-related TEAE and indices of drug withdrawal did not differ appreciably from placebo following kratom administration, supporting the conclusion that kratom or mitragynine alone has low abuse potential at these doses consistent with findings from community surveys of kratom users.

Kratom; Mitragynine; Abuse Potential

L64 The Extraction's Always Greener on the Other Side

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Learning Overview: After attending this presentation, attendees will better understand the concepts of Green Analytical Chemistry and how they relate to forensic toxicology. This presentation will demonstrate how open-source software can be used to quantify the environmental impact during the development of new analytical assays.

Impact Statement: This presentation will impact the forensic science community by demonstrating the concepts of green analytical chemistry and how they can be applied to forensic toxicology, describing what tools are freely available and providing practical examples of implementation.

As forensic science laboratories continue to evolve, analysts are often tasked with updating or creating new assays to complement new technologies and conform to various accreditation standards. The Green Chemistry movement aims to incorporate several principles to help assess both new and existing methods and promote methodology changes such as waste reduction and safer solvent selection. This movement has further evolved into Green Analytical Chemistry and Green Toxicology, where the principles have been adjusted to better fit the scope of analytical extractions. Several open-source software tools have recently been developed that use statistical calculations along with "traffic light" color coding to create simple pictorials to compare methods.

From December 2020 to October 2022, seven Gas Chromatograph/Mass Spectrometry (GC/MS) methods were transferred to Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) instruments. Some methods were combined due to analyte similarities, leading to the validation of five new LC/MS/MS methods using both Agilent® and SCIEX® instrument platforms. In 2023, a retrospective study was conducted to assess the "greenness" or environmental impact observed after the implementation of new sample preparation procedures and instrument methods. AGREE, AGREEprep, and ComplexGAPI open-source software packages were utilized for this study. Each software package assessed several different factors that contribute to analytical greenness, including specimen/matrix volume, reagents used, extraction length (duration and steps), energy usage, and sample throughput.

The average greenness calculated for GC/MS methods was 0.28 and 0.35 for AGREEprep and AGREE, respectively, on a scale of 0.0–1.0. The average greenness calculated for the LC/MS/MS methods was 0.43 and 0.48, respectively, indicating an average increase of 15% and 13%, respectively. As ComplexGAPI does not include a numerical score, greenness was compared using the red/yellow/green color system of the pictogram generated for each method. The areas where the greatest differences were observed included decreased specimen and waste volumes, the removal of derivatizing steps, and the increased number of analytes in each method due to method consolidation. Since AGREE and AGREEprep weight each parameter the same, further analysis will reveal how individual parameter weighting adjustments can impact overall scores and be used to promote specific analytical goals.

An additional study was conducted exploring the changes in helium usage within the laboratory over the past three years. In that time, helium usage decreased approximately 10% each year. This decrease can be attributed to decommissioning three GC/MS instruments and the implementation of gas-saving standby parameters for all remaining instruments.

The forensic toxicology field continues to transition away from lengthy extractions, harmful derivatizing agents, and GC/MS analyses while embracing simplified sample preparation, smaller specimen and solvent volumes, and sensitive LC/MS/MS instrumentation. This shift has not only resulted in improved efficiency and safety, but also lessens the environmental impact of laboratory work.

Green Chemistry; Laboratory Improvement; Method Development

L65   Homoamphetamine: A Cautionary Tale

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Learning Overview: After attending this presentation, attendees will be able to recognize the analyte homoamphetamine as a metabolite of labetalol, as well as a Novel Psychoactive Substance (NPS) of little-to-no activity, that has been seized in a few rare occurrences in the illicit drug market over the past decade. Attendees will learn about cases from the North Carolina Office of the Chief Medical Examiner (NC OCME) where homoamphetamine has been encountered as an analyte and how to evaluate its presence in a decedent.

Impact Statement: This presentation will impact the forensic science community by explaining the nuances of an analyte that is not widely encountered. This presentation will help prevent unsuspecting laboratories from assuming NPS use when therapeutic blood pressure treatment is indicated by the scene investigation or past medical history of the decedent.

Homoamphetamine (1-methyl-3-phenylpropylamine/ 3-amino-1-phenyl-butane) was first encountered by NC OCME as a Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) library match in an untargeted Gas Chromatograph/Mass Spectrometry (GC/MS) screen in 2019. The decedent had a known history of drug use, and a cursory internet search of the analyte under the name homoamphetamine revealed its presence in a few drug seizures and NPS lists in Europe.[1,2] The analyte was assumed to be an NPS about which not much was known at the time. The library match was confirmed by a reference standard under the name 1-Methyl-3-phenylpropylamine, and homoamphetamine was reported out as an analyte.

In 2022, a series of seven homoamphetamine-positive cases were seen in North Carolina in decedents with histories both indicative of illicit drug use and those where illicit drug use did not seem likely, over a wide range of ages, from 35 to 75 years. A homoamphetamine-positive specimen was sent to the Drug Enforcement Administration Toxicology (DEA TOX) testing program at the University of California San Francisco, which tests for a large variety of both novel and common therapeutic drugs. While the primary goal of the sample submission was identification of an unknown substance, a secondary goal was to see if the program would detect homoamphetamine, and how it would be reported. The DEA TOX program confirmed several substances previously identified by the NC OCME, but also the presence of the therapeutic blood pressure medication labetalol, which we did not detect during any of our screening attempts. Upon examination of the structure of labetalol, analysts noticed the similarity to the homoamphetamine structure and determined homoamphetamine to be a likely breakdown product or metabolite of labetalol.

After discussion with other laboratories, it emerged that this phenomenon has created confusion a few times before. In 1995, a paper was published in the *Journal of Analytical Toxicology* detailing how a labetalol metabolite by the name 3-Amino-1-Phenylbutane (APB) caused false positives for amphetamine via immunoassay.[3] In 2011, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reported seizures of the analyte 3-amino-1-phenyl-butane in Belgium and Poland, but also noted that the substance was a metabolite and precursor of labetalol.[4] In 2017, a presentation was given at the American Academy of Forensic Sciences conference in New Orleans, LA, detailing the danger of false identification of metabolites of rarely detected therapeutic substances as novel psychoactive substances, using 3-amino-1-phenylbutane/ homoamphetamine as one of three examples.[5]

To explore this analyte further, analysts at the NC OCME created an approximately 1mg/mL solution of labetalol from a pill and tested it via both untargeted GC/MS and targeted LC/MS methods with the goal of detecting homoamphetamine and/ or labetalol. A small amount of labetalol was detected via untargeted GC/MS, while a simultaneous large homoamphetamine peak indicated the majority of the parent drug does not survive instrumental analysis via this method. NC OCME was able to detect both labetalol and homoamphetamine via targeted LC/MS in a retroactive analysis of every 2022–2023 decedent previously determined to be positive for homoamphetamine via untargeted GC/MS. Area count ratios of the metabolite to parent drug varied widely from around 5% to around 140%.

A structure/ activity relationship study referenced in a publication from 1978 indicates that homoamphetamine is minimally active if active at all.[6] Therefore, it is not likely to become a commonly sold or consumed NPS in its own right. The apparently rare detection of the drug in illicit market seizures supports this theory, as does the lack of amphetamine-like side effects in consumers of labetalol. However, the inconsistency of nomenclature between GC/MS libraries and literature creates difficulty for toxicologists attempting to learn more about this rarely encountered substance.

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Homoamphetamine; Labetalol; Novel Psychoactive Substance

L66   The Bath Salts State: A Comprehensive Review of Substituted Cathinones in Miami From 2011 to 2023

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Learning Overview: After attending this presentation, attendees will be able to discuss the evolution and tenure of substituted cathinones since they were first detected in postmortem casework at the Miami-Dade Medical Examiner (MDME) Department, discuss how the impact of these substances on the cause and manner of death have changed over time, and understand how case demographics changed with the increase of drug-related deaths.

Impact Statement: This presentation will impact the forensic science community by providing a comprehensive study of substituted cathinones in MDME postmortem casework, alluding to the unpredictable evolution of the novel Psychoactive Substances (NPS) market over the past 12 years and demonstrating how local drug trends align with the national landscape.

Since 2011, the MDME has detected NPS in routine postmortem casework. While the MDME has seen a wide variety of NPS over the past 12 years, the most abundant class of NPS reported has been substituted cathinones. Substituted cathinones, derived from the khat plant, are a class of NPS with stimulant-like psychoactive properties similar to methamphetamine and MDMA. When substituted cathinones were first introduced into the drug supply, they were marketed and sold as "Bath Salts" or legal highs to bypass current drug legislation. The MDME detected its first substituted cathinone in 2011, when two postmortem cases were positive for methylone. As these substances increased in popularity, their incidence in MDME cases quickly increased as well.

Due to the high prevalence of these substances in MDME postmortem cases, a comprehensive study analyzed all cases positive for a substituted cathinone between 2011 to 2023. Using the toxicology laboratory's current case management system, case demographics were analyzed for all positive substituted cathinone cases, including age, race, sex, Cause Of Death (COD), and Manner Of Death (MOD). In addition, any quantitative values for the target analytes were assessed, as well as additional analytes detected, especially those from an NPS class.

From Q4 2011 to Q4 2022, 780 postmortem cases at the MDME have contained at least one substituted cathinone, with 170 positive cases detected in 2022 alone. Currently, the first two quarters of 2023 have already had 136 positive cases and are expected to surpass the total cases from the previous year within the next few months. Within the last 12 years, substituted cathinones have been identified among decedents ranging in age from 3 days old to 77 years old, with a mean and median age of 37 years and 35 years, respectfully. Principally, these decedents are Black males, and initially, the primary cause and manner of death in these cases was classified as gunshot wound homicides, with substituted cathinones rarely considered a contributory factor nor listed in the cause of death. However, since 2019, a steady increase in accidental drug-related deaths has occurred, primarily attributed to the abuse of illicit fentanyl. Of these accidental drug-related deaths, 50% of them contained a substituted cathinone, and almost 90% of those cases had the substituted cathinone included in the cause of death. In turn, the COD and MOD in cases containing substituted cathinones shifted to mostly accidental polydrug toxicities, with almost all of these cases containing fentanyl.

Eighteen unique substituted cathinones have been identified in MDME casework, varying in potency and tenure in the local drug market. Of note, the drug supply in Miami exhibits the same volatility as that across the United States when compared to published NPS drug trends. In most cases, the newest substituted cathinone makes its initial appearance in Miami before other parts of the country, likely due to the party culture and drug-trafficking channels. Usually, a substituted cathinone maintains its presence in MDME casework for about a year before a new one is circulated in the drug supply. However, from Q4 2018 to Q4 2021, eutylone was the primary substituted cathinone detected until the introduction of N,N-dimethylpentylone in August of 2021. Two years later, N,N-dimethylpentylone and its metabolite, pentylone, are still the most abundant substituted cathinones and the most detected substituted cathinone to date. As the breadth of NPS continues to grow, substituted cathinones will likely remain not only the most prevalent NPS class in Miami, but also one of the most prevalent psychoactive substances detected in postmortem casework.

Novel Psychoactive Substances; Postmortem; Stimulants

L67 The Search for the K-Hole: A Six-Year Study of Ketamine in Postmortem Cases From the Miami-Dade County Medical Examiner Department, 2018–2023

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Learning Overview: The objectives of this presentation are to offer an overview of ketamine deaths in Miami-Dade County from 2018 to mid-July 2023, including demographics, manner and cause of death, and the concentration range for those cases where ketamine was quantified. A case will also be presented that highlights an extreme example of ketamine abuse.

Impact Statement: Ketamine use has been increasing around the country; therefore, the number of medical examiner cases involving ketamine are rising. This presentation will impact the forensic science community by providing information relevant and important to the forensic science community: an overview of medical examiner cases involving ketamine in Miami-Dade County, FL, in the past six years. It is important for the forensic community to share findings with each other for many reasons, including education and public health.

Background/Introduction: Ketamine is a dissociative anesthetic approved medically as an injectable for the induction or maintenance of anesthesia and as a nasal spray for treatment-resistant depression. It is referred to as a "dissociative anesthetic hallucinogen" because it produces feelings of disconnection from self, distortions in perceptions of sights and sounds, decreased pain sensations, and hallucinations. Consequently, ketamine is an abused drug, particularly popular at dance clubs and all-night dance parties or "raves." It is short acting, lasting approximately 30–60 minutes, which is significantly shorter than the "trips" produced by other drugs like PCP and LSD. In Miami-Dade County, there is no shortage of night clubs and parties; therefore, it is not surprising that ketamine has rapidly gained popularity.

Methods: A query of Miami-Dade County Medical Examiner case data was conducted for all cases from 2018 to mid-July 2023 where ketamine was detected. Data collected and reviewed included cases where ketamine was detected, demographic details, the cause and manner death, and the concentration range for those cases where the drug was quantified. At the MDME, ketamine is initially discovered in any of the laboratory screening methods: a full-scan drug screen by either Gas Chromatography/Nitrogen Phosphorous Detection/Mass Spectrometry (GC/NPD/MS) or Liquid Chromatography (LC) Ion Trap- Multistage Mass Spectrometry (MS^n) or a targeted drug screen by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). If necessary, it is quantified by Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS).

Results: From 2018 to mid-July 2023, 153 cases were received in which ketamine was identified in toxicology testing. Demographically, most of the decedents were White (72%), with males more prevalent than females (75% of cases). The predominant age range for the cases reviewed was between 20 and 29 years of age. Approximately 50% of cases were ruled as accidental, and of these, 34% listed ketamine in the cause of death. All cases in which ketamine was included in the cause of death also listed other drugs such as cocaine, MDMA, and methamphetamine. Concentrations of ketamine (n=30) ranged from detected less than the limit of quantitation (0.05 mg/L) to 52 mg/L.

One highlighted case involves a well-known DJ, record-label owner, and music producer who was found deceased in his Miami Beach residence at the age of 49 years old. He was known to drink alcohol frequently and abuse drugs, including ketamine. Numerous vials of liquid identified as injectable ketamine were found in his home. Multiple drug-facilitated crimes allegations had been levied against him, and he was due in court the following day, suggesting a suicide as a possible manner of death. Lack of evidence to support a suicide; however, led to the case being finalized as an accidental death. The cause of death was listed as Acute Ketamine Toxicity with MDMA and cocaine listed as contributory causes. The concentration of ketamine in the iliac vein blood was 8.8mg/L, and the concentrations of MDMA and cocaine were 0.101mg/L and < 0.010mg/L, respectively.

Conclusion/Discussion: The number of cases involving ketamine has increased substantially in Miami-Dade County, and it appears that this increase will continue to rise in 2023 and beyond. The demographics are consistent with club attendees seeking a euphoric experience while listening to dance music. Additionally, most cases involving ketamine involve other substances, which adds to the complexity of medical examiner cases.

Ketamine; Postmortem; Death Investigation

L68   **Segmented Workflows and the Confrontation Clause: Achieving Congruence Between Constitutional Guarantees, Laboratory Productivity, and Toxicological Expertise**

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Learning Overview: After attending this presentation, attendees will be able to evaluate the legal theories governing the accused's right to confront witnesses in the context of forensic toxicology casework performed in a segmented workflow model, with particular focus on legally differentiating early process witness testimony from toxicologist interpretation and testimony.

Impact Statement: This presentation will impact the forensic science community by evaluating current caselaw and practice in criminal trials involving testimonial and non-testimonial forensic toxicology testimony and by articulating methodologies for upholding the constitutional right of confrontation while maintaining efficient and productive laboratory operations.

As in other complex analytical processes, forensic toxicology practice has evolved over the past 30 years based on practical considerations, resource availability, and increasing costs and demands in laboratories. Laboratories have transitioned from a cradle-to-grave model of testing in which the analyst receives the sample or samples, conducts presumptive and confirmatory testing on the evidence, calculates and reviews data, formulates opinions, and then testifies to the entire process in court. Many laboratories now deploy a model where workflows are segmented to a greater or lesser extent with different individuals receiving, screening, confirming the test results, and formulating conclusions and opinions, often with these processes themselves being sub-segmented.

Against this backdrop, constitutional jurisprudence has similarly evolved to seemingly mandate production of myriad personnel in the testing process at criminal trials pursuant to the Sixth Amendment to the United States Constitution: "In all criminal prosecutions, the accused shall enjoy the right … to be confronted with the witnesses against him, …." The United States Supreme Court (SCOTUS), in the *Crawford, Melendez-Diaz, Bullcoming, and Williams* decisions have created a transference and evolution from an accused's right to confront witnesses to a right to cross-examine every person who produces an entry in a laboratory's testing record. The theory behind such evolution seems to conflate the Sixth Amendment's right to confront witnesses with the premise that all out-of-court declarants in the testing chain provide testimonial statements and must, therefore, be presented for testimony and subject to cross-examination. Per Justice Gorsuch's opinion in *Stuart v. Alabama*, the Court's "fractured decisions have yielded no majority and have sown confusion in courts across the country," resulting in decisions that threaten workflow efficiencies, laboratory production, witness availability, and testifying toxicologists' and analysts' ability to testify to underlying data and information used to reach conclusions pursuant to long-standing federal and state rules of evidence.[1-4]

Two broad questions have arisen in the jurisprudence: (1) How many persons in the chain of testing must appear to satisfy the Confrontation Clause, and what role do those persons play, and (2) May a toxicologist or certifying scientist rely on the underlying work done by testing personnel to opine at trial, and if so, what level of analysis must precede the proffered testimony? For valid legal answers to be discerned and identified to these questions, the science of standard operating procedures, quality control, segmented workflows, and the toxicologist's comprehensive review process must be completely understood and considered. The processes of accessioning, sampling, analysis, data review, and calculation and interpretation, which comprise the science of forensic toxicology, evade lay persons' common knowledge, including the common understanding and knowledge of most judicial officers. SCOTUS cases demonstrate a broad-brush application of the Confrontation Clause as applied to scientific processes, with a particularly broad brush being used by SCOTUS in the *Bullcoming* case.

This presentation will review current forensic toxicology practices with respect to analytical processes and decision-making opinion generation in forensic toxicology casework. Through a careful dissection and deconstruction of the SCOTUS decisions and most probative and relevant state case pronouncements, a formula for articulating the reasons why personnel in the chain of testing produce non-testimonial data and reporting can be made. Similarly, the very words of the Supreme Court decisions can formulate standard operating procedures to assure that the laboratory has blinded and documented processes, reporting requirements, and standard operating procedures to enable the toxicologist or certifying analyst witness to independently review all data and case information to formulate an independent opinion, or as in the case of *Bullcoming*, to formulate a second opinion based on complete, independent review of facts and data.

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Judicial; Expert Testimony; Judge

L69   A Quantitative Analysis of Cannabis Exposure Biomarkers in Exhaled Breath Condensate and Oral Fluid

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Learning Overview: The goal of this work is to present a validated Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) method for detecting and quantifying a variety of cannabis exposure and oxidative stress biomarkers in Exhaled Breath Condensate (EBC) and Oral Fluid (OF) matrices.

Impact Statement: This presentation will impact the forensic science community by presenting a reliable analytical method for the detection and quantification of major and minor cannabinoids and metabolites in the alternative specimen matrices EBC and OF.

The continuous increase in cannabis use for its perceived medical benefits and legalization as a recreational drug in the United States has created a great demand in forensic toxicology for the identification of biomarkers of cannabis exposure, other than the commonly detected delta-9-Tetrahydrocannabinol (Δ9-THC) and its metabolites. In addition, markers that can be sampled non-invasively and with relative ease are needed.

EBC and OF are alternative, non-invasive sample matrices that hold promise for identification of cannabis exposure biomarkers. OF is currently being explored as a matrix for cannabis exposure analysis. EBC is the airway lining fluid from the lungs that can be easily collected. It is an aqueous specimen consisting of condensed water vapor, dissolved volatiles, and water-soluble polar and non-volatile small molecules, including metabolites. Although licit drugs and metabolites have been measured in EBC, it has not been explored as an alternative matrix in forensic toxicology studies, despite its obvious advantages. The most employed sample preparation method for OF is Solid Phase Extraction (SPE), and lyophilization for EBC. However, these approaches require rigorous method development, are time consuming, and utilize instrumentation that may not be widely available.

This work focuses on the assessment of an extraction method and the validation of a previously developed LC/QqQ/MS dynamic Multiple Reaction Monitoring (dMRM) method for the analysis of the cannabis exposure biomarkers from EBC and OF samples. A total of 25 cannabinoids and metabolites were targeted for analysis, including five internal standards. An Agilent® 1290 UHPLC coupled to a 6460 LC/QqQ/MS/MS with Electrospray Ionization in positive mode (ESI+) was utilized for this work. Chromatographic separation was achieved using a Zorbax® 120 EC-C18 column (3.0 x 100mm, 1.8μm) and a step gradient. OF was collected using Quantisal™ and EBC samples were collected using RTube™ devices. Quantisal™ buffer was used as an extraction solvent for both OF and EBC to aid in higher recoveries. For OF, after collection using the provided pads with the device, the pads were placed in 3ml buffer provided, and for EBC, 500μL of buffer was added to 500μL of sample. Sample preparation involved Solid Liquid Extraction (SLE) using of 1ml ISOLUTE SLE+ supported liquid extraction columns. 800μL samples were pretreated with 10μL concentrated formic acid and extracted using MTBE and hexane. The resulting eluent was dried with nitrogen and reconstituted in acetonitrile. A partial method validation will be carried out in compliance with American National Standards Institute/ Academy Standards Board (ANSI/ASB) Standard 036. The method will be assessed for calibration, carryover, Limit Of Detection/Limit Of Quantitation (LOD/LOQ), bias, precision, matrix effects, and recovery.

The method showed sufficient linearity for all analytes at their respective linear ranges. LOD and LOQ for the target cannabinoids biomarkers ranged from 0.2 to 5ng/mL, and from 1 to 14ng/mL respectively, except for 11-nor-cannabinol-9-carboxy-acid, which had an LOD of 26ng/mL and LOQ of 79ng/mL. Recoveries for the SLE method ranged from 14 to 70%, and 10 to 140 %, with most analytes averaging around 38 and 53%, for OF and EBC respectively. The validation of the method is currently ongoing, and the other parameters are being evaluated.

The developed method is capable of extracting, detecting, and quantifying 25 cannabinoids. Once the method has been fully validated, it will be used to analyze OF and EBC samples obtained from a human cohort of cannabis smokers with different user profiles to differentiate between licit vs. illicit, recent vs. past, and episodic vs. regular use.

Cannabinoids; Alternative Matrices; Method Validation

L70   **The Use of Hair Analysis as a Potential Predictor of Tolerance in Decedents With High Fentanyl Concentrations in Fentanyl-Related Deaths: Preliminary Results**

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Learning Overview: Attendees of this presentation will gain knowledge on the usefulness of hair analysis for studying past drug exposures of decedents. Attendees will also understand how the results of these tests may assist in explaining high fentanyl blood concentrations detected at the time of death.

Impact Statement: This presentation will impact the forensic science community by revealing how a non-conventional matrix, such as hair, is capable of providing a complete toxicological picture concerning high blood fentanyl concentrations at the time of death in postmortem casework, offering a hypothesis regarding whether past exposure/tolerance correlates with significant increases in blood fentanyl concentrations.

Fentanyl, a synthetic opioid, is highly toxic even at low doses, with the possibility of sudden deaths occurring at low blood concentrations due to decreased respiratory drive and cardiac arrest or an anaphylactoid reaction in rare cases. Fentanyl-related deaths have been reported in cases resulting from therapeutic use, recreational use, overdose, or tampering with pharmaceutical products.

Over the past few years, there has been an increase in fentanyl fatal overdose cases nationwide.[2] In the United States, from 2013 to 2019, the age-adjusted rate of deaths involving synthetic opioids (primarily fentanyl) increased by 1,040%. A helpful tool for assessing previous exposure to opioids is hair analysis, which can be used to evaluate acquired tolerance to drugs such as opioids or benzodiazepines.

Fentanyl-related deaths in Jefferson County, AL, followed a trend similar to the nation, with a 12.3% increase from 316 deaths in 2021 to 355 deaths in 2022, accounting for 78.5% of all overdose deaths during that period. From early 2023, an increase in peripheral blood fentanyl concentrations has been observed in overdose cases.

Toxicological hair analysis was performed on cases that fell under the jurisdiction of the Jefferson County Coroner/Medical Examiner's Office (JCCMEO) from January 2023 to March 2023. In cases identified as fentanyl-related death (either as a cause or contributing factor), hair testing results were compared to blood analysis to investigate this method's utility in identifying those exposed to high concentrations of fentanyl over time before their death

Hair samples (n=150) were obtained during the examination of decedents at the JCCMEO from January 2023 to March 2023. Urine and blood were also collected and submitted for toxicological analysis. Screening (using the Enzyme-Multiplied Immunoassay Technique [EMIT]) was performed on urine with confirmation in preserved blood by Gas Chromatograph/Mass Spectrometry (GC/MS), where the limit of quantification was 2.5ng/mL for fentanyl. Hair analysis was carried out by the Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) method developed to quantify fentanyl and related analogs. This method was also validated according to the American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*.[4] The results of toxicological testing in all matrices were compared in deaths certified as being related to fentanyl (n=36).

The median blood concentration of fentanyl was 19.5ng/mL (range 3–610ng/mL), whereas the median hair concentration of fentanyl was 293pg/mg (range 0–10,000pg/mg). Spearman's coefficient ($r_s = 0.49$) showed a moderate correlation between fentanyl concentration in blood and hair (p=0.0029).

The population was then divided into two cohorts based on the concentration of fentanyl in the hair: above (high group, n=24) or below (low group, n=12) 1,000pg/mg. The low group showed a median of 11.5ng/mL (range 3–62ng/mL) in blood, while the high group showed a median of 29.5ng/mL (11–610ng/mL). A Mann–Whitney test showed that the difference in fentanyl blood concentration between the two groups was statistically significant (p=0.0026).

Given the scientific basis of toxicological hair analysis, a higher concentration of compounds can mean greater exposure to them in the antemortem period. Data from the present study shows that greater antemortem exposure to fentanyl correlates with higher postmortem blood fentanyl concentrations. In other words, acquired tolerance to fentanyl provides context to the significantly high fentanyl blood concentrations detected in this small sample.

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Fentanyl; Hair; Drug Analysis

L71   A Quantitative Assessment of Five Hair Drug Testing Laboratories With a Commercial Hair Reference Material

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Learning Overview: After attending this presentation, attendees will be aware of a commercially available hair drug testing reference material. Attendees will see that several laboratories were able to obtain quantitative test results in good agreement with the assigned values for the analytes. These results are at least supportive of the accuracy of the assigned values indicating that this material might be useful for laboratories engaged in hair drug testing.

Impact Statement: Quantitative accuracy in hair drug testing remains a challenge. The knowledge gained in this study will impact the forensic science community by possibly leading to improved agreement between laboratories engaged in hair drug testing.

In 2023, the National Laboratory Certification Program (NLCP) at RTI International (RTI) undertook a program to improve performance for laboratories likely to apply for accreditation under federal drug testing guidelines for drugs in hair. This program has been termed the "Hair Lab Readiness Plan" and includes development of Proficiency Testing (PT) samples and general assessment of laboratory quantitative performance and interlaboratory quantitative agreement. To assess laboratory quantitative performance, a preliminary study was performed in which laboratories were supplied with a commercially prepared hair reference material containing 14 drugs or metabolites of relevance to the NLCP. The evaluation of results of laboratory performance with these samples is the first stage in preparing laboratories to participate in the United States federal hair drug testing program once the Department of Health and Human Services (HHS) Hair Mandatory Drug Testing guidelines are in place.

Hair reference material (TricoCheck® Product SSCTR002270 CTRL H 20 multiCONTROL) was purchased from Comedical® inTrento, Italy. This control material contains 36 drug analytes in seven drug classes, 14 of which are relevant to the NLCP: cocaine, benzoylecgonine, cocaethylene, norcocaine, codeine, morphine, 6-acetylmorphine, oxycodone, fentanyl, methamphetamine, amphetamine, methylenedioxymethamphetamine, methylenedioxyamphetamine, and 11-nor-9-carboxy-Δ⁹-Tetrahydrocannabinol (THC-COOH). The material included a certificate with an assigned value and range of acceptability for each analyte. Samples were relabeled to hide the source of the material and submitted to six laboratories. Participating laboratories were instructed to test the samples for the 14 analytes in five separate analytical batches and to submit test results in an Excel® spreadsheet. Lab results were evaluated for agreement with each analyte's assigned value and acceptable range.

Test results were submitted by five of the six laboratories. Two laboratories, Lab A and Lab D, performed all 70 tests. A third laboratory, Lab E, performed testing on all analytes except fentanyl. A fourth laboratory, Lab B, performed tests for all analytes, but did not complete the five replicates for six analytes, reporting 63 total test results. The fifth laboratory, Lab C, reported results for 9 of the 14 analytes.

Lab D showed the best overall results with 58 of 70 tests (82.8%) within the acceptable range. This was followed by Lab E (48 of 65, 73.8%), Lab A (44 of 70, 62.8%), Lab B (37 of 63, 58.7%), and Lab C (6 of 45, 13.3%). All five laboratories reported some results that were outside ±50% of the assigned value for some analytes. The laboratory with the lowest number of 50% errors was Lab A (8 errors). This was followed by Lab D and Lab E (9 errors each), Lab B (10 errors), and Lab C (33 errors).

The four laboratories that completed testing (Labs A, C, D, and E) were provided with a report of their results and a list of references regarding methods for optimizing recovery of different analytes from the hair matrix. Lab B will be provided with the same information upon completion of their remaining seven tests. The next phase of this project will be to re-evaluate the laboratories' performance after they have had sufficient time to investigate the causes of their quantitative errors.

Hair; Drug Analysis; Accuracy

L72   The Gonadotoxic Effects of Tobacco Smoking on Human Seminal Fluid

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Learning Overview: After attending this presentation, attendees will have learned: (1) method development and validation for the quantification of nicotine and cotinine using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS); and (2) the gonadotoxic effects of nicotine and cotinine on human seminal fluid.

Impact Statement: This presentation will impact the forensic science community by helping clinical and forensic toxicologists learn about the detrimental effects of tobacco smoking on the human reproductive system and to prove LC/MS/MS is a highly sensitive analytical tool that could be used for quantification of nicotine and cotinine in biological samples.

Introduction: According to World Health Organization Report in 2019, prevalence of tobacco smoking remains high worldwide, with an estimated 19% of the adult population is using tobacco products: 33% among males and 6% among females. Consistently increasing abuse of tobacco is of alarming concern since tobacco use is unequivocally associated with multiple chronic disorders and adverse health outcomes, including potential gonadotoxic effects in both men and women. Evidence suggests that tobacco affects reproductive health more than alcohol. Tobacco consumption yields approximately 4,000 compounds. Tobacco smoke can be divided into a gaseous phase and a particulate phase. The principal harmful components of gaseous phase are CO_2, nitrogen oxide, ammonia, and volatile hydrocarbons whereas nicotine is the main component of the particulate phase. Nicotine is a natural, volatile, colorless, water-soluble alkaloid. Pharmacological action of nicotine takes place at the autonomic ganglia. Nicotine has dual action (i.e., it acts as a Central Nervous System [CNS] stimulant as well as a depressant). Nicotine is quickly absorbed through the respiratory tract, mouth, mucosa, and skin. It is mainly metabolized into cotinine primarily by the liver, but also by the kidneys and lungs. Nicotine and cotinine levels have not only been detected in urine, serum, saliva, hair, and milk, but also in seminal plasma at significant levels.

Purpose: The purpose of this study is to determine the potential gonadotoxic effects of tobacco abuse on semen quality, seminal plasma antioxidant activity, and sperm DNA integrity and to determine relative concentrations of nicotine and cotinine in human seminal fluid compared to urine and serum.

Methodology: The study included 200 male participants aged 20–40 years. The study requirements were carefully explained to participants, who provided written informed consent before entering the study. The study was conducted in accordance with the Declaration of Helsinki. Urine, serum, and semen samples were obtained from cigarette smokers (having a history of cigarette smoking of at least one year). While the inclusion criteria were experimental subjects with azoospermia, exposure to chemicals, alcohol consumption, and subjects with a history of recreational drug abuse. Seminal parameters were evaluated as per World Health Organization (WHO) guidelines. Quantification of nicotine and cotinine in urine, serum, and semen samples was done by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS). Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-Nick End Labeling (TUNEL) assay was used for DNA Fragmentation Index

Results: In all cases, the corresponding semen sample was positive for nicotine and cotinine. Nicotine and cotinine were detected in human seminal plasma at concentrations as high as in plasma. Urinary cotinine concentrations were highly correlated with serum concentrations. A significant decrease in semen quality (sperm count, sperm progressive motility, etc.) was found for cigarette smokers. DNA fragmentation index was significantly higher for smokers. Nicotine and cotinine also inhibited catalase and glutathione reductase activity, contributing to an accumulation of reactive oxygen species.

Conclusion: The present study suggests that tobacco smoking significantly affects human seminal parameters, seminal plasma antioxidant activity, and sperm DNA integrity. It is therefore recommended that male smokers should stop smoking tobacco well in advance of planning to have children.

Tobacco; Semen; LC/MS/MS

L73   The Identification of the New Synthetic Opioid N-Piperidinyl Etonitazene (Etonitazepipne) in Postmortem Alternative Matrices

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Learning Overview: The purpose of this presentation is to inform attendees of the ability of etonitazepipne to accumulate within alternative biological matrices, particularly in the vitreous humor, nails, and hair. Given the scarce information that is still available in the literature on this substance, knowing more about the mode and characteristics of accumulation may prove important for forensic toxicologists to better understand the effects this substance may have on humans.

Impact Statement: This presentation will impact the forensic science community by helping to disseminate new information on new synthetic opioids, especially nitazenes, which are becoming increasingly prevalent in the United States and European illicit drug markets, making the information available to the European early-warning system.

The new synthetic opioid N-piperidinyl etonitazene, commonly referred to as etonitazepipne, belongs to the benzimidazole group, and was formally notified in Europe to the European Monitoring Centre for Drugs and Drug Addiction's (EMCDDA) Early Warning System (EWS) on Novel Psychoactive Substances (NPS) in February 2021.[1] To date, there are very few published references in the literature regarding the molecule's detection in biological matrices. Furthermore, no scientific article mentions the identification of etonitazepipne in alternative biological matrices. These alternative matrices, often overlooked, play a complementary role to conventional matrices in toxicological analyses.

Alternative matrices, including keratin matrices, are extremely important for forensic toxicology because nails and hair have been shown to accumulate substances within them consistently over time.[2,3] The affinity for the MOR receptor of etonitazepipne is almost equivalent to that of morphine and slightly higher than that of fentanyl.[4] The molecule is more than 50 times more potent and 80% more effective than morphine in activating the receptor. As a result, etonitazepipne can be identified as a substance hazardous to human health. The fact that the molecule is highly effective makes it plausible that even a small dose of the substance is sufficient to achieve the narcotic effect. This makes the detection process difficult in biological matrices such as blood and urine. This presentation aims to comment on the finding of etonitazepipne in postmortem samples of hair, nails, and vitreous humor in a case where the cause of death was ascribed to fatal etonitazepipne intoxication.

Samples taken during the judicial autopsy were stored in special containers at temperature of 4°C until analysis. Hair and nails were washed with H_2O, dried, and cut into small fragments. A validated method for the soughtafter new synthetic opioids has been followed for the extraction.[5] Briefly, samples were fortified with methanol and incubated at 52 C for 24 and 72h for hair and nails, respectively. Then, the samples were centrifuged, the supernatant of each was recovered and evaporated. The extracts were resuspended with 50μL of phase B and submitted to the analysis by liquid chromatography at high-resolution coupled with the Thermo™ Exactive™ Plus Orbitrap™ (Ultra High-Performance Liquid Chromatography-High Resolution Mass Spectrometry [UHPLC-HRMS]). Vitreous humor was subjected to a liquid/liquid extraction. The pH of the sample was adjusted at 9 and a methanol-chloroform solution (50/50 v/v) was added. After extraction, the sample was brought to dryness and resuspended with 50μL of organic phase B for subsequent chromatographic injection.

The finding of the molecule in these matrices leads to several considerations. Since its presence in the vitreous humor can be traced back to the presence of etonitazepipne in the blood, as it is known that morphine-like compounds are present in the vitreous humor in lower amounts than in the blood.[6,7] It follows that positivity for the drug in this matrix could be indicative of acute or fatal intoxication. Furthermore, the presence of the substance in keratin matrices indicates repeated exposures over time that have led to the accumulation of the molecule.

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Novel Synthetic Opioids; Postmortem; Liquid Chromatography

L74   Postmortem Pediatric Forensic Toxicology

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Learning Overview: After attending this presentation, attendees will gain an appreciation for the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

Impact Statement: This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 23rd Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth exists of interpretive information involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation or issue-specific concern, including pharmaco-toxicokinetic data and other relevant ancillary information, followed by audience participation to provide interpretive clarity around case-specific impacts of the toxicological findings. This session, attended by various sections of the Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Five cases will be presented that highlight the difficulty in assessing the role of toxicants in each case or the lengths one must go to in some cases to reach some interpretive value. Katherine Kenerson, MD, Andrew Baker, MD, Sterling McLaren, MD, Diane Moore, PhD, and Liz Zaney, BS, will be reviewing cases from their experiences as forensic pathologists and toxicologists that highlight the issues and confounders in the pediatric population.

Dr. Kenerson will be discussing a case involving a 2-month-old infant in which cause and manner of death must be balanced between the scene and other circumstances versus the presence of a novel psychoactive substance. Such cases are very challenging and raise questions as to the interpretation of toxicological findings.

Dr. Baker will focus on a case of elevated glucose in a 22-month-old. This case highlights the required toxicological testing for differential diagnoses and chasing down a potential offending agent.

Dr. McLaren will report on a 1-month-old with recent illness. Toxicological testing revealed the presence of potentially significant toxic agents. The case highlights the difficulties of natural illness versus toxicology in determining the cause and manner of death and potential pitfalls with the diagnosis.

Dr. Moore will be describing a 4-month-old with multiple drugs found postmortem; however, circumstances are such that interpreting the toxicological findings was not straightforward.

Ms. Zaney will show two independent cases of teenagers who died from classic toxicological agents. Such cases highlight the potential copycat nature of agents versus random coincidence.

Postmortem; Pediatric; Toxicity

LW1   A Forensic Analysis of the 1960 Rome Olympics' 100-Meter Dash Final

John David Bullock, MD, MPH*, Ophthalmic History Research Institute, Longboat Key, FL; Gerald H. Ashworth, MBA, Cape Neddick, ME; David Guyton, MD, The Johns Hopkins University School of Medicine, Baltimore, MD

Learning Overview: After attending this presentation, attendees will have learned NOT to unconditionally trust electronic devices or the judgments and actions of high officials.

Impact Statement: This presentation will impact the forensic science community by ensuring that attendees will once again appreciate that sometimes really good people lose and cheaters win.

At the 1938 Millrose Games, the African American sprinter, Benjamin Johnson, was clocked at 5.9 seconds in the 60-yard dash. The officials rounded his time to 6.0 seconds, arguing that no human could ever break the 6.0-second barrier. At the 1972 Munich Olympics, individual medley swimmer Gunnar Larsson (Sweden [4:31.981]) "defeated" Tim McKee (USA [4:31.983]) by 0.002 seconds (or ~2.94mm at the finish line). This was an absurd decision since the variance in lane length was most probably at least ±10mm and the event required eight transits of the pool. Afterwards the official Fédération Internationale de Natation (FINA) rule (SW 11.2) disallowed timing to 0.001 seconds.

The United States Swimming and Track Trials prior to the 2021 Olympics encountered a number of technical gaffes. The touchpads malfunctioned during the men's 400-meter individual medley, mis-identifying the first and second place finishers. In a number of both men's and women's track events, miscalibrated, ultra-heat-sensitive electronic starting blocks resulted in an unprecedented number of invalid "false" starts. The above facts document the imperfections in electronic timing devices and officials' judgments even in high-level athletic competitions.

During the 1960 Rome Olympics' 100-meter dash final, United States sprinter, Dave Sime, "lost" to Germany's Armin Hary by "less that one inch." The internet-available photographs show a "dead heat, (i.e., inseparable to the human eye). Both sprinters finished in the exact same stopwatch-determined official time (still listed today) of 10.2 seconds, recorded as new Olympic records. Hary's first-place time, remeasured with an unapproved and unofficial (until 1964) photo-finish clock, gave him a 0.03-second lead over Sime and the gold medal. A forensic analysis of the race, based on Sime's terminal velocity (calculated using his co-record 60-yard dash time), reveals a discordance of the finish-line photographs and the photo-finish clock. The 0.03-second lead over Sime translates to an inter-runner distance of 11.6–13.0 inches, which would have been readily discernible by the judges and the photographs. So either the photo-finish clock was wrong, or the officials misread or misstated it.

Hary was well-recognized for false starting as the self-styled "Thief of Starts," modified from the moniker of the Hollywood heartthrob, Rudolph Valentino, the "Thief of Hearts." During this event, Hary had already had two (then allowed) false starts. His British competitor, the bronze medalist Peter Radford (subsequently, Professor of Sports Science), figured out Hary's devious (later declared illegal) technique. "He'd wait until we were all on our fingertips in the set position. Then he'd take up his place, pause momentarily—and run. He might get caught with a false start, but he might also get away with it." Additionally, Hary should have been declared ineligible as a "professional," being in violation of Rule 26 (valid until 1984) of the Olympic Charter, for receiving payments from two different shoe companies. After the Olympics, Hary was suspended by the German Athletic Federation for expense account fraud. In 1980, he was sentenced to 18 months in jail for abusing his real estate trader position and defrauding the Catholic Church out of 3.2 million Deutschmarks.

During his career, Sime set or tied nine world records and appeared on the cover of *Sports Illustrated* as "The World's Fastest Human." Sime graduated in the top 10% of his Duke Medical School class and completed his residency at the Bascom Palmer Eye Institute in Miami, FL. He became a famous ophthalmologist, serving as the ophthalmic consultant for the United States Olympic Committee and the Miami Dolphins. His patients included Richard Nixon, Mickey Mantle, and Ted Williams.

Because Hary's "victory" was based on deception and an unapproved, unofficial, and flawed timing device, Sime should receive a posthumous gold medal.

Quantitative Evidence; Photography; Footwear Evidence

LW2   The Strange Deaths of American Presidents

Lawrence Quarino, PhD*, Cedar Crest College, Allentown, PA

Learning Overview: After attending this presentation, attendees will be informed about the controversies and historical insights into events surrounding lesser-known deaths of certain United States Presidents.

Impact Statement: The presentation will impact the forensic science community by showing how evolving medical practice, scientific investigation, and cultural norms affect the understanding of the deaths of certain United States Presidents.

The circumstances and reasons surrounding the deaths of certain United States Presidents have often been studied, discussed, and debated by historians, scientists, and medical professionals. Although the narratives surrounding the deaths of some Presidents such as Franklin Roosevelt, Abraham Lincoln, and John Kennedy are well known, the deaths of some of our other Presidents offer interesting historical insight and will be the focus of this presentation.

In the case of three pre-civil war Presidents in the mid-19th century, plausible theories have developed that suggest that the historically accepted reason for the death of these three Presidents may not be the full truth, and all three may share a commonality of cause.

In 1841, William Henry Harrison died just 31 days into his Presidency, with the cause of death listed as pneumonia resulting from a two-hour inauguration address given on a cold, rainy March day. James K. Polk had the shortest post-Presidency in history when he died of cholera in New Orleans shortly after he left office in 1849. Zachary Taylor died five days after dedicating the corner stone of the soon-to-be constructed Washington Monument on July 4, 1850. The demise of President Taylor was believed to be due to a lethal form of gastroenteritis brought on by his consumption of copious quantities of cherries and ice milk. Historical debate ensued over the next century over the death of President Taylor with some believing that he was poisoned by arsenic, a claim later dispelled in the 1990s.

Twenty-first century researchers have suggested that all three deaths were either directly or indirectly caused by *Salmonella* poisoning of the Washington, DC, drinking water in the mid-19th century. The state of medicine at the time of certain Presidential deaths may explain the death of George Washington as well as the deaths of James Garfield and William McKinley. Blistering agents and bloodletting to treat a throat infection impacted the death of President Washington, while harmful medical intervention played as significant a role in the death of Presidents Garfield and McKinley as did an assassin's bullet. I

In 1885, Ulysses Grant died after a slow agonizing death due to cancers of the throat and mouth. Although his doctors did their best to prevent the public from knowing the condition of the dying ex-President due to the intense stigma associated with cancer at that time, it was impossible to conceal his illness from the media. What proceeded was the first media "death watch" of a United States President. Media reports surrounding the President's condition bordered on hysteria and, in some cases, were complete fabrications until his death in July 1885.

At least one President succumbed to substance abuse. The life and Presidency of Franklin Pierce was marked by family tragedy, and he is often considered our most melancholy President. President Pierce drank heavily before, during, and after his Presidency, resulting in his death due to cirrhosis of the liver in 1869.

Unfounded conspiracy theories, including death from food poisoning and homicidal poisoning, surrounded the death of Warren Harding in 1923 even though all medical evidence showed a cardiac-related death. Interest and debate over the death of President Harding did, however, prove a long-standing rumor that President Harding fathered a child out of wedlock.

Finally, the deaths of two of the nations' founding fathers, John Adams and Thomas Jefferson, on July 4, 1826, 50 years to the day of the signing of the Declaration of Independence, is perhaps one of the most profound coincidences in history.

Death Investigation; Interdisciplinary; Scientific Evidence

LW3 (De)coronation Jewels: The "Affair of the Diamond Necklace" Between Historical and Forensic Implications

Matteo Borrini, PhD, MS*, Liverpool John Moores University, Liverpool, England, United Kingdom

Learning Overview: After attending this presentation, attendees will understand the impact of prejudices and stereotypes during investigation and how they could negatively influence the pursuing of Justice.

Impact Statement: This presentation will impact the forensic science community by presenting a notorious historical scandal where fraud and forgeries merge prejudices, demonstrating the difficult delivery of Justice for All.

Jewels have always been related to wealth and nobility and have often been a symbol of royalty, even if several legends report the malevolent influences of some treasures on their noble possessors. Among the tales, a real necklace is truly related to the monarchy's fall during the French Revolution.

Charles Auguste Boehmer and Paul Bassenge were two of the finest jewelers in France when King Louis XV commissioned to them an exquisite diamond necklace to please one of his mistresses. For several years and with a great expenditure, the two artists collected stones to satisfy his majesty's desire. After the monarch passed away in 1774, the craftsmen tried to sell the necklace to the new Queen, Marie Antoinette, who always rejected it.

In 1785, a woman of dubious background and self-claimed "Comtesse de la Motte," Jeanne de Valois-Saint-Rémy, embarked on an elaborate plot to obtain the necklace. The first target of De la Motte's plot was Cardinal Prince de Rohan, a former French ambassador, who was seeking a reconciliation with the Queen since he had aroused the dislike of the Queen's mother during a visit to Vienna in 1772.

Jeanne de la Motte pretended to enjoy Marie Antoinette's favor, and by forging a correspondence with the Queen, she made the Cardinal believe that Marie Antoinette had fallen in love with him. A secret nocturnal encounter in the gardens of Versailles, where a prostitute, hired for her resemblance to the Sovereign, convinced de Rohan he had gained Marie Antoinette's endorsement and love.

Now that the Cardinal was under her influence, de la Motte produced other fake letters where the Queen expressed the desire to buy the necklace without interfering with the royal finances. Consequently, she hoped the Cardinal could lend her the money as a personal favor. The Cardinal believed in the letters' authenticity and agreed to pay for the necklace in installments. However, the prelate failed to fulfill the agreement, and Auguste Boehmer complained to the Queen. The scandal unfolded.

Cardinal de Rohan, de la Motte, the prostitute who acted as the Queen, and Rétaux de Villette, who forged the royal correspondence, were arrested. Also, the infamous Italian adventurer and occultist Giuseppe Balsamo, self-proclaimed Count di Cagliostro, was incarcerated and held in the Bastille. After being jailed for nine months, Cagliostro was finally acquitted, as no evidence had been found; nevertheless, he was banished from France, probably due to his dubious reputation. Cardinal de Rohan was also acquitted during the trial but was deprived of his office and exiled to his abbey, while Jeanne de la Motte and Rétaux de Villette were both found guilty. However, only Jeanne was sentenced as she was still in custody.

Despite the result of the trial, the public sympathized with the swindler, and, ultimately, in the eyes of society, the target of the controversy became Marie Antoinette and the Bourbon monarchy. The Queen, who had no knowledge of the plot and had never requested the necklace, was unjustly accused of extravagant spending and contributing to the nation's financial woes. The affair exacerbated the growing resentment against the monarchy and added fuel to the already tense social and political atmosphere: according to historians, it was an additional, non-return step toward the French Revolution.

The author will present how the arrests and the trial following the "necklace affair" have been negatively influenced by prejudices and social stereotypes and the final historical outcomes have been driven by public perception. This historical account demonstrates the difficulties forensic investigators and judicial authorities could encounter when pursuing the truth to guarantee justice for all.

Historical Case; Injustice; Fraud

LW4 Man vs. 'Gator—The Forensic Story vs. The Media Story

Walter F. Zoller, DMD*, Titusville, FL

Learning Overview: After attending this presentation, attendees will have a greater understanding of the alligators' "place" in Florida, with a brief review of alligator-human encounters. One such encounter will be analyzed in detail. Attendees will gain knowledge of the media/press coverage of this event, its forensic investigation and how the media "view" may influence the general public's perception of occurrences such as this.

Impact Statement: This presentation will impact the forensic science community by highlighting a tragic and violent death and the differences with respect to the incidents documentation by the forensic professionals involved in the investigative process vs. how the event was reported by the media/press.

Florida, prior to any human occupation, was, and still is, the home of two very famous water inhabitants. These creatures are perceived quite differently by the public at large. Yet, they live together within the Florida inland waterways in harmony. These are the manatee, known as the gentle "sea cow" and the alligator, considered by many as the fierce predator. Florida residents and visitors alike are warned to keep their distance from both native animals. Yet, many do not listen to these warnings. Most suffer no consequences. Unfortunately, not all.

One such case occurred in Blue Springs State Park. This beautiful sanctuary is approximately 32 miles southwest of Daytona Beach. What draws snorkelers and divers to this retreat is the presence of its underwater caves, combined with many natural springs; meaning the water temperature remains stable nearly all year round. The manatees require warm water to thrive. Thus, this refuge is an ideal fall and winter home for these water dwellers as well as for the park visitors that view them. All year round, irrespective of the water temperature, Blue Springs State Park is also the home of the Florida alligator (gator).

It was a beautiful day, Monday, October 19, 2015. Warnings were issued to swimmers as to the potential presence of gators in the waters that day. The swimmers, snorkelers, and divers arrived, nonetheless. Finally, following reports of definitive gator activity near the swimming/diving areas, park officials began limiting access to the springs at about noon. James J. Okkerse was an experienced diver and was very comfortable in the Blue Springs underwater environment. He did not heed the initial warnings. Mr. Okkerse paid the ultimate price for this decision, not anticipating his violent death that was to follow.

A presentation of the press/media coverage of this event will be reviewed in detail. This will be intermixed with the forensic investigation. That is, the joint efforts of the three forensic odontologists involved, as well law enforcement and the medical examiner team. This probe included a detailed necropsy/dismemberment of the suspect gator. The final forensic findings, both from the lead forensic odontologist and medical examiner, will be presented and dissected, in comparison to the press reports of the same.

An obvious cautionary warning will follow the conclusion of this comparison.

Violent Deaths; Underwater; Dismemberment

LW5   The First Full-Service Criminalistics Laboratory: Scientific Detection Laboratory, Chicago, IL

Peter Striupaitis, MS*, PS Forensic Consultants, Northville, MI

Learning Overview: After attending this presentation, attendees will have learned that Dr. Calvin H. Goddard, MD, a fireman examiner, identified the fired evidence from the 1929 St. Valentine's Day Massacre (at the S-M-C Cartage Co., 2122 N. Clark Street, Chicago, IL) and subsequently opened the Scientific Crime Detection Lab (SCDL), which arguably became the first, full-service, criminalistics laboratory in Chicago, and trained law enforcement personnel. Dr. Goddard's impact on the criminalistics was considerable.

Impact Statement: This presentation will impact the forensic science community by informing attendees of the historical facts of criminalistics/forensic laboratories in the United States.

On February 14, 1929, in Chicago, IL, seven members of the Bugs Moran's gang were killed by gun fire. Although the notorious Al Capone was the primary suspect, both he and his group all had alibis as to their whereabouts. A blue ribbon of individuals was sworn in; they were well-to-do businessmen who hired an expert in forensic "ballistics" from New York City to come to their city to solve these killings. Dr. Calvin H. Goddard, MD, came at their request.

Dr. Goddard had a medical background but was also keenly interested in firearms and fired evidence. He had spent years of research documenting, measuring, and developing instrumentation to determine via comparison micrography and other methods and techniques whether those specific firearm(s) had fired the specimens recovered from the crime scene. Goddard was able to determine that there were 125 discharged .45 auto caliber and 12-gauge discharged shotshells. Dr. Goddard concluded there were separate shell casings from two Thompson submachine guns and one shotgun.

In December of that same year in Michigan, there was an auto accident, and the driver was killed. When a police officer approached the suspect and his vehicle, Fred Burke shot and killed the officer. Burke fled, only to later disable his vehicle. A search of the abandoned, suspect vehicle led to Frederick Dane (Burke's alias), which led to the recovery of two Thompson submachine guns and one shotgun. The guns were brought to Goddard who positively identified the guns as being the ones used in the Massacre. Impressed by his work, the captains of industry, who also were the blue-ribbon jury members, requested that Dr. Goddard stay and establish a crime detection laboratory. He obliged them and the laboratory became the Scientific Crime Detection Laboratory at Northwestern University, Chicago, IL. The laboratory was composed of several sections and worked thousands of cases during its tenure. It also provided training to hundreds of police personnel, including the Federal Bureau of Investigation (FBI). Subsequently, it was sold/transferred to the Chicago Police Department in 1938. It was the first, to my knowledge, full-service criminalistics laboratory in the United States.

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[1.] Fingerprinting Bullets: The Expert Witness, by Wesley W. Stout from *The Saturday Evening Post*, June 13,'25, pp. 66, 67,102-04, 197.
[2.] Chicago Police Department, *Firearms section records ledger:1975-1979*.
[3.] Dr. Calvin Hooker, "Father of Ballistics" pioneered system that traces bullets to guns, by Frederick N. Rasmussen, T*he Baltimore Sun*, February 12, 2011.

Forensic Science; Ballistics; Firearms

LW6   Will Hatred Never Die? The Murder of Bishop William Alexander Guerry

J.C.U. Downs, MD*, ForensX, LLC, Johns Island, SC

Learning Overview: After attending this presentation, attendees will be informed about the life and work of W.A. Guerry and the Social Justice movement, his efforts at continuing integration of his church diocese, and his tragic martyrdom.

Impact Statement: This presentation will impact the forensic sciences community by familiarizing the audience with the ministry of Bishop Guerry at the hands of one of his priests and the similarity of this case to the Mother Emanuel murders 87 years later, both at the hands of avowed racists.

Progress, far from consisting in change, depends on retentiveness…. [W]hen experience is not retained, as among savages, infancy is perpetual. – George Santayana[1]

It was a warm day on June 17, 2015, in Charleston, South Carolina, home of the secession that led to the United States Civil War in April 1861, when a lone White male entered the church grounds of the Mother Emanuel AME Church and proceeded to engage with the people there, including the pastor. Without warning, this man, a troubled 20-year-old and an avowed racist and segregationist, brandished a handgun and shot and killed nine parishioners and injured a tenth victim at an evening prayer meeting. The murdered included Senior Pastor and South Carolina State Senator Clementa C. Pinckney. That this unspeakably evil act occurred is awful. That a similar act occurred in the same city 87 years earlier is largely forgotten.

On June 5, 1928, the bishop of the Episcopal Diocese of South Carolina, William Alexander Guerry, was in his office at the mother church of the Diocese, St. Philip's Church, a mere eight blocks from Mother Emanuel. A lone gunman joined Guerry under the pretext of discussing his retirement. Suddenly, J.H. Woodward produced a firearm and shot the bishop, then himself. The shooter died immediately, while Guerry survived for four days. The shooter was opposed to the bishop's support for the Social Gospel, a pre-World War I Protestant religious movement aimed at ending injustice and racial inequity, a movement that was very active in Denver, Colorado. Guerry specifically sought integration in the Church by installing an African American suffragan bishop to keep the denomination integrated, which Woodward saw as damaging to White supremacy. Despite his murderer's motivation, Bishop Guerry lived his faith to the end, as his reported last words were, "Forgive him, Father, he knew not what he did." As did the Mother Emanuel shooting, Guerry's murder made national headlines—not surprising, since the shooter was one of Bishop Guerry's own diocesan priests. A century later, the act has barely been remembered, even in his own church.

The senseless murder of Guerry left him a martyr to the cause of diversity, equity, and inclusion. Less than a century later, at very nearly the same location, another disturbed lone gunman attempted impose his racist views on the world. One is reminded of Santayana's admonition: *Those who cannot remember the past are condemned to repeat it.[1]*

Reference:
1. Frank Daniels III. Teachable moments: Santayana brought critical eye to field of philosophy. The Tennessean. 15 December 2013. https://www.tennessean.com/story/opinion/columnists/teachable-moments/2013/12/16/teachable-moments-santayana-brought-critical-eye-to-field-of-philosophy/1579552/.

Social Justice; Handguns; Religion

LW7   The Unknown Child on the Titanic: Identified! Identified?

Colleen M. Fitzpatrick*, Identifinders International LLC, Fountain Valley, CA

Learning Overview: After attending this presentation, attendees will have learned how forensic analysis, in conjunction with historical research, can be used to discover a cohesive narrative about historical events in spite of conflicting eyewitness accounts and overwhelming hearsay.

Impact Statement: This presentation will impact the forensic science community by demonstrating the synergy that exists between forensic and historical investigation. This presentation will demonstrate how a variety of scientific disciplines, including meteorology, accident investigation, and DNA identification, can be used to create a coherent picture of historical events.

On Sunday, April 14, 1912, the SS *Titanic*, on her maiden voyage to America with 2,207 souls onboard, struck an iceberg and sank in less than three hours. Only 705 passengers survived. Of the 328 bodies recovered by the salvage operation sent from Halifax, only one was that of a child. All that forensic identification of that time could indicate was that the baby was male, about two years old, and a third-class passenger. He was buried along with so many other victims in Fairview Lawn Cemetery in Halifax; his grave has become a memorial for the dozens of children who lost their lives that night.

The identity of the Unknown Child on the *Titanic* was known only to God for nearly a century. That changed in 2002, when Dr. Alan Ruffman and Dr. Ryan Parr announced that they had identified the remains of the Unknown Child as Eino Panula, a 13-month-old Finnish baby who had perished in the accident along with his mother and four older brothers.

But was this identification correct?

In 2007, a controversy arose because the shoes of the child, held in the Maritime Museum off the Atlantic in Halifax, were too large for a 13-month-old baby. It was then revealed that the results of the DNA test had been tied between Eino and 19-month-old Sidney Leslie Goodwin, so that the final identification had been based on estimates of the maturity of the three tiny teeth found in the grave. Could that estimate be wrong?

This presentation will discuss how forensic tools of the 21st century were applied to discover the true nature of the 1912 *Titanic* disaster, focusing on how the controversy over the identity of the baby who died in 1912 was finally resolved so the Unknown Child on the *Titanic* is unknown no longer.

DNA; Disaster; Accident

Y1 The Forensic Acquisition and Inspection of Aftermarket Vehicle Infotainment Systems

Jett Brandes, MD*, Marshall University, Elgin, IL; Andrew Clark, Marshall University, Huntington, WV; Robert Boggs, Associates Police Science, West Virginia State Police, Huntington, WV

Learning Overview: After attending this presentation, attendees will better understand the forensic value of information that can be obtained from an in-vehicle infotainment system. This presentation will also provide a basic approach to obtain the data through a non-destructive processes such as In-System Programming (ISP) and Chip-off.

Impact Statement: This presentation will impact the forensic science community by offering a better insight into the potential forensic evidence that can be contained, acquired, and inspected from popular aftermarket infotainment systems commonly found in vehicles.

Many modern vehicles contain infotainment systems that provide users with access to features such as Apple® CarPlay, Android® Auto, Bluetooth® audio, and radio, among others. These systems have been standard in the car industry since 2016 and are available across all manufacturers. For vehicles manufactured before 2016 or those that do not come equipped with infotainment systems, aftermarket options are available, allowing individuals to purchase and replace their Original Equipment Manufacturer (OEM) stereo. Popular brands offering aftermarket infotainment systems include Sony®, Pioneer®, and Google®. These infotainment systems are designed to pair with an individual's phone, facilitating data transfer between the mobile device and the vehicle through either a wired or wireless connection.

Previous research on this topic has uncovered that OEM infotainment systems from manufacturers store specific data in both the mobile device and the infotainment system's internal storage, which include flash memory and Universal Flash Storage (UFS), embedded Multimedia Cards (eMMC), or traditional hard drives.[1-3] The mobile device retains information such as APP in list use, Bluetooth® MAC (BT MAC) address of Paired Vehicle, Name of Paired Vehicle, Last Used Time, Projection Activation Time, and Disconnection Time. On the other hand, the infotainment system's internal storage contains data such as Bluetooth® Paired Device List, Phone Number of the Bluetooth® Paired Device, Pairing Time of the Bluetooth® Paired Device, Vehicle Location, **International Mobile Equipment Identity** (IMEI), and International Mobile Subscriber Identity (IMSI). This data becomes valuable in establishing the relationship between an individual and a vehicle as it serves as a link connecting the individual's mobile device to the infotainment system.

This presentation focuses on popular aftermarket infotainment systems, as mentioned earlier. While it has become a new standard for the car industry, many vehicles lacking "upgrade packages" and older models do not come equipped with infotainment systems featuring Android® Auto or Apple® CarPlay. In such cases, individuals often opt to purchase these aftermarket options. These systems offer various apps connected to an individual's mobile device for entertainment purposes, including music apps, contacts, and text messaging apps. Android® Auto also provides a developer option that allows the addition of extra text messaging apps like Skype, Whatsapp, and Facebook® Messenger. Additionally, some apps have the capability to record GPS data, such as Google® Maps, Apple® Maps, and Waze.

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Digital Evidence; Forensic Imaging; Motor Vehicle

Y2   The Impact of Zoom on Smart Phone Camera Identification

Gavin Norton*, The Center for Statistics and Applications in Forensic Evidence, Knoxville, IA; Stephanie Reinders, PhD, Research Scientist, Center for Statistics and Applications in Forensic Evidence, Ames, IA

Learning Overview: The goal of this presentation is to educate attendees about whether the zoom at which a photo was taken influences the reliability of the source camera identification.

Impact Statement: Currently, there is little research exploring whether a photo's zoom will impact the reliability of camera identification. This presentation will impact the forensic science community by gathering photos from telephoto cameras on smart phones to analyze the impact of zoom. These photos will be released publicly without charge for other researchers to use in their studies.

As with all feature-based forensics, camera identification includes a photo from an unknown source (like a latent print) and a known camera (like a suspect's fingerprint). Forensic scientists then attempt to determine if a photo came from a specific camera based on the similarities between the photo and the camera's "fingerprint." A study by Lukas, Fridrich, and Goljan found that the noise of a photo can be used to identify the camera that took it.[1] A noise residual is made from a photo with an unknown source and compared with a known camera's "fingerprint." The fingerprint is an average of the noise residuals from reference photos taken by the known camera to generalize the camera's typical noise residual. Miroslav, Fridrich, and Filler found that the Peak-to-Correlation Energy (PCE) score can be used, with high accuracy, to compare and classify the image from the unknown source and the camera fingerprint from the known camera to determine if they are from the same camera or different cameras.[2]

As camera technology improves and almost everyone carries a smart phone, the relevance of smart phone camera identification also grows. This study seeks to determine if zooming in while taking a photo (not editing or enhancing the photo after it's taken) affects the reliability of the comparison. The Center for Statistics and Application in Forensic Evidence (CSAFE) at Iowa State University purchased 60 smart phones for camera identification studies. This project evaluates the impact of zooming on ten iPhone® 14 Pros—a phone with a dedicated telephoto camera for zoomed-in photos. Data collection involves taking 1,000 photos for each iPhone®, 500 of which are of a blank white wall at different zooms, and 500 of subjects in a natural, everyday setting. The natural photos are taken in sequences of five, at 3x, 6x, 9x, 12x, and 15x zoom. This image database will help determine the impact of zoom on identification and will be released for free to the public for any researcher to use.

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Imaging; Camera; Digital Evidence

Y3   **Known Hash Filtering: An Efficient Way to Eliminate Irrelevant Files and Show Files of Interest in Digital Examinations**

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Learning Overview: The presentation will highlight the steps needed to perform known hash filtering with validated digital forensic tools utilizing known hash sets, such as the Reference Data Sets from the National Software Reference Library (NSRL), Project VIC, and custom hash sets. After attending this presentation, attendees will have a better understanding of how known hash filtering can be used to ignore irrelevant flies and successfully target files of interest, thus reducing the total number of files to be examined in casework.

Impact Statement: This presentation will impact the forensic science community as the implementation of known hash filtering can be used to efficiently eliminate known irrelevant files and locate known files of interest, essentially decreasing examination time and the overall amount of evidence digital forensic examiners parse through.

Digital forensics is defined as a branch of forensic science that focuses on identifying, acquiring, processing, analyzing, and reporting data stored electronically.[1] This includes everything from computers, tablets, phones, vehicles, and more, resulting in extensive amounts of data for forensic examiners to parse through. While the already-large volume of digital evidence continues to increase, there are ways to reduce the number of files needing to be examined. Some of these ways include techniques such as filtering out known, irrelevant files and displaying files of interest. These actions can be done through the use of known hash set filtering. Hash sets are collections of data that are compiled of hash values, or unique digital fingerprints, that match known files. Such hash sets include Reference Data Sets of the National Software Reference Library (NSRL) that are compiled from traceable, default files, and Project VIC, which are known contraband files from prior investigations involving child exploitation. By utilizing validated examination tools with the use of known hash sets, examiners are, hypothetically, able to effectively filter known files to eliminate the time needed to parse through irrelevant data or the time needed to search for known illegal content.

Therefore, this project has three goals. The first goal was to determine if different software tools were able to effectively filter known computer files using the NSRL hash set and to create protocols to produce repeatable results for each software program utilized in the laboratory.[2] The second was to import the Project VIC hash set into the programs and create custom hash sets to simulate the application of Project VIC to display files of interest. The last goal was to apply the methods of hash filtering to mobile devices. In order to complete these objectives, a total of six forensic tools, five for general examination and one specialized for image search, were employed and tested using five test cases. Subsequently, it was determined that five of the six programs could ingest and apply the known hash set to five test cases to hide known files from the total number of files parsed by the software. Additionally, four of the six programs were able to import Project VIC and custom hash sets, successfully filtering the files of interest. This process can also be applied to mobile devices. Hash values of known files were subject to change when saved on Apple® devices versus Android® devices, which did not change when comparing the known hash values to the files saved to the device.

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NSRL; Known Hash Set Filtering; Digital Forensics

Y4   Producing Datasets: Capturing Images on Multi-Camera Smart Phones for Source Camera Identification

Megan McGuire*, Center for Statistics & Applications in Forensic Evidence, Ames, IA; Stephanie Reinders, PhD, Center for Statistics and Applications in Forensic Evidence, Ames, IA

Learning Overview: This presentation shows how images on multi-camera smart phones were collected as reference images for source camera identification research. After attending this presentation, attendees will better understand the methods used to produce this dataset and the reasoning behind those methods.

Impact Statement: This presentation will impact the forensic science community by informing attendees about an image database for source camera identification and other research topics. This database will show images from all cameras available on recent models of smart phones, and it provides the public with access to data that is more representative of actual casework. This image database will be open and free for public use sometime next year.

The lack of data available in the forensic science community proves to be an ongoing problem. This project's main objective was to create a dataset with a sizeable number of images from all available cameras on multi-camera smart phones that will be publicly available and representative of images potentially found on a person of interest's smart phone.

The digital evidence team at the Center for Statistics and Applications in Forensic Evidence (CSAFE) worked with 60 recent models of multi-camera smart phones to create this dataset. Among the 60 smartphones were ten Samsung™ Galaxy Note 10s, ten Samsung™ Galaxy s20s, ten Samsung™ Galaxy s21s, ten iPhone® 11 Pros, ten iPhone® 12 Pros, and ten iPhone® 14 Pros. Each of these 60 smart phones contains four individual cameras: the front selfie camera, the rear telephoto camera; the rear wide-angle camera; and the rear ultra-wide-angle camera. Other existing datasets, including VISION and StegoAppDB, also provide substantial amounts of data, but do not include images from each available camera, and only contain images taken using the main rear camera.[1,2]

For each of the four cameras in each phone, the team at CSAFE captured 100 natural scene images and 100 flatfield images. Natural scene images contain indoor and outdoor scenes such as plants, furniture, and architecture but exclude all personally identifiable information such as people, brands, and logos. The flatfield images are photos of evenly lit blank white walls. These images allow each photosite in a camera's sensor array to be given similar information while the natural scene images provide examples of an image that could potentially be found on a person of interest's phone. Each photo was taken with specific camera settings to ensure consistency throughout the images. The most important settings include no use of flash, using auto-exposure to capture images, keeping location tags turned on, and capturing the images in JPEG format.

While conducting data collection, it was found that the Samsung™ Galaxy Note 10s had two separate sensors in the rear wide-angle camera, one used to capture images in dim light and the other to capture images in bright light. The dim light sensor has an aperture of 1.5 and the bright light sensor has an aperture of 2.4. This finding resulted in the CSAFE team capturing an extra 100 images for the rear wide-angle camera for all ten Samsung™ Galaxy Note 10s to ensure that the dataset included 100 images for each sensor. This dataset will be available and free for public use at some point this year and will be used by research teams at CSAFE to develop and test source camera identification methods to further forensic science research.

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Digital Evidence; Camera; Imaging

Y5 The Quantification of Alcohols and Acetaldehyde in Hand Sanitizers Using Headspace/Gas Chromatography/Mass Spectrometry

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Learning Overview: Attendees of this presentation will gain valuable insights into the safety and composition of hand sanitizers. This presentation will explore the presence of toxic chemicals with a focus on how these products align with Food and Drug Administration (FDA) requirements for safety. Attendees will access the advantages and disadvantages of using hand sanitizers in various storage conditions. This presentation will provide an analytical technique involving Headspace/Gas Chromatography/Mass Spectrometry (HS/GC/MS). Attendees will learn how to identify and analyze the chemical compositions of samples using this advanced method. This knowledge will empower them to assess product safety and quality independently.

Impact Statement: This presentation will impact the forensic science community as forensic scientists will benefit from improved analytical skills, particularly in utilizing advanced techniques like HS/GC/MS for chemical analysis. This expertise can be directly applied in forensic investigations involving a wide range of substances beyond hand sanitizers, particularly in toxicology and seized drug units. Forensic experts will be better equipped to evaluate the composition and safety of products submitted as evidence, ensuring that they meet regulatory standards and providing more accurate information to legal proceedings. The research methodology and technique discussed can be adapted and applied to forensic investigations, expanding the research capabilities, and developing new methods for analyzing unknown synthesized chemicals.

Since the declaration of COVID-19 as a global public health emergency, hand sanitizers have become a crucial commodity for rapid disinfection in public spaces, primarily relying on ethanol and isopropanol as their key chemical components. Ethanol is typically manufactured through a fermentation process, which entails the conversion of sugars into ethanol and carbon dioxide, facilitated by the action of yeast or bacteria. Within this production process, it's possible for unintended byproducts to emerge, including organic acids, aldehydes, and higher alcohols such as n-propanol.[1] Furthermore, there exists a potential risk of microbial contamination during either the fermentation or distillation phases of ethanol production, which can result in a blend of ethanol and methanol.[2] Additionally, acetaldehyde can be generated during the oxidation of ethanol by yeast and acetic acid bacteria within the ethanol fermentation process.[3] These impurities have the potential to become mixed with ethanol and water, subsequently finding their way into hand sanitizer formulations.

Due to the direct application of hand sanitizers onto the skin without rinsing with water, concerns have arisen regarding potential health issues with prolonged exposure, driven by the skin's adaptability to these ingredients. In response to an increasing number of reports documenting a wide range of adverse events linked to the use of alcohol-based hand sanitizers, the FDA issued a cautionary advisory.[4] In 2020, the FDA also established specific concentration requirements for each ingredient in hand sanitizers.[5] Notably, these products have been found to contain impurities such as methanol, n-propanol, and acetaldehyde, all of which are known for their toxicity and potential to cause severe harm.

As a result, this research project was carried out to detect the presence of impurities in commercial hand sanitizers. The study aim is to quantitatively identify methanol, n-propanol, and acetaldehyde and determine whether their quantities meet the limit requirements approved by FDA. An HS/GC/MS method was developed and validated to detect and investigate the concentration of methanol, ethanol, isopropanol, n-propanol, and acetaldehyde in eight samples of different gel-typed hand sanitizers. All samples had the methanol and n-propanol limit under control, which the highest samples retained 0.0218mg/mL and 0.152mg/mL, respectively. Two out of eight samples exceeded the FDA limit for acetaldehyde concentration (average ACH of 0.08605mg/mL > 0.005mg/mL of FDA). Furthermore, a correlation exists between the levels of ethanol and acetaldehyde and the temperature, which will be studied in our future study.

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HS/GC/MS; Drug Analysis; Alcohol

Y6 **The Development and Validation of a Biotage® Extrahera™ Automated Extraction Method of Opioids and Their Metabolites in Human Urine Using LC/MS/MS**

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Learning Overview: After attending this presentation, attendees will be aware that the opioid epidemic in the United States describes the increased prescription of opioid medications, which has led to the vast misuse of both prescription and illicit opioids. As urine samples arrive at a toxicology laboratory to be tested, opioids and other drugs of interest must be extracted from the matrix in order to identify and quantitate the compounds present. To make sampling and extraction easier, cleaner, and more efficient, the Biotage® Extrahera™ (BE) automated extraction apparatus electronically aliquots and extracts the drugs of interest from the given matrices. The automated Solid-Phase Extraction (SPE) instrumentation and quantitative analysis of 21 opioids and their metabolites can be applied to forensic and clinical toxicologists or medical examiners in either autopsy cases, pre-employment drug tests, pain-management therapy programs, or drug toxicity cases.

Impact Statement: Typically, toxicology laboratories manually extract opioids and other compounds from human urine samples, which is described to cause frequent challenges for the analyst and the analytical instrumentation. With an automated extraction apparatus, this validated method can help high-throughput laboratories with the application of a relatively quick and clean extraction of the targeted opioids and their metabolites. This presentation will impact the forensic science community by informing attendees that the quantitative parameters obtained from this study, and the method overall, can be applied to forensic and clinical toxicologists/ analysts/method developers, and other laboratory personnel.

In this study, a BE automated SPE instrument is utilized to easily and cleanly extract 21 opioids and their commonly detected metabolites from human urine samples. A 96-well cation exchange SPE plate is used to remove the analytes from pre-treated and hydrolyzed urine samples, followed by two sequential wash steps with 4% phosphoric acid and 50% (v/v) methanol. Elution of the analytes from the sorbent is completed with additions of 78:20:2 dichloromethane: isopropanol: ammonium hydroxide and 78:20:2 dichloromethane: methanol: ammonium hydroxide and analyzed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) on an AB SCIEX™ Triple Quad™ 4500MD with Multiple Reaction Monitoring (MRM) transitions. A five-concentration linear calibration model spanning 1ng/mL to 2,500ng/mL as dependent on the analyte, is used to evaluate three control levels, and various fortified and patient matrix samples. The developed method is validated to the American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 036 required parameters for quantitative analysis including bias and precision, calibration model, carryover, common drug interference, ionization suppression/enhancement, limit of detection, limit of quantitation, dilution integrity, and processed sample stability.[1]

Bias studies of three concentration levels of fortified matrix sample over five runs produced less than 10% measurement error for each analyte, while within-run and between-run precision produced less than 20%. All analytes presented a linear correlation across their established working range and random distribution of residuals with coefficients ≥ 0.98. Carryover was not detected above any analyte's Limit Of Detection (LOD), and only buprenorphine, norbuprenorphine and acetylfentanyl showed interference or ion suppression with compounds in the interference mixture, where further analysis would need to be conducted. Fortified matrix samples possessing concentrations above the working linear range were verified with 1:2 and 1:20 dilutions of blank matrix, and low- and high-level analyte concentrations were shown stable up to three-days for all analytes within ± 10% accuracy. The obtained results can be of use for forensic and clinical toxicology laboratories and meet the fit-for-purpose extraction of these opioids from urine samples under this standard.

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Toxicology; Opioids; Solid-Phase Extraction

Y7 The Death of an Accused Person in a Court After the Announcement of the Verdict

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Learning Overview: The goal of this presentation is to highlight the importance of conducting a thorough forensic autopsy to determine the true cause of a prisoner's death, particularly in cases where the cause of death is uncertain or appears suspicious. In this case, the forensic autopsy determined the exact cause of the woman's death, which prevented erroneous conclusions and ensured that the woman's family received clear answers about the circumstances of her death.

Impact Statement: This presentation will impact the forensic science community by informing attendees that the forensic autopsy is essential to ensure that the rights of all individuals are respected and to identify the causes of deaths. In this case, the autopsy revealed that the woman had ingested a toxic substance, leading to an awareness of the need to protect prisoners from exposure to potentially dangerous substances.

When an accused is found guilty by a judge and sent to prison, it is important to ensure that their health is adequately taken care of. However, in some cases, tragic events may occur, such as the death of the accused shortly after the verdict. In such a case, a forensic autopsy may be essential to determine the exact cause of the accused's death.[1-10]

In a case study presentation, we examined the history of a woman accused of drug-related crimes who died shortly after being sentenced to prison. Initial information suggested that the death was due to a heart attack, but the forensic autopsy revealed that the woman had actually ingested Paraphenylenediamine (PPD), a toxic substance used in hair dyes. PPD is a dangerous chemical that can cause severe allergic reactions in some people resulting in kidney, liver, and nerve damage, as well as death. In this case, the forensic autopsy identified PPD as the cause of the woman's death.

This case highlights the importance of conducting a thorough forensic autopsy to determine the true cause of a prisoner's death, particularly in cases where the cause of death is uncertain or appears suspicious. In this case, the forensic autopsy determined the exact cause of the woman's death, which prevented erroneous conclusions and ensured that the woman's family received clear answers about the circumstances of her death.

The announcement of the verdict constitutes a critical moment for the accused and is a period when the risk of a suicidal crisis is very high; for this reason, in special situations measures must be taken.

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10. Autopsy after the death of an accused in court: a question of transparency and impartiality, *La Revue du Barreau*, vol. 69, no. 3, 2021.

Autopsy; Cause of Death; Toxicity

Y8   The Use of Novel Psychoactive Substances (NPS) in Italy: A Comprehensive Meta-Analysis

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Learning Overview: The goal of this presentation is to present a comprehensive meta-analysis on data relating to the use of NPS in Italian society in the period between the years 2013–2023 (including the COVID era) with a special focus on four different aspects: drug seizure data, survey-based studies, toxicological studies, and wastewater-based epidemiology. Official data have been collected from Scopus®, PubMed®, Science Direct®, Google® Scholar, the United Nations Office on Drugs and Crime (UNODC), and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) websites and statistically analyzed using JMP Pro 16.2.0. With this investigation, a deeper knowledge about the use of new drugs in Italy will be reached.

Impact Statement: NPS are new compounds available for consumption that have not been extensively researched. Each NPS has relatively unknown adverse effects, habit-forming potential, withdrawal symptoms, and a lethal dose. Their misuse presents a huge challenge to the health care system and may silently contribute to untold fatalities in emergency settings.[2] These substances, chemically engineered to mimic the effects of illicit drugs, present unique challenges to society due to their constantly evolving nature and the difficulties associated with their regulation and control. This presentation will impact the forensic science community by providing an in-depth knowledge on new formulations and trends that could be of particular interest to forensic toxicologists, drug analysts, and legal experts, and it could help to update the legislative framework related to NPS integrating the list of substances to control.

NPS seizures in Italy have changed over time, with some notable fluctuations and trends. During 2013–15, a total of 162 NPS were observed, with 3-Methylmethcathinone (3-MMC), 4-Methylethcathinone (4-MEC), Methylenedioxypyrvalerone (MDPV), Pyrolidinovaleropgenone (α-PVP), and alpha-Pyrrolidinohexiophenone (α-PHP) being the most common.[3] Notably, there was a staggering 200% increase in NPS seizures in Italy during the COVID-19 lockdown. In 2020, 479 postal parcels were seized, with NPS identified in 92.6% of these samples. Surveys revealed that 3-MMC, carfentanil, ocfentanil, MPF-47700, JWH-250, and α-PVP are the most abused NPS in Italy. The data showed that 8.21% of individuals have tried opioids at least once. According to the World Drug Report 2023, a total of 16 deaths have been reported due to NPS overdose in Italy. Wastewater-Based Epidemiology (WBE) was employed to monitor NPS use throughout Italy, with synthetic cathinones and tryptamines, particularly methcathinone, being detected in all major cities.

From this analysis, it is evident that in Italy, as throughout the world, NPS abuse represents a serious threat. This study reports the most abused NPS among Italians: some NPS are marketed as legal alternatives to traditional medicines, exploiting legal loopholes and online platforms for distribution. Addressing the problem of NPS abuse requires a coordinated effort by policymakers, health care professionals, and researchers to mitigate their harmful consequences.

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Novel Psychoactive Substances; Drug Abuse; Seized Drugs

Y9   Monitoring the New England Drug Supply Through Novel Drug Checking Methods

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Learning Overview: After attending this presentation, attendees will have analyzed trends in recreational drug markets in the northeastern United States and discussed the benefits of laboratory-based confirmatory testing to community drug checking programs and public health agencies drug surveillance programs.

Impact Statement: This presentation will impact the forensic science community by demonstrating how surveillance testing of drug materials can provide real-time insights into regional drug markets, informing testing practices in forensic drug testing laboratories.

The United States is still suffering through an epidemic of opioid use that has resulted in the deaths of over one million people. Most opioid-related deaths are linked to the use of illicitly manufactured fentanyl. The recent proliferation of xylazine and other sedatives as adulterants in the recreational drug supply has become an area of concern as reports of their contribution to combined drug toxicity and death have emerged. The appearance of previously unreported drugs as novel adulterants and Novel Psychoactive Substances (NPS) in the drug supply create the need for effective messaging about the health implications and possible dangers associated with recreational drug use.

The Center for Forensic Science Research and Education (CFSRE) through its NPS Discovery program has partnered with the Opioid Policy Research Collaborative (OPRC) at Brandeis University to perform qualitative and quantitative analysis of drug samples submitted as part of the OPRC's StreetCheck program. StreetCheck is a community-based drug checking initiative where individuals or organizations can submit samples for testing and receive analytical results to better inform decisions about drug use and drug use habits. Trend analysis and descriptive statistics of the data are used to inform local stakeholders on changes within their drug supply. Samples are collected from several states across the Northeast, including Massachusetts, Connecticut, Rhode Island, and Vermont.

Samples began being collected and analyzed in December of 2022. Initial qualitative analysis is performed by Gas Chromatography/Mass Spectrometry (GC/MS). Results were confirmed by Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). To date, qualitative analysis has been performed on 571 samples. The majority of samples (48%) contained fentanyl as the primary drug, followed by cocaine (29%), methamphetamine (6.5%), and heroin (4%). One advantage of a community-based approach to drug monitoring is that the collaborating agency may receive contextual information about the sample in question, which provides valuable insights. For example, 348 of the 571 samples were reported to have been consumed, resulting in 33 reported overdoses, 10 of which were fatal. Being able to quickly associate chemical findings to local public health scenarios allows harm reduction agencies to create plans to mitigate the negative impacts a drug product may cause.

If sufficient sample remained following qualitative analysis, quantitative analysis was performed by GC/MS to determine the concentrations of nine drugs, including cocaine, fentanyl, and xylazine. Quantitative analysis has been performed on 183 samples to date, of which 84 samples were found to contain fentanyl as the primary drug. The average purity of fentanyl by mass was 8.0% ($\pm$7.6%). Notably, 37 of the fentanyl-dominant samples contained xylazine adulterants, comprising 8.7% ($\pm$11.0%) of the sample by mass. In stimulant samples, the average purity of cocaine and methamphetamine by mass was 71.3% ($\pm$24.3%) and 37.5% ($\pm$28.0%), respectively.

Local public health and safety organizations need to be apprised of emerging drug trends in order to prepare for potential overdose outbreaks. The combination of NPS Discovery's comprehensive drug analysis workflows with OPRC's StreetCheck nimble data analysis and communication frameworks has enabled the pinpointing of particularly vulnerable regions, with the goal of potentially predicting and preventing overdose clusters.

Drug Checking; Novel Synthetic Opioids; Overdose

Y10   An Evaluation of Postmortem Redistribution of Δ9-Tetrahydrocannabinol (THC) and 11-Nor-9-Carboxy-THC (THC-COOH)

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Learning Overview: After attending this presentation, attendees will be informed that a review of 49 postmortem cases from Ontario, Canada, showed evidence of low to moderate Postmortem Redistribution (PMR).

Impact Statement: This presentation will impact the forensic science community by contributing to the understanding of the effects of PMR on the interpretation of postmortem drug concentrations.

PMR is a phenomenon that can complicate toxicological interpretation of drug concentrations in postmortem samples. This process results in a change in drug concentration following death, can occur at various anatomic sites, and can be influenced by many physicochemical properties of a drug such as lipophilicity, pKa, volume of distribution, and protein binding affinity. Many cannabinoids, including Δ9-THC have lipophilic properties and high pKa values, and therefore may be susceptible to PMR.

In this study, 49 postmortem cases which had confirmed quantitative results for THC and THC-COOH in both central blood (i.e., heart blood) and peripheral blood (i.e., femoral blood) were reviewed. The central blood to peripheral blood concentration ratio (C:P) for THC ranged from 0.14–2.8 with a mean of 0.72 (Standard Deviation [SD]: 0.54, median: 0.54) and 13 of the 49 total samples had a C:P ≥1. With respect to THC-COOH, the C:P ratio ranged from 0.03–5.5 with a mean of 1.18 (SD: 0.87, median: 1.11), and 24 of 49 cases had a C:P ratio greater than or equal to 1. The cases were further divided into categories in which samples contained other drug findings and those without. Out of the 49 cases, 37 contained other drugs and the C:P ratio for THC ranged from 0.16–2.83 with a mean of 0.70 (SD: 0.56, median: 0.49). In those same cases, the C:P ratio for THC-COOH ranged from 0.03–3.35 with a mean of 1.25 (SD: 0.72, median: 1.13). Of the 12 cases with no other drug findings, the C:P ratio for THC ranged from 0.14–1.35 (mean: 0.81, SD: 0.43, median: 0.87), and for THC-COOH ranged from 0.36–5.05 (mean: 1.30, SD: 1.35, median: 0.98). Antemortem (AM) blood samples were available in six cases and concentrations were compared to Postmortem (PM) femoral blood concentrations. The PM blood to AM blood concentration ratio (PM:AM) for THC was greater than 1 in two cases. The PM:AM for THC-COOH was greater than 1 in four cases. Results indicate that low to moderate PMR can occur; however, no trends were observed regarding other drugs impacting the PMR of THC.

Cannabinoids; Postmortem; Drug Analysis

Y11 Chemiluminescent Detection Limitations of Cannabinoids in Edibles and Urine Samples

Cristina Elisabeth Lemus*, Davenport, FL

Learning Overview: The goal of this presentation is to teach attendees about the application of chemiluminescence in cannabinoids and their metabolites. This research addresses the challenges and limitations associated with this method, especially the potential for adulteration of urine samples with substances like vinegar, bleach, or food dyes to produce false negative results. Ultimately, this presentation will cover the novelty of chemiluminescence as a rapid and accurate presumptive test for Δ^9-Tetrahydrocannabinol (Δ^9-THC) in urine samples and aqueous solutions.

Impact Statement: In current forensic toxicology casework, there is no application of chemiluminescence in presumptively testing cannabinoids and their metabolites in complex matrices like edibles products and urine samples. This impact extends to addressing the issue of sample tampering and will have a significant impact on the forensic science community. This research will not only advance the field but also address practical challenges, enhance detection, and contribute to the education of future forensic professionals.

Chemiluminescence, characterized by its high sensitivity, offers a robust means of detecting pharmaceuticals and controlled substances such as cannabinoids and their metabolites. In forensic science applications, chemiluminescence can provide a rapid and accurate presumptive test for the presence and concentration of Δ^9-THC in urine samples and aqueous solutions.[1] However, current literature has raised concerns that individuals could tamper with urine samples using adulterants such as bleach, vinegar, or food dyes to produce false negative results. These developments present a significant challenge for forensic toxicologists, necessitating a deeper understanding of the limitations inherent in current drug testing processes.

Continuing from an ongoing project, this presentation aims to address these challenges by evaluating the limitations of a chemiluminescent detection method for the presumptive detection of natural cannabinoids. This is achieved by utilizing a tube luminometer to detect natural cannabinoids and their metabolites in aqueous solutions, both in the presence and absence of common adulterants. Cerium (IV) sulfate and rhodamine B were used in the chemiluminescent detection of Δ^9-THC and tartrazine in water.[2] The Δ^9-THC exhibited a linear correlation of 0.0636–3.18μM and tartrazine of 7.71–0.964μM across the compounds. When spiked with vinegar, the chemiluminescence of Δ^9-THC diminished, while spiking with bleach and tartrazine inhibited chemiluminescence.

Additional work to be presented will show the impact of these adulterants on the detection of other cannabinoids. This research holds the potential to equip forensic toxicologists with a cost-effective and rapid method for detecting cannabinoids. These results indicate that chemiluminescent detection of cannabinoids may be deterred in the presence of adulterants or ingredients in edible products. The continuous pursuit of optimized chemiluminescent detection methods for cannabis edibles and biological samples, improvements in matrix compatibility, and the establishment of standardized protocols will undoubtedly contribute to the accuracy and reliability of forensic analyses in this evolving and complex field.

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Chemiluminescence; Δ^9-Tetrahydrocannbinol; Edibles

Y12   Method Development for a Quantitative Panel of Psychoactive Adulterants in Biological Matrices Using LC/MS/MS

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Learning Overview: The goal of this presentation is to show the development, validation, and application of an original Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method to quantitate nine adulterants commonly identified with illicit drugs of abuse. This presentation will also discuss the negative impact of these psychoactive adulterants and why the development of this method was necessary to expand upon existing analytical procedures.

Impact Statement: This presentation will impact the forensic science community, as well as the larger community, by describing an efficient LC/MS/MS method to detect and quantitate potentially harmful adulterants of common drugs of abuse.

An adulterant is a substance that is added to an illicit drug product for its pharmacological effects to provide the effect of a higher-quality drug.[1,2] The adulteration of illicit drugs of abuse with psychoactive substances, such as veterinary or unscheduled prescription drugs, has been commonplace for many years. These adulterants can lead to unpredictable effects, which may increase the toxicity of the primary drug. Some adulterants may also cause toxic effects in drug users.[1,3] Despite this, a comprehensive panel for psychoactive adulterants within drugs of abuse has not previously been published. A single analytical procedure aimed at isolating common adulterants would enable the comparison and tracking of current trends in adulteration of illicit drugs of abuse. In addition, it will provide a standardized method for identifying compounds that may increase the toxicity of illicit drug products and may help prevent the underreporting of adulterants.[1]

The objective of this presentation is to introduce a quantitative panel for the analysis of the most prominent psychoactive adulterants: levamisole, xylazine, lidocaine, benzocaine, procaine, phenacetin, quinine, and tramadol and O-desmethyl tramadol. These adulterants were selected for their psychoactive properties and because they have been established in the toxicology literature as frequent adulterants of drugs of abuse. Comparison of two sample preparation techniques, Solid Phase Extraction (SPE) and Liquid-Liquid Extraction (LLE), was completed to study the recovery of the analytes in various extraction conditions. The quantitative panel was applied to the following biological matrices: blood, serum/plasma, urine, and oral fluid to fill the existing gaps in literature for the analytes in such matrices.

Instrumental analysis was performed on an Agilent® 6495 Triple Quadrupole Liquid Chromatography/Mass Spectrometry (LC/MS) system. Chromatographic separation was achieved with an Agilent® InfinityLab Poroshell EC-C18 (3.0 x 100mm x 2.7μm) analytical column combined with gradient elution with 10mM ammonium formate and 0.1% formic acid in methanol for an overall run time of five minutes.

LLE was performed using 80:20 dichloromethane:isopropanol as the extraction solvent. The LLE results were compared to an SPE performed with the UCT Clean Screen DAU 3mL SPE cartridges using 78:20:2 dichloromethane:isopropanol:ammonium hydroxide as the elution solvent. During development of both extractions, factors including the organic solvent and pH were altered to optimize analyte recovery. Recovery studies were performed for both extractions and used to determine that the LLE performed better for the overall panel of analytes. While SPE provided cleaner samples and better recovery for some analytes, recovery was not consistent among the whole panel of analytes due to their different chemical properties.

Method validation was completed in accordance with Academy Standards Board (ASB) Standard 036 guidelines, including calibration model evaluation, carryover evaluation, matrix matching, bias, precision, determination of the limit of detection and limit of quantitation, evaluation of interferences, and evaluation for ion suppression. The validated method will then be used to analyze authentic case samples provided by NMS Labs. Finally, results from the application of the method to case samples will be discussed with a focus on the impact of psychoactive adulterants on drug users.

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Adulterants; Method Development; LC/MS/MS

Y13   Body Fluid Identification From Mixed Samples and Male DNA Confirmation Utilizing SERATEC® PSA and PMB Tests

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Learning Overview: After attending this presentation, attendees will know how mixed body fluid samples containing menstrual blood and semen can be detected utilizing both the SERATEC® PMB and the SERATEC® PSA Semiquant Tests. The ability to successfully extract male DNA from mixed samples using the test membranes from the immunochromatographic tests will also be discussed.

Impact Statement: This presentation will impact the forensic science community by filling in specific gaps in the current research regarding the SERATEC® PMB and the SERATEC® PSA Semiquant immunochromatographic tests by specifically highlighting body fluid identification from semen and menstrual blood mixtures and the ability to obtain a male DNA profile from relevant casework mixture type samples.

Evidence collected as part of a sexual assault evidence kit may contain multiple body fluids from different sources since body fluids from the perpetrator and victim may mix due to the nature of the assault. It is important to distinguish between present fluids through presumptive or confirmatory tests as this can aid and direct further downstream analyses, such as obtaining a DNA profile of the perpetrator. Results of screening and distinguishing between present body fluid types can guide the investigation, contribute to crime scene reconstructions, and corroborate events.

The SERATEC® PMB Test, which detects the possible presence of peripheral blood and/or menstrual blood, and the SERATEC® PSA Semiquant test, which detects the possible presence of semen, are the presumptive immunochromatographic assays used in this study.[1-3] While previous studies have performed mixture studies with menstrual blood and semen on the SERATEC® PMB Test, the mixtures were prepared in limited ratios. Currently there are no studies that have tested performance of these mixture types on the SERATEC® PSA Semiquant test. This study seeks to fill this gap in the current research by assessing the performance of both tests using an expanded range of mixture ratios of menstrual blood and semen and evaluate body fluid identification in unbalanced male-to-female mixtures. In situations where the original sample is limited, extracting DNA directly from the sample pad of immunochromatographic tests can be a particularly useful method for cases where both serological and DNA testing are of importance. This study assesses the ability to obtain male DNA profiles from these mixed samples when extracting directly from the test membranes.

Samples of menstrual blood were collected from donors (n=3) from multiple menstruation days (ranging from 1 to 5 days) and tested on SERATEC® PMB test kits using several tested dilutions. We determined the optimum test dilution (1:50) for the SERATEC® PMB Test and donor collection day (day 2). Samples of semen were collected from donors (n=3) and tested on SERATEC® PSA Semiquant test kits using several tested dilutions to determine the optimum dilution (1:500). A scale developed and used by SERATEC® was used in this study to rank intensities of all test results.[4] Buccal swabs were collected from the donors in this study for reference profiles.

After determining the optimal test dilutions, mixtures of menstrual blood and semen were made in several proportions (1:10, 10:1, 1:100, and 100:1) and tested on both the SERATEC® PMB and SERATEC® PSA Semiquant tests in duplicate. Each male donor sample was prepared as a mixture with each female donor sample (and vice versa). A cutting was taken of the test membranes and stored in microcentrifuge tubes for DNA analysis. DNA testing included an automated extraction method on the QIAGEN® EZ1® Advanced XL instrument to extract DNA from the test membranes. Following extraction, quantification (with the Quantifiler® Trio kit), amplification (with the Powerplex® Fusion 6C and PowerPlex® Y23 System kits), and DNA typing of the extracts (using the Applied Biosystems® 3500 Genetic Analyzer) were performed following the Center for Forensic Science Research & Education's (CFSRE's) guidelines. Analysis was performed using GeneMapper® ID-X Software v1.4. The DNA profiles were compared to reference profiles from collected buccal swabs.

We report on the observed body fluid identifications using serological tests when working with mixtures of menstrual blood and semen in different proportions, which is an informative evidence sample type in sexual assault cases. Additionally, we discuss the ability of obtaining a male DNA profile from a presumptive positive or negative result by comparing the SERATEC® PSA Semiquant test results with the percent allele recovery observed from the male DNA profiles.

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Serology; Mixture Analysis; Forensic DNA

Y14   Exploring the Performance of an Initial Set of DIP-STR Markers for Biogeographic Ancestry Prediction in United States Populations

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Learning Overview: After attending this presentation, attendees will have a better understanding of the performance of a set of Deletion-Insertion Polymorphism-Short Tandem Repeat (DIP-STR) markers to infer major biogeographic origins of individuals.

Impact Statement: This presentation will impact the forensic science community by providing investigators with an alternative intelligence tool for biogeographic ancestry inference.

DIP-STRs are genetic markers that combine low mutation DIPs with closely linked high-mutation STRs.[1] Originally developed for targeting the minor donor in imbalanced two-person mixtures, DIP-STRs use specific primers that overlap the DIP (S or L) sequence to create allele-specific amplifications of one/two minor DIP-STR haplotypes that include the non-shared DIP allele with the major donor. These markers have recently been tested as ancestry informative markers to predict an individual's ancestry and have proved effective in separating the major continental regions.[2,3] This study was designed to investigate the performance of a set of 23 DIP-STRs in their ability to predict the ancestry of individuals from four major United States population groups.[4]

An initial set of 100 samples from individuals of self-reported African American, European American, Asian American, and Southwest Hispanic origin was selected. The samples are quantified using the Quantifiler® Trio Quantification kit on a QuantStudio™ Real-Time Polymerase Chain Reaction (PCR) system. The genotyping workflow requires a first round of PCR amplification and separation of DIP markers to determine the target DIP allele, followed by a second round of PCR amplification of DIP-STRs using allele-specific PCR amplifications.[1] Fragments are separated on the SeqStudio™ Genetic Analyzer and profiles analyzed using GeneMapper® ID-X. To characterize and visualize the genetic structure of the populations of interest, population genetic data is analyzed, and clustering analysis is performed using STRUCTURE software and Principal Component Analyses (PCA).

The set of 100 samples was quantified and diluted down to 0.5ng/ul. DNA samples were first PCR amplified for the 23 DIP markers using a DIPplex assay and the S/L DIP allele calls were reported. Samples were further amplified for the 23 DIP-STR marker-set using allele-specific PCR amplifications and DIP-STR haplotypes were determined. The extensive dataset of allele frequencies generated from the 23 DIP-STR markers-set is in the process of being compiled to elucidate their overall distributions across the four United States population groups. Lastly, summary input files of DIP-STR haplotypes will be assembled and run through STRUCTURE software and PCA to determine the ancestry affiliations of the selected population samples, which are expected to cluster within the major continental regions.

In conclusion, a comprehensive DIP-STR profiling analysis of the generated population dataset is ongoing to reveal the genetic structure of the four United States population groups tested.

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Biogeographic Ancestry; DIP-STR; United States Populations

Y15   An Internal Validation of the Janovsky Color Test and Testing the Platinum Chloride Microcrystalline Method for Benzodiazepines

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Learning Overview: The goal of this presentation is to outline the findings for an internal validation of the Janovsky color test for benzodiazepines at the North Carolina State Crime Laboratory (NCSCL) as well as testing methods for a microcrystalline test. This project examines the need for, and increased efficiency of, a preliminary test method for benzodiazepines in a crime laboratory setting. This project examined both pharmaceutical preparations and designer benzodiazepines.

Impact Statement: This presentation will impact the forensic science community by demonstrating how a preliminary test method for novel benzodiazepines can be utilized in a crime laboratory.

Benzodiazepines are commonly seen in forensic samples; thus, presumptive tests are useful for the efficient analysis of seized drugs in a crime laboratory. The minimum standard for a targeted approach of analysis set forth by American Standards of Testing Materials (ASTM) and Organization of Science Area Committees (OSAC) adopted by the NCSCL requires a series of tests that support one another to identify controlled substances. Currently, the NCSCL lacks a preliminary test for benzodiazepines. Validation of an efficient, selective, robust, and rugged color test and/or microcrystalline test would improve efficiency in the crime lab, help reduce cost, and reduce the wait time for the consumer. This study will explore the effectiveness of the Janovsky color test and the platinum chloride microcrystalline test as potential preliminary methods to identify benzodiazepines.

Appropriate guidelines for validation were utilized in this study. The Janovsky color test consists of m-dinitrobenzene and potassium hydroxide solutions. An approximate 2% dinitrobenzene solution (Solution A) and 5N potassium hydroxide solution (Solution B) were prepared.

Pharmaceutical and designer benzodiazepines (17 tested) had consistent chemical reactions resulting in an obvious purple color, except for lorazepam and oxazepam. Ten forensic science practitioners tested unknown samples using the Janovsky color test and correctly observed the expected color changes. The Limit Of Detection (LOD) was between one part benzodiazepine with 500 to 750 parts diluent. Three drops of Solution A to one drop of Solution B were needed to yield an obvious purple color. Color observation was less obvious when Solution A and Solution B were added and approximately two minutes elapsed to perform the test and when Solution A and Solution B were added approximately two minutes apart from one another. When Solution B was added first, the reaction was weaker. The more material in the well, the more prominent the reaction. When comparing acetone and methanol for cleaning, there was no difference in the results of the test.

Five controlled substances most encountered by the NCSCL were tested to determine if they produced similar effects. A purple color change was observed in the presence of fentanyl but took longer to develop and was not as prominent as with benzodiazepines. The color change reaction for diazepam did not appear to be affected by cocaine base, cocaine HCl, fentanyl, heroin, or methamphetamine. The Janovsky color test will be implemented into the analytical scheme of the NCSCL drug chemistry section due to this validation.

The platinum chloride reagent microcrystalline test produced distinctive crystals in the presence of some benzodiazepines that can be observed with a polarized light microscope. An approximate 5% w/v chloroplatinic acid hexahydrate solution was prepared. Delorazepam, diazepam, flunitrazepam, and phenazepam resulted in distinct crystal formations. The LOD was between 250 to 500 parts diluent to one part benzodiazepine. The diluents/common controlled substances inhibited crystal formation.

The microcrystalline test is not currently recommended for implementation. Future studies can include testing more benzodiazepines and potentially validating the Janovsky color test for other controlled substances.

Designer Benzodiazepines; Janovsky Color Test; Platinum Chloride Microcrystalline Test

Y16   The Use of Leaf Spray PSI-MS for the Detection of Kratom (*Mitragyna speciosa*) Leaf

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Learning Overview: The goal of this presentation is to determine if kratom can be detected using leaf spray Paper Spray Ionization-Mass Spectrometry (PSI-MS). This research will advance the forensic science community in that it will allow for a method of detection for kratom that will not require any preparation of the sample. This is because the kratom leaf can be placed on the instrumentation with no prior manipulation. Kratom has been detected using MS and, therefore, a spectrum of kratom is known. This advancement will allow for more methods of detection for kratom that will limit the possibility of contamination as the same can be removed directly from the evidence bag and placed on the instrument for analysis.

Impact Statement: Currently, there is much that is unknown about kratom. This includes the interaction and similarities of this drug with others, the dependency of the drug, the short- or long-term effect of taking this, and how to effectively detect kratom.[1-3] This presentation will impact the forensic science community by determining if kratom can be detected using leaf spray ionization MS. The Organization of Scientific Area Committees (OSAC) has deemed kratom research at a status assessment I.[4] This means that there is a "major gap in current knowledge" and "no or limited current research is being conducted."[4] Resolving one of these gaps could greatly impact the forensic and investigative communities.

Mitragyna speciosa is a plant that produces a metabolite called mitragynine. This substance has been used for centuries as a traditional pain remedy, primarily in Southeast Asia. Recently it has become available in the United States and is commonly known as kratom. Kratom has become a popular substance for relieving pain, fatigue, and opioid withdrawal. In addition, people have reported an enhanced mood and decrease in panic attacks. Kratom is currently not considered an illegal substance in the United States; however, the Drug Enforcement Administration has deemed kratom a drug of concern. There is much unknown about kratom, including the effects it has on the human body along with a standardized detection method of the substance. The purpose of this study is to use the kratom leaf on a PSI-MS instrument for the detection of the substance. The detection of *Mitragyna speciosa* using leaf spray ionization MS was proven to be successful. Kratom was detected in all three strains tested, meng da, white meng da, and red meng da. All initial scan contained a parent ion peak at 399 m/z. This is indicative of the [M+H] peak of kratom as it has a molecular weight of 398.5g/mol. The fragment ion peaks that were present in the CID scans of 399 were 364m/z, 238m/z, 226m/z, and 174m/z. All four of these peaks are also specific to kratom. From these results, the hypothesis stands that kratom can be detected using a kratom leaf saturated in methanol to perform leaf spray PSI-MS for the detection of kratom. Future experimentation will include identifying possible unique components between strains to determine if the specific strain of kratom can be determined using this method. In addition, the identification of the particular fragmentation pattern of kratom will be examined using Electrospray Ionization/Mass Spectrometry (ESI/MS) with kratom extracted in methanol.

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Kratom; Drug Analysis; PSI-MS

Y17 The Effects of Fingerprint Development Techniques on Forensic Cartridge Case Identification

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Learning Overview: After attending this presentation, attendees will have learned how fingerprint development techniques impact the ability of making a cartridge case comparison and optimal cleaning methods to employ prior to comparison.

Impact Statement: This presentation will impact the forensic science community by displaying the inconsistencies that exist among crime laboratories in that some will develop fingerprints on cartridge cases and others will not. The majority of the cartridge cases were successfully compared following development techniques, and acetone was determined to be the optimal cleaning method.

When a fired cartridge case comes into a forensic science laboratory, there are various pathways it can go through to be analyzed. The more frequent process of analysis is done by the fingerprint section, followed by the firearm section, but some laboratories will analyze them in the opposite way or will not attempt to develop fingerprints. There is no standard that exists among all forensic science laboratories as to which section should analyze the evidence first, if at all.

The purpose of this study is to determine what effects certain fingerprint development techniques may have on cartridge cases and if these techniques impact cartridge case comparisons. Brass, steel, and aluminum cartridges in a 9mm and .45 caliber were fired using a Taurus® 708 and Remington® 1911, respectively. The cartridges were processed using cyanoacrylate fuming, gun bluing, basic yellow 40, black powder, and a sequence of these techniques. Microscopic markings left on the breech face of the cartridge cases from the firing process were compared using the comparison microscope both before and after processing with each development method and a sequence of them. Markings on the cartridge cases were identified following development with cyanoacrylate fuming, basic yellow 40, and black powder while some markings on the cartridge cases did not contain enough detail for an identification to be made, yielding an inconclusive result. Cartridge cases that came in contact with gun blue were not able to be identified prior to cleaning. Cartridges that underwent gun bluing were cleaned using acetone, an alcohol wipe, and soapy water to remove the gun blue that accumulated on the headstamp.

Acetone is known to be a cleaning agent and proved to be the optimal cleaning technique with the majority of those cases for all of the metal types being identified when compared to the alcohol wipe and soapy water. Some of the markings were not of enough detail for an identification to be made and others were unable to be cleaned enough for an identification to be made.

Firearms; Latent Prints; Cartridge Casings

Y18   The Impact of Age on Strength and Fracture Patterns of the Human Hyoid Bone

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Learning Overview: This research study will utilize 3D-printed hyoid bones from living patients of various ages to determine how much force is necessary to produce fractures on the hyoid bone. After attending this presentation, attendees will better understand why fractures may or may not occur during manual strangulation, how manual strangulation can be simulated, and how age influences the force necessary to fracture hyoid bones.

Impact Statement: This presentation will impact the forensic science community by better defining the relationship between age and hyoid bone fracture for victims of manual strangulation. This could potentially be useful in the field of forensic pathology when determining the manner of death in suspicious cases.

The human hyoid bone has been studied in conjunction with suicide and homicide since the 19th century. Suicide and homicide are two leading causes of death in the United States, with hanging being a frequent method of suicide worldwide.[1] Fractures of the hyoid bone are significant indicators of hanging, strangulation, or blunt force trauma to the neck, causing them to garner great importance and relevance in the forensic science community.

Several studies have elucidated that fracture is most common in manual strangulation events and least common in hanging events.[2-5] Furthermore, numerous studies have examined the relationship between age, sex, and the morphology of the bone with the occurrence of hyoid bone fracture, but there is no existing data on the amount of force required to fracture hyoid bones of different ages in simulations of manual strangulation.[6-9] The differences in shape, size, and rigidity of this bone throughout the human lifespan suggest that different amounts of force are required to fracture hyoid bones in the various stages of life.[10,11]

In this study, digital imaging files (.stl) of hyoid bones were collected from living patients of the University of Pittsburgh School of Dental Medicine. Four female bones were collected for each age group of 18–30, 30–45, and 45–80 years old. The digital imaging files were sent to a 3D printer to create four copies of each hyoid bone for a total of 48 bones. Several morphological measurements were made before encasing the 3D-printed hyoid bones in collagen and chamois. The collagen and chamois casing served to mimic the structure and skin of the human neck to better simulate manual strangulation. The collagen-encased hyoid bones were subjected to a Torbal FT Odyssey force gauge to determine the amount of force necessary to produce fractures. The locations of fractures were noted as well. The data collected will better define the relationship between age and strength of the hyoid bone, manual strangulation fracture patterns, and will aid in manner-of-death conclusions.

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Fracture; Asphyxiation; Homicide

Y19   Surveillance Efforts to Track Drug Market Changes in Philadelphia, Pennsylvania

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Learning Overview: After attending this presentation, attendees will be able to describe and implement analysis techniques used by the authors to study current trends in, and composition of, the Philadelphia drug supply and will be able to evaluate important developments within the local drug supply.

Impact Statement: This presentation will impact the forensic community by communicating the findings of the Center for Forensic Science Research and Education's (CFSRE's) Novel Psychoactive Substances (NPS) Discovery drug-checking program, emphasizing the value of timely monitoring of recreational drug samples for the benefit of public health and awareness.

The complex dynamics of today's drug markets must be met with real-time collection, purposeful chemical analysis, and advanced data interpretation and reporting. Drug checking is a harm reduction strategy that utilizes a variety of analysis modes to provide insights into drug markets for the benefits of public health and safety agencies. Comprehensive drug market surveillance in near real-time by forensic laboratories has the opportunity to impact current and future forensic practices, especially as it relates to novel analytical workflows, scope of testing, and information sharing.

The CFSRE, through its NPS Discovery drug early warning system, has been formally partnered with Philadelphia Department of Public Health (PDPH) since 2020 to provide confirmatory testing on drug materials collected within various parts of the city. Our laboratory has analyzed drug samples collected since 2019 with 47 samples analyzed that year and similarly 46 samples the next year in 2020. However, our drug checking program has grown over the past few years, with a total of 289 samples in 2021 and 505 samples in 2022. In 2023, the samples continue to grow with more than 390 samples through August 2023. For the first half of 2023, qualitative analysis showed that over half (56%) of the samples submitted contained fentanyl as the primary drug, 28% contained cocaine as the primary drug, and the remaining 16% containing other primary drugs such as methamphetamine, *para*-fluorofentanyl, PCP, *N,N*-dimethylpentylone, bromazolam, and others. For the first half of 2023, quantitative analysis showed the mean purity of fentanyl was $14.5\% \pm 8.2\%$; xylazine was $44.8\% \pm 15.4\%$; cocaine was $54.5\% \pm 24.8\%$; and methamphetamine was $63.6\% \pm 13.8\%$.

Polydrug, or polysubstance, use refers to the recreational consumption of multiple drugs, either by using a mixture of drugs concurrently (e.g., speedball, fentanyl and cocaine) or using different drugs (e.g., fentanyl and methamphetamine) within the same time frame. Either can cause drug interactions and increased risks of toxicity. Depending on the jurisdiction and its drug supply, polydrug use may be more or less common. This may increase the risk of unintentional drug overdose. In 2019, death certificates and toxicology reports showed that 50% of all drug overdose deaths involved more than one drug.[1] Out of all the drug samples analyzed in 2023, only 23% were comprised of one component and 19% contained four or more components, with all analytes of interest considered as components. Drug samples containing multiple components can lead to harmful effects, especially when the consumer is unaware of the purity of the drug sample, drugs present, and/or possible drug-drug interactions. Another concerning trend is the increasing positivity of xylazine combined with fentanyl. In 2019, 67.4% of fentanyl drug samples tested from Philadelphia also contain xylazine; however, in 2023, 98.9% of Philadelphia drug samples contain fentanyl and xylazine, with xylazine frequently as the principal component in the sample.

Drug checking is an important tool and resource to better understand the current drug epidemic and evolving drug markets. Collecting, analyzing, interpreting, and communicating drug checking data and trends in real-time allows for timely, informed drug user education and countermeasures to help reduce adverse effects and overdose.

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Fentanyl; Novel Psychoactive Substances; Overdose

Y20   **The Detection of Low Concentration Ignitable Liquid Residues From Fire Debris Using Gas Chromatography/Mass Spectrometry and an Ignitable Liquid Detection Canine**

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Learning Overview: This presentation will allow attendees to form a better understanding of how Ignitable Liquid Residues (ILRs) are detected by Ignitable Liquid Detection Canines (ILDCs) and Gas Chromatography/Mass Spectrometry (GC/MS) instrumentation, as well as the discrepancies between the two methods. This study demonstrates a need for the advancement of laboratory instrumentation in the analysis of fire debris evidence.

Impact Statement: This presentation will impact and benefit the forensic science community, especially the fields of fire investigation and fire debris analysis, by displaying the discrepancies between ILDCs and GC/MS instrumentation in the analysis of fire debris for ILRs. The importance of this research stems from being unable to confirm the presence of ILRs in fire debris with GC/MS after identification by an ILRC. Bridging this gap would allow for advancements to be made in the collection and analysis of fire debris.

Fire crimes are difficult to investigate because physical evidence tends to be destroyed by fire; however, ILR is one type of physical evidence that can be left behind in fire debris. The presence of ILRs at a fire scene can indicate criminal activity and aid investigators in the classification of the fire. A fire can be classified into four distinct categories based on the results of the investigation: accidental, natural, incendiary, and undetermined. ILDCs are commonly used in fire investigations to assist in detecting potential remains of an ILR and direct investigators to notable locations for sample collection. This saves investigators and forensic scientists time and resources when collecting and analyzing fire debris samples. After collection, the fire debris samples are sent to forensic laboratories where GC/MS analysis is performed to determine the presence and nature of the ILRs. There have been instances where GC/MS analysis has been unable to confirm the presence of ILRs after an ILDC has alerted. These instances become more complex when additional factors, such as witness statements, fire dynamics analysis, fire patterns, etc. indicate the potential introduction of an ignitable liquid. This leaves a gap in the field of fire debris analysis as to the difference between the detection limit of ILDCs and GC/MS instrumentation for ILRs.

This research assessed the difference in detection limit by conducting test burns on nine samples of wood and carpet with varying volumes of gasoline and varying times of burning. Gasoline was used as the ignitable liquid in this study because it is readily available to the public and is commonly used as an accelerant in incendiary fires. The samples contained either 1/8 liter or 1/4 liter of gasoline and were burned for one minute or five minutes. Control samples were made with varying amounts of gasoline and no heat application, varying amounts of heat application and no gasoline, or no gasoline and no heat application. All samples were run in triplicate for a total of 27 samples. An ILDC was then used to analyze the samples. Each sample was collected in a metal paint can and brought to a forensic laboratory for GC/MS analysis. The heated passive headspace concentration technique with activated charcoal strips was utilized in order to pre-concentrate the samples, which were then analyzed using GC/MS instrumentation. The detection limits of both methods were noted and compared using statistical analysis. By understanding this gap in fire investigation, future research can be done to enhance the collection and analysis methods of fire debris.

Fire Debris Analysis; Ignitable Liquid Detection Canine; Gas Chromatography/Mass Spectrometry

Y21 An Analysis of Drug Content Distribution on Paper Using Both Soak and Spray Methods by Gas Chromatography/Mass Spectrometry (GC/MS)

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Learning Overview: This presentation will demonstrate the distribution of drugs on different paper types using both soak and spray methods with analysis from GC/MS. After attending this presentation, attendees will better understand the relevance of these methods in relation to the emerging issue of illicit drug transport into prisons using the mail system.

Impact Statement: This presentation will impact the forensic community by improving the understanding of drug distribution on varying paper types. The data found will aid in the future development of a method to analyze drug-infused paper being sent into prisons unknowingly. Such research will help bring light to a newly evolving issue and its urgency.

In recent years, illicit substances have been clandestinely introduced into the prison system via the mail.[1] The presence of these drugs can be a hazard to any individual within correctional facilities, including inmates, guards, and mailroom staff. In order to obtain these illicit drugs, inmates require the assistance of an individual outside the prison. This outside connection will acquire the drug of choice and dissolve it in acetone to create a solution.[2] Any paper type can be either soaked in or sprayed with the solution, then disguised as a regular letter when dried. Despite this being an emergent issue heavily impacting jails and prisons, little to no research on this topic has been completed.

This study aims to investigate the distribution of drugs on different paper types while observing both soak and spray methods. For this research, a 3.33mg/mL solution of the desired surrogate drug type was created by dissolving the drug in acetone. The solutions were used to test both soak and spray methods on three paper varieties, including cardstock, drawing, and computer paper. Thirteen one-by-one-centimeter squares were marked on the paper type according to a template to ensure consistency for later in the experiment. In order to properly observe distribution factors, each paper was either dipped in or sprayed with solution two, four, or six times, depending on their respective trial. Between each dip or spray, the paper dried completely. The paper squares were then soaked in methanol to extract the drug from the paper matrix. After extraction, the solution was transferred into a GC/MS vial using a nylon syringe filter. The samples were run in the instrument utilizing a developed GC/MS method for the detection and quantification of the target analyte. The chromatographic data provided by the GC/MS were used to observe how the surrogate drugs were distributed throughout different paper types.

By understanding how drugs are distributed on the paper, further research can be conducted to help identify when a piece of mail has been tampered with and avoid these illicit substances making their way into prisons while protecting those who may unknowingly come into contact with these illicit drugs.

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Drug Analysis; Drugs on Mail; Drugs on Paper

Y22   **Examining the Effects of Thermal Stress on the Structure and Downstream DNA Analysis of Human Teeth**

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Learning Overview: After attending this presentation, attendees will have learned more about how DNA quality may be compromised under thermal stress in human teeth. In many forensic cases, human identification can be performed using the teeth of victims.

Impact Statement: This presentation will impact the forensic science community by advancing knowledge on the heat transfer and ability to extract DNA from human teeth that may explode under thermal stress. In forensic cases stemming from major disasters, terrorist attacks, fires, or car accidents, human teeth can be used to identify victims. In these cases, human remains can become extremely damaged or degraded. This poses a challenge to forensic laboratories and can hamper Short Tandem Repeat (STR) analysis when using DNA to identify an individual. Therefore, in most cases, teeth and bones are the only source of viable DNA.

Teeth are often recovered from events such as mass disasters and are a viable source of DNA for identification purposes. Examples of these events include human identification cases stemming from natural disasters, terrorist attacks, fires, and car accidents.[1] However, sufficient DNA from teeth may be a challenge to obtain if they are exposed to extreme heat, especially for an extended time period. This study demonstrates that the DNA quality is compromised under thermal stress. It was hypothesized that a heat transfer occurs within human teeth, causing the teeth to explode and fragment. Very few teeth were found at the site of 9/11 to aid in the identification of the missing. So, it has become a theory that teeth can explode into fragments when heat transfer occurs within human teeth. However, no one has been around to see this process happen in real time. We know that DNA is much harder to recover from burned remains, but we do not have an exact temperature where DNA actually becomes unrecoverable from human teeth.

To test this hypothesis, 44 human wisdom teeth were collected following extraction by an oral surgeon between 2019 and 2020. Each tooth was received without any cleaning process and contained small amounts of blood and tissue related to the extraction. DNA profiles of the remaining blood or tissue on each tooth were created as a reference. This study used two male and two female individual wisdom teeth at each extreme temperature. The extreme temperatures used were 100°C, 200°C, 300°C, and 500°C. A heat-resistant firefighter body camera was placed inside a kiln with the teeth to observe the changes that occurred during the time that they were exposed to heat. After the heat exposure, each tooth and its fragments were pulverized using a freezer mill, extracted using a Hi-Flow® Silica-Column Extraction, and quantified using quantitative Polymerase Chain Reaction (qPCR). The DNA profiles were analyzed for allele drop out, allele drop in, Relative Fluorescence Units (RFU) values, and heterozygote peak balance. Degraded DNA was analyzed by measuring the allele dropouts. This study advances the knowledge on the heat transfer and ability to extract DNA from human teeth that may explode under thermal stress.

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Mass Disaster; Thermal Damage; Human Remains

Y23   An Analysis of the Transfer of Drugs to the Envelope in Mail Over Time and Varying Conditions

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Learning Overview: The goal of this presentation is to use gathered data to show how substances from substance-soaked paper transfer to the envelope in the mail when exposed to different conditions. After attending this presentation, attendees will better understand how substances soaked onto paper in the mail will transfer to the envelope and how different conditions affect this transfer.

Impact Statement: This presentation will impact the forensic science community by demonstrating how the substances transfer from the substance-soaked paper to the envelope, which can aid in developing better methods for analyzing drug-soaked mail sent into prisons. Learning to test the envelopes will allow for analysts to have less exposure to dangerous substances. Additionally, this research can bring awareness to the fact that substances from these substance-soaked papers do transfer to the envelope, so proper safety procedures need to be in place to protect all individuals coming into contact with this mail.

According to the National Institute on Drug Abuse, 85% of the prison population has an active substance abuse disorder or was incarcerated for a crime including drugs/drug use.[1] This high percentage attributes to the large amount of drugs that are smuggled into prisons and jails, which continues to be a problem for prison staff. One method smugglers use is sending substance-soaked papers through the mail. In this method, the smugglers soak a piece of paper in a liquid mixture that contains the illicit substance of interest. This piece of paper is then placed in an envelope and mailed into the prison or jail. These pieces of mail are received by the prison staff, and, in several cases, the correctional officers who come in contact with these pieces of mail have fallen ill as a result of the substances soaked onto the paper mail. Because of this, correctional facilities have been faced with the difficult challenge of regulating the mail without breaching the inmates' First Amendment rights. Several prisons have turned to only allowing mail to be sent on white printer paper or using electronic services to deliver mail.[2]

The purpose of this research is to investigate the transfer of illicit substances from the substance-soaked paper mail onto the envelope, and how different conditions affect this transfer. Thus far, minimal research has been done investigating substance-soaked papers, especially when sent through the mail. The research was accomplished by dipping pieces of paper into a mixture of acetone and the desired substance, made to a concentration of 3.33mg/mL. These substance-soaked papers were then placed in manila or white paper envelopes and exposed to different temperatures or allowed to sit for various periods of time. The samples were then extracted and analyzed using Gas Chromatography/Mass Spectrometry. Results thus far have shown that mail samples allowed to sit for longer amounts resulted in a larger transfer of substance. Future research will include using different substances as well as exposing the mail samples to different temperatures and creating samples to be actually sent out in the mail.

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Chromatography; Drug Analysis; Transfer

Y24 Investigation and Detection Methods for Digital and Penile Penetration With No Ejaculation

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Learning Overview: After attending this presentation, attendees will better understand the importance of collecting Sexual Assault Kits (SAKs) from victims who have been digitally penetrated or penetrated with no ejaculation. This presentation will discuss optimized analysis of both vaginal and external female genitalia samples analyzed with Y-chromosome Short Tandem Repeats (Y-STRs) to obtain male DNA profiles at established time intervals.

Impact Statement: This presentation will impact the forensic science community by showing an optimized method to analyze sexual assault samples, thereby improving the quality of the final male DNA profile. The use of mock sexual assault samples provides realistic samples typically received by the crime laboratory.

In sexual assault cases, the examiner is often looking to identify seminal fluid as a biological matrix housing male cellular material. In cases devoid of seminal fluid, the analysis can be challenging because the analyst is reliant on the typically small amount of male epithelial cells present in a highly concentrated female DNA sample. As a consequence, SAKs may not be examined if case histories suggest that body fluids from a male source will not be present, as when digital penetration assault is alleged to have occurred due to the belief that male DNA will be insufficient in quantity. There have been studies that review old sexual assault casework samples and have reported successful detection of male epithelial cells after penile penetration up to 48 hours post deposition.[1,2] A further study used Y-STRs to identify that the window of survival and detection of DNA from male epithelial cells deposited from digital penetration can extend as far as 72 hours.[3]

The objective of this research is to study the collection of non-sperm samples deposited in the vaginal cavity via digital and penile penetration, as well as external genitalia swabs, 24 and 72 hours after deposition. To obtain samples of this nature, mock sexual assault samples were created by couples who abstained from sexual activity prior to collection. The samples collected from the couples are digital penetration samples with and without saliva as lubricant in addition to penile penetration samples with no ejaculation. All collections were performed at a controlled time interval and before each collection control swabs were taken. To date, initial studies from both vaginal and external genitalia samples at 24 hours post-deposit using saliva as a lubricant for digital penetration resulted in nine positive profiles. For penile penetration 24 hours post-deposit, vaginal samples have resulted in five positive profiles.

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Sexual Assault; Digital Penetration; Y-STR

Y25   Identifying the Presence of Semen Through the Detection of Fructose

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Learning Overview: This presentation will discuss original research exploring the use of fructose concentration as an identifying characteristic of seminal fluid compared to other bodily fluids. After attending this presentation, attendees will better understand the importance and role of fructose in seminal fluid and its potential as a target molecule in a novel identification method for bodily fluids.

Impact Statement: This presentation will impact the forensic science community by aiding in the development of a potential new method for bodily fluid identification that circumvents known issues presented by currently utilized methods, thus improving the range of testing methods available for serologists to accurately identify the presence of seminal fluid.

Seminal fluid is among the most valuable sources of biological evidence that can be recovered from a crime scene. Current testing methods for seminal fluid, such as the Prostate-Specific Antigen (PSA) test and microscopic examination for spermatozoa, are non-comprehensive and have a high potential for false positive or false negative results. Microscopic examination and identification of spermatozoa is often utilized as a confirmatory testing method, but relies on the presence of spermatozoa in the sample.[1] PSA testing is an attractive alternative that should return positive results for seminal fluid even in the absence of spermatozoa, such as in cases where a male subject is vasectomized.[2] However, this test is known to produce false positives in response to urine, breast milk, and condom lubricants.[2-6]

Fructose is a naturally occurring sugar that serves as the main energy source for sperm in semen.[7] Fructose is present in all seminal fluid at varying levels, depending on the presence or absence of spermatozoa.[7-9] There is currently no testing method for seminal fluid that utilizes fructose as the target molecule. However, it is possible that quantification of fructose in semen under different conditions, and compared to other bodily fluids, may aid in identification of crime scene samples, forming an alternative method to PSA testing.

To explore this potential, a fluorometric fructose assay kit was used to quantify fructose levels in semen samples as well as other common biological fluids, including saliva, urine, and vaginal fluid. Samples were absorbed into cotton swabs, allowed to dry, then re-extracted from the swab in an attempt to reflect forensically relevant sample collection methods. Additionally, comparisons of fructose levels were made between semen samples from vasectomized and non-vasectomized men under the hypothesis that the presence of sperm will decrease fructose levels over time as the sugar is metabolized. Preliminary results of this research indicated that fructose levels in seminal fluid were much lower than expected based on literature values from a medical context, providing an important indication of the potential impact of the forensic sampling methods utilized. The differences in fructose concentrations observed across the sample groups, as well as potential implications for the discriminatory power of fructose-based identification of body fluids, will be discussed.

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Semen; Serology; Bodily Fluid

Y26   Addressing Sexual Assault in Pakistan: Insights From an Analysis of Female Survivors at Lahore General Hospital

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Learning Overview: After attending this presentation, attendees will be informed about the demographics and circumstances surrounding sexual assault cases in Lahore, Pakistan, including age distribution, examination timing, and perpetrator relationships. The study highlights the need for policy reforms and improved infrastructure to address sexual assaults effectively in the region.

Impact Statement: This presentation will impact the forensic science community by providing valuable insights into the challenges and patterns of sexual assault cases, offering guidance to the forensic science community on the need for enhanced forensic infrastructure and improved victim support. This presentation underscores the importance of evidence-based policy reforms to better address and prevent sexual violence, ultimately contributing to the advancement of forensic science practices in this critical area.

Background and Objective: Rape is a severe kind of sexual violence that represents a significant violation of human rights. This act is widely recognized as an infringement on human rights, and its victims experience severe physical, psychological, and social ramifications. The objective was to investigate the demographic and clinical features of female survivors of sexual assault in Lahore, Pakistan.

Method: A retrospective study was conducted at the Department of Forensic Medicine of Lahore General Hospital, analyzing records of female sexual assault survivors investigated by affiliated police stations from November 2020 to November 2022. Data were collected from medicolegal certificates and medical notes and analyzed using SPSS software.

Result: 282 female survivors reported to Lahore General Hospital's Forensic Medicine Department, aged 5–55 years, with a mean age of 22.74 ± 8.30. A significant proportion (50.4%) were aged 16–25 years. Most survivors were examined between 6 and 24h (34%) and 2–7 days (37.9%) after the assault, with the majority (95%) exhibiting well-developed secondary sexual characteristics. Sexual assault and physical abuse were both reported in 56.4% of cases. In 77% of cases, the perpetrator was known, and in 36.5% of cases, a weapon was used. No external body injury was observed in 74.8% of cases, and 2.8% showed signs of fresh hymen rupture. The study identified 92.2% of cases lost to follow-up.

Conclusion: This study highlights the pressing need to implement effective measures to prevent and address rape in Pakistan. Survivors and those close to them should be informed about the significance of reporting such an occurrence immediately and having the survivor examined so that appropriate documentation and evidence can be collected. It is distressing to note that 92.2% of cases were lost to follow-up, highlighting potential gaps in pursuing these reports. The findings emphasize the need for education, improved forensic infrastructure, and policy reforms to address sexual assault effectively.

Sexual Crimes; Rape; Sex Offender

Y27 A Gunshot Residue Analysis: The Development of a Combined Procedure for the Detection of Inorganic Elements and Organic Compounds in Traditional and Clean Range Ammunitions Using Spectroscopic Methods

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Learning Overview: After attending this presentation, attendees will have learned about Gunshot Residue (GSR) analysis, types of instrumentation used for analysis, and how they work. Attendees will also be informed about the limitations of current testing methods and why it is necessary to improve testing methods. Proposal of new methods and the obtained results from my research show how this method could exceed current testing methods.

Impact Statement: This presentation will impact the forensic science community by informing attendees that current GSR analysis testing standards rely strictly on the presence and detection of three specific heavy metals: lead, barium, and antimony. As ammunition companies are creating cleaner ammunition free of heavy metals, they go undetected, creating false negative results. The proposed method exceeds current testing time frames to produce results and can detect metals present in both traditional ammunition and ammunition free of heavy metals. This method also looks at the detection of organic compounds seen in both types of ammunition that are not tested for currently. Proposing a method that can identify both metal and organic compound signatures in both types of ammunition within a short time frame will provide a positive or negative result within minutes. The instrumentation used to identify organic compounds also has portable capabilities, and results can be obtained within minutes on the scene of a crime. Overall, this method would provide forensic scientists and crime scene investigators a method with low error rates that can be completed rapidly in order to identify a potential shooter.

When a firearm is discharged, gases, vapors, and other particles are discharged from the primer and propellent components of the bullet that adhere to the skin and clothing of the shooter. This GSR is an essential way for forensic scientists to determine if a firearm has been discharged and to link a potential suspect to a firing. Traditionally, ammunition primer has been comprised of lead styphnate, barium nitrate, and antimony sulfide. Currently the standard testing method is scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy (SEM/EDS). SEM/EDS analysis relies on the distinct morphology and presence of inorganic elements, including lead, barium, and antimony particles, to produce a positive result. In addition to inorganics, ammunition also contains various organic compounds; however, these are not tested for using current testing methods.

Aside from health concerns related to exposure of heavy metals from the inorganic components, there is motivation to develop more environmentally friendly ammunition that uses primers free of heavy metals and is known as clean range ammunition. However, these ammunitions can also go undetected using SEM/EDS and result in a high false negative testing rate since lead, barium, and antimony particles are not present. Limitations of SEM/EDS include time-consuming analysis, several-day turnaround time for results, and the method is limited to only the detection inorganic residues.

Proposed in this study is a combined procedure for the detection of inorganic and organic residues in both traditional and clean range ammunitions using spectroscopic methods. Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) is a spectroscopic method that aims to atomize and ionize the sample creating ions that are measured and characterized based on their respective masses. ICP/MS can measure inorganic elements at trace levels within the parts per million to parts per billion range. In these studies, ICP/MS has been used to detect inorganic residues. Surface-Enhanced Raman Spectroscopy (SERS) has been used to detect organic residues. SERS is an inelastic light scattering method that uses a high-powered laser and colloidal silver nanoparticles. The nanoparticles interact with the sample analyte, and light becomes scattered as the laser interacts with the sample. Raman peaks observed in the SERS spectra correspond to characteristic Raman shifts and can be used to identify various organic compounds seen in GSR. SERS offers many benefits, including quick measurements that can produce results within minutes. Raman instruments also have portable capabilities allowing for the instrument to be taken out into the field for on-site analyses.

Overall, this method has shown promising results for the detection of inorganic and organic GSR seen in both traditional and clean range ammunition, at low concentrations with high sensitivity, exceeding the time and method capabilities of current testing standards.

Gunshot Residue; Raman Spectroscopy; Mass Spectrometry

Y28 The Recovery of Human DNA in an Aqueous Environment Using Novel Technology

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Learning Overview: The goal of this presentation is to allow attendees to form a better understanding of the influence of environmental conditions on the degradation and migration of Deoxyribonucleic Acid (DNA) within a freshwater environment. This study showcases the need for a forensic DNA testing technique that is able to assist in the recovery of human DNA from aqueous environments in cases of missing individuals.

Impact Statement: Through the exploration of a novel technology, known as Akadeum Microbubble Extraction, an attempt to recover human DNA from an aqueous environment was made to advance the recovery of missing persons linked to bodies of water. The importance of this research stems from the prevalence of missing persons cases today. Current methods of human recovery in water are time costly and greatly rely on chance. This presentation will impact the forensic science community by informing attendees of a novel extraction method that would allow for a more efficient search for missing individuals.

Through this study, it was anticipated that a trendline may be generated to allow for the area in which a missing individual is likely present within a water source to be discovered by studying the natural degradation of human tissue in a body of water. In an attempt to generate this trendline, a tissue sample was studied in a spring-fed pond located in Kane, PA. The tissue was placed within a mesh-lined suet feeder and submerged within the pond approximately three feet deep. The tissue went through the natural decomposition process. Water samples were collected at 0ft, 3ft, 6ft, 9ft, and 12ft from the tissue source each week for three months (June–August). A control chamber was also set up indoors at a location where outside environmental conditions would not affect the tissue sample within the chamber. Sampling of the control occurred at the same measured increments as the pond collection to further account for the impact of current on DNA migration. The Microbubble technology was used to extract the human DNA from the water samples collected. Extractions were performed in triplicate. Any recoverable DNA within the collected samples was analyzed via quantitation. Downstream, the most favorable samples will be amplified and genotyped. The trial was performed in the summer months to allow the impacts of temperature, pH, oxygenation, wildlife activity, bacterial activity, depth, and water current to be examined in regard to the rate of DNA degradation and migration.

By accounting for environmental factors and analyzing any recoverable human DNA, it is intended that the location, presence, and identity of a potential missing person may be discovered. Through the use of DNA extraction techniques on water samples, the recovery of missing individuals may become more efficient in the future. This research project intends to implement a novel method of human detection in bodies of water to assist in missing persons cases.

Forensic DNA Analysis; Microbubbles; Missing Persons

Y29   The Optimization of DNA Extraction Methods From Alternative Reference Materials

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Learning Overview: After attending this presentation, attendees will understand how DNA can be successfully and efficiently extracted from alternative reference materials such as rootless hairs and fingernails.

Impact Statement: This research will impact the forensic science community by describing an optimized nuclear DNA extraction method for low-template samples that enables these samples to be used as alternative reference materials in cases where buccal swabs are not readily accessible or when high-quality DNA samples are unable to be collected.

A common type of biological exhibit in crime scenes is naturally shed hair.[1] Although hair has low levels of DNA and is not readily used to generate profiles from evidence, it is easy to collect in a non-invasive manner for references. Fingernails may be an uncommon sample used in forensic investigations but do contain adequate quantity and quality of DNA.[2] Extraction of DNA from fingernails is challenging because DNA is encased within keratinized cells, resulting in low recovery and fragmented DNA.[2] Similar to hair, fingernail collection is a painless and non-invasive procedure; for instance, a scenario where an elderly person suffering from a painful mouth condition may prefer offering hair or fingernail samples over a buccal swab.

This research aimed to optimize DNA extraction methods for three alternative reference types: (1) hair with root, (2) rootless hairs, and (3) fingernails. Male and female samples were collected for each sample type. Through this project, an optimized extraction method for all sample types was developed, and the relationship between hair thickness, number of hair strands, and DNA yield was evaluated. Additionally, a concentration step for the extraction of rootless hairs was investigated.

To avoid contamination from exogenous DNA, all samples were washed with a 5% Tergazyme™ cleaning solution three times followed by two ethanol rinses and three deionized water washes. Variables tested were incubation time (3 to 24 hours), concentration of proteinase K and DTT (25% increase). The extraction methods used the trace protocol from the QIAGEN® EZ1® DNA Investigator® Kit. DNA was quantified using the Quantifiler® Trio kit on the QuantStudio™ 5. The results showed the rooted hair had a high DNA concentration average of 3.3ng/µL, rootless hair had a low DNA concentration averaging 0.03ng/µL, and fingernail samples produced a high average DNA concentration of 0.6ng/µL. There was little to no change in improvement of the DNA yield from the rootless hair, but sufficient DNA yield was obtained for rooted hair and fingernails when proteinase K and DTT was increased to 25% to aid in digestion during incubation.

To test whether hair thickness and number of strands are correlated with DNA yield, 11 volunteers were selected, and the diameters (micrometer) of three different hair strands per volunteer were measured via microscopy with a stage micrometer and reticle. Three volunteers with varying hair thickness (8.6, 9.2, and 11.0µm) were selected to proceed with downstream processing. For each donor, 5, 10, and 15 hair strands were extracted using the optimized extraction method. The thickest hair samples had a low average DNA quantity of 0.0003ng/µL, while the thinnest hair samples had a high average DNA quantity of 0.03ng/µL. No correlation between hair thickness and DNA yield was observed; however, extracts with 15 hair strands produced the highest DNA yield for each volunteer. Finally, to concentrate the DNA, Vivacon® filtration columns were used to reduce the extract volume from 50µL to 17µL. On average, there was an increase in DNA concentration to 0.15ng/µL, which rendered the extracts from rootless hair suitable for amplification.

The results of this project may be used for the successful extraction of DNA from alternative reference materials. Future work aims to utilize these methods to develop DNA extracts for **Single Nucleotide Polymorphism (**SNP) testing with custom targeted panels. These findings can also serve as a foundation for refining techniques that enable the utilization of hair and fingernails as DNA evidence or alternative reference materials. These methods are invaluable for establishing genetic profiles of potential suspects, verifying investigative leads, or identifying unknown remains of missing persons.

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Forensic DNA; Hair; Fingernail

Y30   Assessing the Impact Dynamics of Less Lethal Bean Bag Ammunition

Brooke Fontaine*, Essex Junction, VT

Learning Overview: After attending this presentation, attendees will be informed about the purpose of less lethal bean bag ammunition in law enforcement and the potential risks of using them.

Impact Statement: This presentation will impact the forensic science community by providing data to determine whether or not this type of ammunition should be used by law enforcement, as there have been many reports of extreme injuries.

Less lethal ammunition is used to control situations without deadly force.[1] However, bean bag ammunition has been reported to be the cause of life-threatening injuries, some of which lead to long-lasting effects.[1,2] This study investigates some of the potential issues of bean bag ammunition in an effort to determine whether they are an effective option for law enforcement.

In order to make this determination, both regular 12-gauge shotgun ammunition and the less lethal ammunition were compared. Both types of ammunition were weighed while completely intact, then disassembled to weigh the individual components to determine if there is any significant variability in weight. After weighing each ammunition type, both intact and their individual components, it was determined that there was no significant variability in weight between the different rounds. The bean bag ammunition used in this study only contained lead shot, unlike some of the other brands that contain other material. The next step was to fire both ammunition types at ballistic gelatin, a skin simulant (chamois cloth), and a cotton t-shirt to analyze accuracy, precision, changes in velocity, impact force, and damage. An adjustable gun mount was used to stabilize the shotgun during testing. The bean bag ammunition was fired at multiple distances, including 4, 21, and 30ft. The less lethal bean bag ammunition did not penetrate the ballistic gelatin; however, some rounds ripped through the chamois cloth. Using a chronograph, a potential variance in velocity was observed. A regular 12-gauge shotgun round was fired at a distance of 30ft at the ballistic gelatin for comparison. The round passed through the ballistic gelatin entirely.

Further testing is ongoing to determine whether there is a definite issue with inconsistent velocity and to come to a conclusion as to whether or not less lethal bean bag ammunition is an effective option for law enforcement officials.

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Criminalistics; Ballistics; Forensic Science

Y31   A Comprehensive Examination of STR and Y-STR Profiling in Samples With Limited or Negligible DNA Quantities

Chih-Wen Su*, Taipei, Taiwan

Learning Overview: After attending this presentation, attendees will be informed of the process for conducting biological samples with low or negligible DNA quantities in the future.

Impact Statement: This presentation will impact the forensic science community by demonstrating a thorough investigation into Short Tandem Repeat (STR) and Y-chromosomal Short Tandem Repeat (Y-STR) profiling within samples characterized by a scarcity of template DNA. The insights gleaned from this study have the potential to facilitate the formulation of appropriate procedural guidelines for forensic DNA scientists in their management of such challenging sample types.

For general criminal cases, the analysis of biological samples still primarily relies on autosomal DNASTR (autosomal DNA-STR) profiling. Therefore, when human DNA quantification results indicate limited or negligible DNA quantities, deciding whether to proceed with DNA analysis on the sample becomes an essential issue for forensic analysts.[1] Additionally, in sexual assault cases, biological evidence collected from the female victims or their intimate clothing often contains not only potential DNA from the male suspects but also a substantial amount of DNA from the female victims themselves. The male-specific Y-chromosomal DNA-STR profiling method significantly increases the likelihood of successfully identifying the male suspect's Y-STR DNA profile.[2] Furthermore, with advancements in DNA profiling techniques and instrumentation, the sensitivity for detecting trace amounts of biological samples has substantially improved. This has presented a critical challenge for forensic analysts when dealing with cases where the DNA quantification results indicate an absence of detectable DNA.[3] This study explores the correlation between DNA quantification results and the detection outcomes of DNA-STR profiles and Y-STR profiles, respectively, aiming to provide reference information for DNA forensic scientists conducting samples with low or negligible DNA quantities in the future.

A total of 206 non-probative, burglary case-type samples (including crime tool transfer swabs, gloves, and trace bodily fluid transfer swabs) and 205 sexual assault case-type samples involving trace amounts of evidence were selected in this study. No DNA was detected (undetermined) for the above samples from DNA quantification using Quantifiler® Trio DNA Quantification Kit with ABI® PRISM® 7500 Sequence Detection System. DNA samples with/without concentration were then amplified using AmpFLSTR® Identifiler® Plus PCR Amplification Kit (for burglary case-type samples) or PowerPlex® Y23 System (for sexual assault case-type samples), and DNA profiles were analyzed using ABI® PRISM® 3500 Genetic Analyzer.

Results demonstrate that out of all 206 burglary case-type samples, 134 samples did not show any peaks above the Allelic Threshold (AT) (100RFU) value in the profiles.[4] Seventy-two samples were observed with at least one signal at a single genetic locus exceeding the AT value in the profiles. However, when further analyzed using a Stochastic Threshold (ST) (500 RFU), only three out of the 72 samples exhibited peaks above the ST value.[5] They could be associated with eight or more identifiable allelic loci, which means only 1.5% (3/206) could be identifiable for further analysis. For the 205 sexual assault case-type samples subjected to Y-STR analysis, even after concentration, 88.8% of the samples (182/205) still could not yield any detectable Y-STR profiles (with a detection count of 0). When applying a minimum standard of 12 Y-STR profiles for issuing an identification report, as many as 97% of the samples (199/205) failed to produce results, with only six samples capable of yielding reportable profiles.

Based on the results of this study, it was observed that the DNA-STR profiling detection rate is only 0.5% when quantification results are undetectable using real-time quantitative PCR, and it is less than 3% for sexual assault case-type samples from the comparison in subsequent Y-STR analysis. These findings can serve as a reference for forensic laboratories when conducting trace evidence.

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Low DNA; Quantitative Evidence; Trace Analysis

Y32   A Landscape Study: Investigating Potential Links Between Adoption and Serial Killers

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Learning Overview: This presentation will use data analysis of serial killers in the United States to highlight the need to understand adopted serial killers and how it expands the Radford/Florida Gulf Coast University Serial Killer Database. Attendees will gain a greater understanding of the significance of American serial killers who were adopted, placed in foster care, or raised by a non-biological parent as well as the circumstances that may have led them to conduct serial murders.

Impact Statement: This presentation will have an impact on the forensic science community by emphasizing the significance of the data analyzed from the American serial killers who were adopted. This type of data, as well as the expansion of the Radford/Florida Gulf Coast University Serial Killer Database, will assist researchers, law enforcement, forensic psychologists, and behavioral analysts in their primary investigations of serial killers, which may be used to create statistical studies and profiles to learn more about that serial killer's particular crime.

While serial killers can be found everywhere in the world, they became increasingly prevalent in the United States throughout the 1960s to present day. The cases of Ted Bundy and Jeffrey Dahmer sparked a public fascination with serial killers. According to the Federal Bureau of Investigation (FBI), a serial killer is someone who kills two or more people at various times. Anger, pleasure, money, and attention are typically the motives behind serial killing; however, there are other motives. This study examines several elements, some of which may have resulted from circumstances imposed on these individuals throughout their early childhood, that can further our understanding why these serial killers killed and kept killing. This study primarily focuses on serial killers who were adopted, placed in foster care, or raised by their non-biological parents. Approximately 90% of children in foster care, according to the Department of Health and Human Services, have experienced trauma, which may affect how they behave in the future.[1]

This study makes use of the Radford/Florida Gulf Coast University Serial Killer Database to broaden research on American serial killers as well as their database's coverage of adopted serial killers.[2] Out of 500 serial killers in the United States researched so far, 10% were adopted, placed in foster care, or raised by their non-biological parents. In order to see if adoption and serial killers have a connection, the two questions that the study aimed to address were: Why is it critical to add information about adoption status to the United States serial killer database? How does this advance our knowledge of serial killers?

This study hypothesized that the Radford/Florida Gulf Coast University Serial Killer Database will be able to expand as a result of the research found on adopted serial killers in this study. This study advances knowledge in the field of forensic science and psychology by identifying the serial killers in the United States that were adopted and the circumstances they experienced that drove them to commit such heinous crimes. This information will be used in the future to expand research on American serial killers who were adopted and to provide resources to forensic psychologists and behavioral analysts using the Radford/Florida Gulf Coast University serial killer database.

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Serial Killer; Adoption; Trauma

Y33 **The Recovery of Nuclear DNA From Skeletal Remains Decomposed in Freshwater**

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Learning Overview: After attending this presentation, attendees will be informed about the effects of wet and dry decomposition environments on skeletal remains. Attendees will see how the quantity of DNA in skeletal remains changes over a three-month time period. Attendees will see that the average quantity of DNA recovered from dry decomposed skeletal samples is higher than that of the wet decomposed samples when using an updated extraction method based on the PrepFiler® BTA extraction kit by Applied Biosystems®.

Impact Statement: This presentation will impact the forensic science community by providing a foundation for further research into evidence collection and processing time frames for skeletal remains. Understanding how the quantity of DNA changes over time can inform crucial time points for DNA sample collection. Additionally, the updated extraction method exhibited in this presentation will provide the forensic science community with a way to improve DNA recovery from skeletal remains.

Recovery of DNA from skeletal remains may be difficult under normal circumstances, but environmental degradation can make this endeavor more challenging. In cases when antemortem dental records are not available and there are no identifying items recovered, DNA analysis may be the only pathway for identification of an individual. This research compared freshwater and dry decomposition environments and their impact on DNA recovery from *Sus scrofa* femora over a three-month period. Prior to decomposition, initial cuttings were taken from "fresh" bone samples. Cuttings were then collected at three-week intervals from waterlogged and dry bone samples. Samples from both decomposition environments were extracted using the recommended protocol from the PrepFiler® BTA extraction kit by Applied Biosystems® and an updated method of the PrepFiler® BTA protocol that excludes the use of proteinase K.[1] DNA quantitation was performed using custom primer sets specific to *Sus scrofa*, using the QuantStudio™ 5 Real-Time PCR System by Applied Biosystems® and PowerUp™ SYBR® Green Master Mix.[2] Results of DNA quantitation showed that dry decomposition samples extracted using the updated protocol had a higher average quantity of DNA recovered than those extracted with the original PrepFiler® BTA protocol. At week three, both decomposition conditions showed a decrease in DNA recovery using both extraction methods. At week six and onward, DNA was recovered only from the dry decomposed samples extracted with the updated protocol. Wet decomposed samples and samples extracted with the PrepFiler® BTA protocol yielded no DNA.

The data suggests the updated protocol improves DNA recovery from skeletal elements decomposed in dry conditions and may be easily implemented in a forensic laboratory for analysis of decomposed remains. This research improves our understanding of how the quantity of DNA changes over time in specific decomposition environments and may provide a framework for analysts to further investigate and develop time frames for sample collection and analyses.

References:
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2. Thermo Fisher Scientific, *PowerUp™ SYBR® Green Master Mix User Guide* (Pub. No. MAN0013511 Rev. D.0), https://assets.thermofisher.com/TFS-Assets%2FLSG%2Fmanuals%2FMAN0013511_PowerUp_mastermix_UG.pdf.

Bone; Decomposition; Forensic DNA

Y34   Examining the Presence of Foreign DNA on Neck Swabs

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Learning Overview: The goal of this presentation is to determine if extraneous DNA is present on necks after performing daily activities. Attendees will learn about the factors that impact the presence of DNA on necks and at what concentration DNA is present in this area. They will also be able to view the preliminary results of genotyped samples and see if there is extraneous DNA present in the profiles.

Impact Statement: This presentation will impact the forensic science community by determining the validity of neck swabs as forensic evidence. It is important to know what could be present on a victim's neck before testing for touch DNA and how daily activities could impact the presence of DNA.

Strangulation is the compressing of blood vessels and/or air passages through external pressure on the neck. Growing research and anecdotal information suggest the increase of strangulation in sexual assault cases. Across four studies, an average of 9.7% of survivors of sexual assault experienced strangulation.[1-4] In a study conducted by the Maine Coalition to End Domestic Violence with 151 survivors, 72.8% of participants had been previously strangled.[5] In a similar study conducted by the Georgia Coalition Against Domestic Violence with 115 participants, 80% had been previously strangled.[6]

In suspected strangulation cases, it may be possible to retrieve touch DNA from a survivor's neck and detect the DNA profile of the perpetrator. However, it is imperative to first establish if foreign DNA is present on an individual's neck even without a strangulation event. This study looks to determine the presence of foreign DNA on a subject's neck after exposure to everyday activities. The goal is to determine if there is a buildup of foreign DNA on our necks even without direct contact.

Reference and neck swabs were obtained from numerous volunteers across three separate visits to a DNA lab. DNA from the swabs was extracted with a QIAGEN® QIAamp® DNA Mini and Blood Mini kit, quantified using a QuantiFiler®®HP kit, amplified with the Polymerase Chain Reaction (PCR) GlobalFiler® kit and genotyped on the SeqStudio™ Genetic Analyzer. Volunteers also filled out a questionnaire related to their daily life and activities they had performed in the 24 hours prior to the collection of the swabs. This questionnaire included their relationship status, living arrangements, social activities performed, and the time since their last shower. Reference profiles were compared to the DNA profiles obtained from the neck swabs to identify the presence of foreign DNA.

Preliminary results show that DNA is present in very low quantities in the neck area. Genotyped DNA samples displayed a variety of results, including single-source profiles matching the reference, two-person mixtures, and incomplete profiles. The relationship status, diversity of living arrangements, social activities performed, and the time since last shower does not seem to affect the quantity of DNA present in the neck area.

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6. Georgia Coalition Against Domestic Violence, *Strangulation Screening and Assessment Survey Results* (2014).

Forensic DNA; Low DNA; Genotypes

Y35   Allied Dental Professionals' Identification Accuracy of Burned Teeth

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Learning Overview: After attending this presentation, attendees will understand how registered dental hygienists and dental assistants performed when using transferrable skills to match burned teeth with simulated antemortem and postmortem photographs and radiographs. During mass fatality incidents, these professionals are supervised by forensic odontologists and serve as supplemental personnel to provide comparative phase consultation; however, little is known about their skills when serving in this capacity. Results of the research helps to fill a gap.

Impact Statement: This research assessed simulated forensic odontology skills performed by registered dental hygienists and dental assistants. Disaster preparedness and response organizations as well as coroner and medical examiner offices may benefit by knowing how these professionals perform when recruiting them for support. This presentation will impact the forensic science community by informing attendees of educational best practices for programs targeting auxiliary dental professionals who choose forensic-based service.

Aim: During Mass Fatality Incidents (MFIs), allied dental professionals are recognized as auxiliary personnel with skills applicable to Disaster Victim Identification (DVI). However, available literature lacks data regarding allied dental professionals' skills that may be transferable for DVI tasks. The aim of this research was to statistically quantify the accuracy of dental hygienists and dental assistants to determine match accuracy of burned teeth with simulated Antemortem (AM) and Postmortem (PM) photographs and radiographs.

Methods: This study was supported by the American Society of Forensic Odontology Research Grant, and the Institutional Review Board (IRB) #1851510-1 exempt approval was granted. Fifteen extracted permanent human teeth (five each of incisors, premolars, and molars) were placed on a ceramic tile, then heated in a muffle furnace at 400°F for 15 minutes. Before and after heat alteration, each tooth was photographed with a digital camera to record occlusal, buccal, lingual, mesial, and distal surfaces, then digitally radiographed; these served as simulated AM and PM images. Finally, the 15 burned teeth were placed in small clear acrylic containers for storage and protected viewing purposes. A convenience sample of dental hygienists (n=15) and dental assistants (n=15) matched for years of work experience were recruited and provided informed consent. Participants were given the simulated AM and PM radiographs and photographs in a predetermined shuffled order, then instructed to correctly match them with the 15 burned teeth. Following the match activity, participants completed an electronic survey to report demographic data and answer items regarding their perceived accuracy for tasks performed.

Results: Each participant (N=30) completed 60 total matches for the 15 burned teeth with the following: one set of pre-burned photographs, one pre-burned Periapical (PA) radiograph, one set of post-burned photographs, and one post-burned PA radiograph. A one-sided one-sample binomial proportion test revealed that, on average, participants were able to demonstrate more than 80% match accuracy of the burned teeth with AM and PM images (p=0.0003). Additionally, average match accuracy per tooth type was: incisors (80.5%), premolars (88.6%), and molars (80.0%). When compared by profession, the average match accuracy was 82.1% for dental hygienists (n=15) and 72.7% for dental assistants (n=15); however, a two-sided two-sample binomial proportion test revealed that, on average, there was no statistically significant difference of performance between the two professional types (p=0.8505). Participants were also assessed based on matched years of work experience: 1–5 years (516 accurate matches), 6–10 years (462 accurate matches), and 11+ years (517 accurate matches). However, a one-sided linear trend test gave p=0.2371, indicating no significant association between identification accuracy and years of work experience. Most participants indicated a moderate level of confidence in their match accuracy (46.6%–66.6%) and reported root morphology as most helpful (66.6%). When asked to choose if formal education or work experience was most helpful in their ability to complete the match accuracy activity, 3.3% indicated formal education and 46.6% indicated work experience as most helpful.

Conclusions: Registered dental hygienists and dental assistants who participated in this study demonstrated that, on average, they could transfer their professional skills to match burned teeth with simulated AM and PM images. Differences in professional background and work experience did not have a significant impact on the participants' accuracy. More research is needed to best understand allied dental professionals' transferrable DVI skills when needed as supplemental personnel during MFIs.

Forensic Odontology; Human Remains; Dental Identification

Y36 Pursuit of Justice: Mapping the Future Landscape of Forensic Psychiatry

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Learning Overview: The goals of this presentation are to help attendees: (1) learn about the novel technologies that may change the landscape of forensic psychiatry: (2) gain an understanding of the proposed purposes and benefits of Artificial Intelligence (AI), genetic testing, and Virtual Reality (VR) on forensic psychiatry; (3) explore the potential benefits and ethical concerns of using these technologies in forensic psychiatry, and (4) highlight the areas for future research to ensure the ethical applicability of these tools.

Impact Statement: This presentation will impact the forensic science community by providing an overview of the potential impact of AI, genetic testing, and VR on forensic psychiatry and how these technologies can improve the accuracy of mental state assessments, identify risk factors for violence, and develop personalized treatment plans for people with mental illness, thus encouraging the forensic science community to be proactive in exploring the potential benefits of these technologies while also being mindful of the ethical concerns.

Introduction: The growing prevalence of mental illness is also likely to significantly impact the field of forensic psychiatry as more people with mental illness encounter the criminal justice system. The field of forensic psychiatry is rapidly evolving as new technologies are developed that can be used to assess mental state and criminal responsibility and develop appropriate treatment plans and rehabilitation. This presentation will explore the potential impact of AI, genetic testing, and VR, aiming to reshape the forensic psychiatry landscape.

Methods: We conducted a literature review of peer-reviewed articles published in the past ten years that explored the use of these technologies in forensic psychiatry.[1-6] We comprehensively surveyed ongoing trends using sources including PubMed®, Cochrane reviews, and other databases. Employing keywords such as "forensic psychiatry," "artificial intelligence," "genetic testing," and "virtual reality," we used the insights from six papers that illuminate the emerging methodologies for their possible application within forensic psychiatry.

Discussion: Our findings suggest that AI, genetic testing, and VR can potentially revolutionize the field of forensic psychiatry. Neuroprediction AI technologies can be used to assess mental states accurately and identify risk factors for violence. Genetic testing has been postulated to identify genes that predispose to violent behavior. VR can be used to develop personalized treatment plans for the forensic population such as cognitive rehabilitation, aggression prevention training, social skills training, and recreating crime scenes for the courtroom. However, the current courtroom applicability of these modalities at the time of this presentation is limited. We identified areas of future study as potential benefits and ethical concerns, exploring the questions surrounding their reliability, privacy implications, and potential bias of these uncharted territories.

Conclusion: These novel technologies promise to reshape the landscape of forensic psychiatry. However, their integration mandates rigorous ethical scrutiny and an understanding of their potential implications before they are widely adopted. As mental health intersects with the pursuit of justice, this presentation highlights the need for cautious optimism, proactive exploration, and ethical implementation to ensure an equitable future.

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Forensic Psychiatry; Artificial Intelligence; Virtual Reality

Y37   **The Makings of a High-Profile Case: How Media Bias Impacts Forensic Investigations in Missing Person Cases**

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Learning Overview: The goal of this presentation is to focus on the possibility of implications within missing person cases in the media in reference to the level of media attention received in conjunction with the forensic investigative process. The use of high-profile missing person cases was compared to cases that did not receive media attention to emphasize the discrepancies that occur when someone goes missing as an underrepresented individual and places attention on how this may impact forensic investigations.

Impact Statement: This presentation will impact the forensic science community by not only showing if there are implications in media attention in relation to forensic investigative work, but additionally driving the discussion of biases within the forensic community. This will ensure justice for all and a push for procedural change to better adjust for underrepresented groups.

As the consumption of true crime cases continues to capture the attention of viewers, listeners, and readers, issues arise when adequate media coverage is lacking for those deemed socially excluded. Bias can present itself as an issue when the media focuses on whom they value the most in society. This dilemma has been described as the "Missing White Woman Syndrome," in which the media prioritizes White women who are missing or murdered rather than underrepresented individuals with regard to race, sexuality, ethnicity, or gender. Those who do not fit the "ideal image" are not necessarily prevalent in the media. However, the implications that this lack of media attention on socially excluded individuals has on the investigative process has not been explored. This leads to the question: How does medial bias impact forensic investigations?

A case comparison study was conducted using high-profile missing persons cases versus a set of cases that were also supplemented by a series of interviews involving experienced forensic investigators and media representatives who work in a multitude of different platforms to properly understand if there are implications, and if so, their professional opinions on the impacts that media has within investigations. Preliminary results have emphasized the fact that media is essentially a revolving door that publishes what their viewers continue to consume, and although many outlets are aware of the bias issues, they implicitly fail to adjust to the critiques of these biases. In addition, missing persons with a support system who are especially willing to talk to the press have shown more push for broadcasting this missing person. In the cases of socially excluded individuals, that support system is lacking and therefore fails to be found within the media.

There are so many factors that play into a missing person case, but the main problem at this time is seemingly a procedural issue. A standard process could not only solve all issues surrounding bias and if there are implications in forensic investigations involving media but ensure everyone gets the rightful attention they deserve. In determining if the media impacts the job that forensic investigators take on, conclusive results could not only improve both media and investigators' roles in missing person cases, but work toward collaborative procedures when needed, for the cause of bringing those missing persons home safely. In addition, this research could draw attention to holding media accountable when needed to adequately spotlight missing persons who are seemingly missing in day-to-day news reports. Increases in advocacy and discussions about the missing person crisis is needed to promote justice for all, regardless of media coverage.

Media Bias; Forensic Investigation; Missing Person Case

Y38   **Using an Indirect Personality Assessment to Determine the Psychopathy of Timothy McVeigh**

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Learning Overview: After attending this presentation, attendees will better understand how the childhood and behaviors of Timothy McVeigh, the Oklahoma City bomber, may lead him to be considered a psychopath using an indirect personality assessment.

Impact Statement: This presentation will impact the forensic science community by validating the indirect personality assessment method to determine if the behaviors of an individual reflect psychopathy.

On April 19 of 1995, the Alfred P. Murrah building in Oklahoma City was bombed by Gulf War veteran Timothy McVeigh using a deadly cocktail of fertilizer, diesel, and other chemicals. McVeigh had an extreme hatred and disgust for the government after the events at Waco, TX. The Waco incident was a 51-day siege between the United States government and the Branch Davidians that resulted in the deaths of four federal agents and 82 Branch Davidians. Additionally, he feared that the government would take away his right to bear arms.

This research aimed to analyze the life of Timothy McVeigh using an indirect personality assessment and a series of interview tapes to determine if he exhibited psychopathic behaviors. In order to complete the indirect personality assessment, many factors were examined, including details of McVeigh's family dynamic and personal relationships throughout his life, the environmental conditions of his childhood, and his physical characteristics/health.

The details of McVeigh's behavior, the most important factor, are discussed in the biography *American Terrorist: Timothy McVeigh and the Oklahoma City Bombing* by Lou Michel and Dan Herbeck.[1] The biography discusses thoughts and feelings of McVeigh throughout his life, from childhood up to his execution. Additionally, the interview tapes, conducted by Herbeck and Michel during McVeigh's prison stay (1997–2001), were utilized to gain further information and listen for emotional inflection in the voice. The combined information mentioned above was used to complete the indirect personality assessment. To conclude or disprove psychopathy, McVeigh's behaviors were rated on a scale from one to five. On the scale, behaviors under the one category are considered to be psychopathic behaviors while five are toward the social norm. Based on the assessment, a presentation of Timothy McVeigh's psychopathic behaviors was determined.

Through this research, the method of using an indirect personality assessment was furthered validated. In the future, the indirect personality assessment will allow us to determine if someone is a psychopath, whether alive or dead, based on interviews and other materials rather than a conductive interview with the individual.

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Psychological Behavior; Personality Disorder; Mass Murder

Y39   Traumatic Brain Injury and Criminal Behavior

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Learning Overview: This presentation aims to provide a review of the literature in regard to the relationship between Traumatic Brain Injury (TBI) and criminal behavior. This presentation will explore the underlying factors that influence criminal behavior within the TBI-affected population. By shedding light on this topic, this presentation hopes to inform attendees of the significance of understanding this relationship, as it holds the potential to inform legal and medical systems, guide interventions, and shape policies to enhance the well-being of individuals with TBI.

Impact Statement: This presentation will provide attendees with a better overview and understanding of how TBI can potentially cause criminal behavior, what the literature has identified as risk factors in this population, and will increase awareness of the link between TBI and criminal behavior.

Introduction: TBI can significantly affect cognitive, emotional, and behavioral functions, leading to altered decision-making capacities and impulsive behaviors. TBI may be relevant to criminal conduct as it can affect an individual's understanding of the consequences of their actions and their ability to conform to societal norms. The prevalence of TBI cases within the judicial system is high and often overlooked, underscoring the urgent need for a deeper understanding of this issue.

Methods: This literature review investigates various aspects of TBI and its potential role in criminal behavior through an analysis of relevant studies and scholarly works. We conducted a comprehensive search of databases to identify peer-reviewed articles and studies related to TBI and criminal behavior. The selected literature was critically reviewed to synthesize key findings and insights.

Discussion: The impact of TBI on criminal responsibility is complex and depends on the specific circumstances of each case. Individuals involved in legal matters may seek to establish causal links between TBI and their behavior as a defense against criminal culpability or to determine civil liability. In some cases, TBI may mitigate responsibility for the individual's actions. In other cases, TBI may be considered an aggravating factor that increases the individual's risk of reoffending. Recognizing the necessity of assessments in forensic settings is crucial to properly evaluate the role of TBI in criminal cases.

Conclusion: This review contributes to the growing body of knowledge concerning the psychiatric forensic assessment of TBI and its implications for criminal responsibility. It highlights the pressing need for a deeper understanding of the relationship between TBI and criminal behavior, especially given the increasing prevalence of TBI cases within the judicial system.[1] Furthermore, it emphasizes the importance of assessments in forensic settings and the potential of rehabilitation programs to manage TBI-related behavioral problems, ultimately enhancing social reintegration and reducing recidivism rates.

Reference:

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Forensic Psychiatry; Head Trauma; Traumatic Brain Injury

Y40 **Determining the Viability of Breathwork and Meditation as Intervention Techniques for Stress in the Forensic Science Community**

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Learning Overview: After attending this presentation, attendees will understand the influence of intervention techniques such as breathwork and meditation on the forensic science community. The principles of the intervention techniques and the workings of the analyses gathered through psychological surveys and salivary cortisol measurements will be discussed.

Impact Statement: This presentation will impact the forensic science community as it could lay the groundwork for future research studies and the possible implementation of these interventions in the workforce and coursework for students to help mitigate the impact of the stressors experienced in the field of forensic science.

Forensic science as an area of study and career that has fascinated many in society over the past few decades. While the field is constantly changing over time, it is lagging in introducing effective intervention techniques that can aid in stress reduction. Practitioners in many disciplines of forensic science are exposed to unique stressors seldom encountered in other careers. However, minimal research has been performed to analyze the physiological response to stress within the forensic science community. In this context, the forensic science community includes degree-seeking students in universities and working professionals in various sectors of the field.

The objective of this study is to determine the viability of breathwork and meditation as intervention techniques in a comparative study to alleviate stress within the forensic science community. While each member of the said community may experience the stressors of the field differently, all must be supported to ensure mental well-being in the field of forensic science. The implementation of the intervention techniques will be assessed for the impact on the reduction of stress in participants by measuring salivary cortisol levels and comparing said data to stress-related quantitative surveys.

The participants will be randomly assigned to groups that practice either breathwork or meditation. The specific breathwork pattern that will be studied is cyclic sighing, which is represented by two consecutive inhalations to maximally fill the lungs, followed by one prolonged exhalation. The group performing cyclic sighing will be requested to practice the breathwork exercise daily for five minutes for a month. The meditation group will be requested to meditate by focusing on their breath for 15 minutes every day for a month.[1] Both groups will be provided with instructional videos and soundtracks prepared for this study to guide them through the activity. Saliva samples and quantitative survey data will be collected from the participants before the implementation of the intervention techniques and after the month-long implementation period is complete.

Salivary cortisol levels will be analyzed with the use of a Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS) in a local laboratory external to the university.[2] The quantitative surveys that will be administered include the Perceived Stress Scale (PSS), the State-Trait Anxiety Inventory (STAI), and the General Well-Being Schedule (GWBS).[3-5] The questions for this study will be compiled together in a Qualtrics form. The research proposal will be submitted to the Institutional Review Board of the university for approval before the sample collection phase of this study begins. The participants will be required to fill out consent prior to participation in this study. Any personal and/or demographic information gathered will only be accessible to the researcher and the primary advisor. The surveys and the saliva samples will be coded with a combination of letters and numbers as unique identifiers that will be formulated by the researcher and primary advisor. All the data gathered from the consent forms and surveys will be disposed of digitally once the project is complete, and all the saliva samples used for testing will be discarded in the laboratory.

The results of this study will provide insights into the viability of these intervention techniques for the forensic science community. Administering these practices within the active workforce can provide information about the real-time impact of breathwork and meditation to combat the unique stressors of this field. The data collected from observing university students could further highlight the importance of implementing interventions for stress earlier, leading to a future career with improved longevity. This information could be useful in helping us find more ways to support the forensic science community and limit high employee turnover in the workforce.

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Stress; Mental Health; Forensic Professionals

Y41   The Extraction and Subsequent Analysis of Gel Pen Ink

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Learning Overview: This presentation will utilize gel pen ink and Thin-Layer Chromatography (TLC) to demonstrate how gel pen ink can be used as an important piece of evidence. After attending this presentation, attendees will understand the importance of ink analysis and why it is important to devote more time and effort into furthering research into this field.

Impact Statement: This presentation will impact the forensic community by drawing more attention to the integrity of ink-based evidence and the ways it can be analyzed using TLC.

Document examination, an important subdivision of forensic science, has many kinds of evidence that yield important results. One such type of evidence is ink analysis. Ballpoint ink, one of the most used mediums, has been analyzed multiple ways. Due to the simple nature of its composition, ballpoint ink can be separated and identified to be analyzed as evidence. There are multiple existing methods of analysis for ballpoint ink that demonstrate the specific banding pattern and separation of the ink sample.

A common method for ballpoint ink analysis involves TLC, which employs the use of a stationary and mobile phase to separate the components of a sample. Another popular medium, gel ink, is very secure and complex, which makes it difficult to wipe from important documents or analyze in general. Therefore, the ability to extract gel pen ink using similar methods to ballpoint ink extraction will be studied.

There have been past attempts to extract gel ink, but none have yet been successful. A new extraction attempt has been made to produce the best possible results. Three different manufacturers of gel ink were extracted using solvents and TLC. The expected results will demonstrate the comparability of gel ink when the source is unknown. So far, an existing method for gel ink extraction using common ballpoint ink extraction methods has not been found to successfully extract all evidence of the gel ink. Results obtained so far began to show distinct differences between the pen manufacturers, when visualized on the TLC plates and under Ultraviolet (UV) lighting.

The most successful extraction method identified so far involves an extended extraction period of two days using methanol before application to a plastic-backed TLC plate with a mobile phase consisting of acetone, n-butanol, and deionized water. The three manufacturers could be individually identified from each other under both natural and UV lighting and by the banding on the TLC plate; however, minute differences between the pens have yet to be identified.

If a concrete extraction method was identified, the impact of evidence gained from gel ink analysis would have greater significance. The opportunity to develop more gel ink extraction and analysis methods would also become relevant. Once a method of analysis is developed, the extracted ink can be used to further analyze questioned documents such as counterfeit notes, ransom notes, and forged currency. A full extraction will allow greater analysis of questioned documents and increase the importance of this evidence in the field. Therefore, further method development would ensure a full separation of all components within the sample. If a method is identified to extract gel ink and allow for analysis, it can be used as an identification tool as the exact composition of an ink pen is never the same between two pens, even from the same manufactures.

Gel; Questioned Documents; Thin-Layer Chromatography

Y42   The Ethical Considerations of Teaching Skeletons in West Virginia

Kristy Henson, MS*, Fairmont State University, Fairmont, WV; Jay Bow, Fairmont State University, Fairmont, WV

Learning Overview: This presentation discusses the importance of ethical treatment and storage of teaching skeletons in university settings. After attending this presentation, attendees will understand problems surrounding "antique" teaching skeletons and implications for how these individuals were acquired, how they are currently treated, ways to improve storage and care for these human remains, and how they may end up in civilian possession.

Impact Statement: This presentation will impact the forensic science community by being of assistance when one encounters skeletal remains used previously for teaching purposes but that have ended up in civilian possession. This presentation may also inspire a reexamination of teaching skeletons at one's institution.

Human skeletal remains have commonly been subjected to the medicalization of science through purchasing or acquiring after cadaveric dissection. From the late 1800s until 1985, teaching skeletons were acquired by American universities and K-12 school systems from India.[1-3] The skeletal trade stopped when the public learned these skeletons were acquired illegally.[2] These teaching skeletons are now considered antiques, and these individuals tend to be forgotten, damaged, and poorly stored as instructors switch to plastic teaching models. Skeletons no longer used may end up in storage or liquidated by appearing for sale online or at oddities shows, allowing these individuals to end up disarticulated, damaged, and in civilian hands.

When cleaning out unused laboratory space, we discovered teaching skeletons stored at our university, spearheading this project. This project has three parts: we examined West Virginia's laws on human skeletal remains, inquired with 12 undergraduate universities in the state about their use and storage of antique teaching skeletons, and, finally, collected all of the skeletal material in our department and applied museum-quality curation to the remains while determining osteological profiles for the individuals. Results were as follows. (1) West Virginia has one state law, §29-1-8a, that outlaws grave robbing. There are no corpse abuse laws. It is legal to buy, sell, and alter non-medicolegal human skeletons in this state. If law enforcement encounters someone with human skeletal remains, they will respond as medicolegal until proof of purchase is shown, and it is not explored further. (2) Four out of 12 institutions responded. These institutions still house their antique teaching skeletons. Two have switched to plastic skeletons and two still use human skeletons. (3) The Minimum Number of Individuals (MNI) for this collection is 42. Age, sex, ancestry, height, and pathology were determined.[4]

A protocol for antique teaching skeletons needs to be established in the United States. It should follow the protocols being created by many osteological museum collections as we take more time to examine the ethical acquisition, repatriation, and ownership of these individuals when consent was not involved.[3]

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Anthropology; Skeletal Remains; Ethics

Y43   Restoring Demographic Information of Antique Teaching Skeletons at Fairmont State University

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Learning Overview: As a case study involving the osteological profiling of antique teaching skeletons, the goal of this presentation is to emphasize the process of restoring personal information lost due to the lack of associated records as well as the overall condition of the remains. Methods of macroscopic identification of demographics of skeletal remains will be introduced and attention will be drawn to antique teaching skeletons as they often are a remnant of crime and ethical violations that gain little to no attention due to being very difficult or impossible to trace.

Impact Statement: This presentation will impact the forensic science community by covering the detrimental effects of the medicalization of teaching skeletons, emphasizing the dehumanizing aspects of their care and the depersonalization that occurs with the lack of records for many of them. This promotes osteological profiling of collections and promotes ethical practices.

Fairmont State University's antique teaching skeleton collection has a Minimum Number of Individuals (MNI) of 43, containing highly damaged partial skeletons. These skeletons have no associated records; however, due to being purchased in the mid-1900s, we can safely assume that they were obtained from India as that was the primary supplier of skeletons to American institutions during this time period.[1,3] Additionally, 12 of the individuals were identified as having Asian ancestry and two had Caucasian ancestry, supporting the above assumption. These skeletons have been mistreated, resulting in postmortem damage and dissociation during their period of use, and they therefore required sorting and extra care along with osteological profiling. Osteological profiling can provide demographic information such as skeletal age, sex, ancestry, height, and pathology, allowing the restoration of personal information of these individuals and giving them the respect they deserve.[1,2,4] Of the 43 individuals, only 28 had enough bones present for analysis. All individuals were adults at their time of death, with 7 probable females, 7 probable males, and 4 intermediate and therefore indeterminate sex, and another 10 individuals missing any potentially sexually dimorphic bones.

These efforts allow for the humanization of these individuals, and proper storage will be provided for each individual to prevent further damage and keep these individuals from being dissociated and lost within the medicalization of the system.[1,2] More guidelines and restrictions should be put in place for teaching skeletons to prevent damage from mishandling and improper storage like that present in this collection. Even if there is no legal requirement to treat these skeletal remains in a certain manner, there are ethical requirements that should be adhered to. It is important to remember teaching skeletons and other archived skeletal remains are the remains of a person who deserves respect and must be treated as a person just as much as other remains are.

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Anthropology; Skeletal Remains; Ethics

Y44   Injury Recidivism as Embodied Evidence of Structural Violence: A Trauma Analysis Examining Fracture Location, Severity, and Occurrence in a New Mexico Forensic CT Sample

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Learning Overview: After attending this presentation, attendees will understand that adverse social experiences may be embodied within skeletal remains in ways that can be interpreted postmortem.

Impact Statement: This presentation will impact the forensic science community by testing the hypothesis that the number, severity, location, and occurrence of ante- and perimortem fractures correlate with the axes along which experiences of social marginalization are structured in the contemporary social demographics (i.e., sex, social race, and class).

Injury recidivism, or the reoccurrence of injuries, has recently become an intersectional space for fields such as biomedicine, biological, and forensic anthropology to analyze and understand trauma.[1] However, few works have adequately mapped these injuries, intentional or accidental, over the course of an individual's life while also contextualizing them within the individual's lived experiences. Specifically, social experiences of structural or systemic inequality can become embodied in an individual's bones and thus can be captured by traditional biological anthropology methods.[2,3]

Structural vulnerability refers to the processes by which social phenomena like oppression or exploitation can come to have real, biological effects in peoples' bodies, producing patterns of suffering, disease, and early death among human populations who live through experiences of marginalization.[2] In keeping with the *violence continuum*—a body of theory that describes various forms of violence that can impact human bodies—inequalities and the systems that reinforce them perpetuate further violence on their bodies as they attempt to heal.[4] This research aims to highlight that structural vulnerability may correlate with an increased risk of injury and the exacerbation of existing injuries.

This poster presents fracture data collected from Computed Tomography (CT) imagery from 400 forensic decedents (n=200 female; n=200 male) who: died in New Mexico; identified as Black, Hispanic, Indigenous, or White; and whose casefiles included information about their Socioeconomic Status (SES). These images originate from the New Mexico Decedent Image Database (NMDID).[5] All anonymized medicolegal CT images in the current sample were authorized for research purposes by next-of-kin and thus provide contextualized demographic data to further articulate skeletal data with lived experiences.

This research tests the hypothesis that experiences of interpersonal and accidental trauma will correlate with demographic data (i.e., sex, social race, SES), given that social inequity in the United States is often distributed along lines of sex, social race, and class. The presentation will present data on the number, location, severity, and occurrence of antemortem and perimortem fractures for the sample decedents and analyze observed patterns to test for potential correlations between fracture data and the decedents' demographic data that contextualize the lived experiences of sex, social race, and class. Fractures will also be interpreted while acknowledging the limitations of inferring interpersonal violence or accidents. This research strives to illuminate the precarity that injuries add to everyday lives and their probable contribution to premature death.[6] By contextualizing skeletal injuries within other biomarkers of inequality, this work intends to illustrate how injuries must be understood within an individual's positionality and lived experiences even after death. In interpreting the experiences of those whose injuries have been overlooked or devalued, this work hopes to demonstrate their resilience and support their fight for equality and justice.

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Anthropology; Trauma; CT Scan

Y45   **An Evaluation of Calcium Removal During Demineralization of Bone for Stable Isotope Analysis of Collagen: A Determination of Optimal Treatment Time**

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Learning Overview: After attending this presentation, attendees will learn optimal treatment time for demineralizing bone samples for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotope analysis of collagen.

Impact Statement: This presentation will impact the forensic science community by providing quantitative evidence to support best practices for sample preparation of bone collagen samples for isotopic analysis.

Demineralizing bone is a crucial step in the sample preparation of bone collagen for isotopic analysis of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. This step is typically done in multiple rounds of acid treatment. However, parameters such as acid molarity, duration, and temperature vary across methods. While many labs conduct method validations for internal standard operating procedures, the methods used often begin by adopting a published or handed-down method. Often the experimental evidence supporting each step of these methods is difficult to find due to lack of publishing in-house method optimizations. An optimal method would demineralize modern bone rapidly without affecting the isotopic integrity of the sample. This study quantified the rate of calcium removal throughout acid treatment to determine optimal treatment time for bone demineralization.

For this study, five rib sections from a cow were demineralized at room temperature with a commonly used reagent, 0.25M hydrochloric acid (HCl). Aliquots of the demineralizing acid were taken at increasing timepoints through demineralization, and the concentration of calcium (Ca) in solution was measured via inductively coupled plasma optical emission spectroscopy. Calcium concentrations were evaluated to estimate when the reaction had reached maximum efficiency.

Ca concentration of the demineralizing reagent increased sharply up to 48 hours (>20% increase in solution concentration in each 24-hour period), with one sample still displaying moderately increasing concentrations up to 96 hours, although at a slower rate (increase of 20% over a 48-hour period). Beyond these timepoints, the rate of Ca extraction drops by more than half, indicating less Ca is being removed.

Based on the Ca concentration data, demineralizing efficiency decreases beginning at 48 hours. Although several rounds of acid treatment are necessary for complete demineralization of bone, this study suggests the optimal treatment time for each round is 48 hours.

Bone Collagen; Best Practices; Stable Isotope Analysis

Y46 Evaluating the Effectiveness of Quantitative Skeletal Age Estimation Methods Compared to Traditional Qualitative Methods

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Learning Overview: The goal of this presentation is to utilize data from the Louisiana State University (LSU) Forensic Anthropology and Computer Enhancement Services (FACES) Laboratory Donated Skeletal Collection; attendees will evaluate the effectiveness of quantitative skeletal age estimation methods, such as Dirichlet Normal Energy (DNE) and Orientation Patch Count (OPC), compared to traditional qualitative methods for adults over 50. Attendees will also consider the impact of practitioner experience and technology on age estimation accuracy and recognize the importance of advancing user-friendly quantitative techniques in forensic anthropology.

Impact Statement: This presentation will impact the forensic science community by highlighting the potential limitations of traditional age estimation methods for older adults in forensic anthropology and emphasizing the need for accessible and accurate quantitative techniques, contributing to advancements in identification processes and law enforcement efforts.

Forensic anthropologists have long faced the difficulty of producing effective age estimations for adults over 50 years of age that are broad enough to encapsulate the range of degenerative but are also narrow enough to aid law enforcement agencies with identification. Many commonly used adult age estimation methods rely heavily on the visual assessment of gross macroscopic features indicative of stages or phases of skeletal joint deterioration. A significant problem with these methods is their reliance on an observer's level of experience with the wide range of variations that can occur throughout the human aging process. With advancements in technology, researchers have developed new quantitative methods for skeletal age estimations that rely on a user's familiarity with technology as opposed to age variation. Most recently surface Complexity Measurements (SCM) such as DNE and OPC have been used by researchers to quantify the increased variation observed in older adult skeletal joint surfaces. This project seeks to examine the effectiveness of SCM at quantifying age-related changes of two pelvic joint surfaces, the Pubic Symphysis (PS) and the Auricular Surface of the ilium (AS), from a regional population and compare the results against the commonly used qualitative methods for the same joint surfaces.

A total of 42 os coxae from the LSU FACES Laboratory Donated Skeletal Collection were selected for this study. The sample consisted of 31 males and 11 females, with ages ranging from 20 to 85 years (a mean age of 51.162). The PS and AS were scanned with a FARO® Design Scan Arm 3D Scanner. All scans underwent post-processing in $^{Geomagic\ Wrap®}$ software to isolate the PS and AS and were converted into mesh models. Mesh models were imported into R statistical software to calculate DNE and OPC values following the methods described by Pampush et al.[1] Additionally, the PS and AS from each os coxa were scored by a practitioner with over five years of experience with the qualitative methods. Spearman's correlations were conducted to identify relationships between age and each of the SCMs. The results from the Spearman Correlation found DNE values and age for the AS have a negative correlation $r = -0.24490$, with a P-value of 0.1440; this shows that DNE value with age has a weak negative correlation. Additionally, DNE values with age for the PS is $r = 0.13257$, with a p-value of 0.4341; this shows that DNE values with age have a weak positive correlation. When examining OPC values with age for the AS and PS, the results are similar. OPC values and age for the AS are $r = 0.09344$, with a p-value of 0.6171, and for the PS OPC values and age $r = 0.17619$, with a p-value of 0.2969.

From the results, both DNE and OPC values had weak correlations with age. Comparatively, the qualitative methods found moderate to strong correlations; this could be the result of the practitioners' experience and knowledge with using qualitative pelvic aging methods. Furthermore, with the advancements in technology, tools like FARO® Design Scan Arm 3D Scanner are paving the way for regular approaches to quantifying age-related changes in skeletal joint surfaces. Nevertheless, it is imperative to acknowledge that some studies utilize similar cutting-edge technology and have reported robust correlations with age-related changes. Discrepancies in results may be influenced by limitations such as the utilization of relatively small sample sizes or potential sources of user error. While this study's findings may not be without imperfections, they underscore the need to explore additional quantitative methods that offer improved accuracy and user-friendliness in the age estimation process.

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Age Estimation; 3D Technology; Method Validation

Y47 "Known Only to God": Rediscovering a Historic Freed Slave Cemetery Through the Use of Fluxgate Gradiometry and Its Application to Forensic Science

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Learning Overview: After attending this presentation, attendees will have learned the basics of using a fluxgate gradiometer through an example of a historical cemetery survey. Attendees will also learn how this instrument and others like it can be used in forensic search and recovery missions.

Impact Statement: This presentation will impact the forensic science community by demonstrating the impact of using applied archaeological and geophysical methods to aid in forensic search and recoveries. Many tools, methods, and processes from other disciplines greatly aid in forensic casework, encouraging inter-discipline cooperation that leads to an increased likelihood of locating clandestine burials.

The Amasa Stone Cemetery is a small, historic cemetery outside of Stoneboro, Mercer County, PA. This cemetery is found near the location of a historic town, dubbed "Liberia" by its inhabitants, which was a significant stop along the Underground Railroad. In 1818, the land where the cemetery sits was purchased by a freed slave, Richard Travis, Sr., who helped establish this location as a safe haven for freed and escaped slaves fleeing the American South.[1] Until the late 1970s, the cemetery was used as a final resting place for formerly enslaved peoples and their descendants. Through historical documentation and headstone information, only ten individuals are confirmed to be buried here, but it is estimated that there are likely many more.

The goal of this project was to collect surface and subsurface data from the area using non-destructive surveying technology with the intention of locating potential unmarked burials and emphasizing the significance of this area as a landmark for the National Register of Historic Places for the Underground Railroad. An initial geophysical survey was conducted to estimate the potential number of clandestine graves both within and outside the modern boundaries of the cemetery.[2] Microtopography, magnetometry, ground penetrating radar, and fluxgate gradiometry were employed, and several anomalies were observed. The presenting authors were responsible for the fluxgate gradiometer survey. From this survey, a minimum of six anomalies were identified, possibly indicating the location of clandestine graves. The six paired anomalies appeared in a rectangular-like formation measuring approximately 7.5–11 feet in length, similar to the configurations of old wooden boxes or coffins joined by metal nails at each corner.

This preliminary data is promising and indicates a need for further studies of this area and others like it. Because this research demonstrates the utility of the fluxgate gradiometer in potentially locating clandestine graves, the authors will argue for its applicability to the pursuit of forensic search and discovery.

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Burial; Survey; GIS

Y48   A Digital Microfluidic Approach to Processing Forensic Samples

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Learning Overview: After attending this presentation, attendees will have learned about our proposed method of processing sexual assault evidence in an automated fashion with the use of a microfluidic platform and the motivation behind this project.

Impact Statement: This presentation will impact the forensic science community by informing attendees that valuable efforts are being made to make a robust system that can successfully identify the perpetrators from sexual assault evidence.

Integrated Rapid DNA Analyzers can perform sample-to-answer forensic DNA Short Tandem Repeat (STR) -typing in under two hours in a form-factor that can be operated outside of the laboratory. These systems are incompatible with samples containing DNA from multiple sources (as is the case for sexual assaults), which must be sent to a laboratory for sample processing by Differential Extraction (DE) or Differential Digestion (DD) to separate the male DNA from the female DNA.[1] We automated some of these preliminary steps using Digital Microfluidics (DMF). While other forms of microfluidics have been used for forensic applications, this represents, to our knowledge, the first report of using DMF in forensic science.[1]

In DMF, an array of electrodes is used to manipulate discrete droplets of liquids, enabling moving, heating, and mixing functions in an automated fashion. DMF devices were fabricated and controlled using a customized version of the open-source DropBot control system that has previously been validated for work outside of the laboratory.[2]

The two sample types we worked with were buccal swabs spiked with diluted seminal fluid (mainly used to assess extraction efficiency) and vaginal swabs collected post-coitus (more representative of sexual assault samples). The non-sperm DNA digestion and sperm lysis portion of DD was automated using DMF. The sperm fractions were purified using QIAamp® DNA Investigator Kit and multiplex quantitative Polymerase Chain Reaction (qPCR) was performed to quantify DNA via PowerQuant™. DNA quantification was followed by conventional lab-based amplification with the Identifiler® Plus STR Kit and capillary electrophoresis on the Applied Biosystems® 3500 XL to generate STR profiles. The sperm fractions of four vaginal swabs, collected up to 12 hours post-coitus, were loaded into a Rapid DNA Analyzer, which performed all of the purification, amplification, and capillary electrophoresis steps automatically in under two hours.[3]

DE (adapted from Alderson et al.) and DD (adapted from Wong et al.) were compared off-chip; DD had higher extraction efficiency than DE.[4,5] Low Autosomal/Y ratios (below the threshold of 2) are comparable with single-source samples from males. Low Autosomal/D (below the threshold of 2) ratios for sperm fractions indicates that exposure to DNase did not degrade the target sperm DNA. From our investigations we can conclude DMF DD and off-chip DD performance are analogous with respect to male DNA purity and DNA degradation; both methods produced sufficiently pure male DNA without significant amounts of degradation. DD of vaginal swabs (collected 1 hour, 3 hours, 6 hours, and 12 hours post-coitus) on DMF yielded enough DNA for downstream processing.

Performing STR analysis on post-coital samples processed on DMF was the main validation step in this study. No female DNA carryover to the obtained male fraction was observed, and the allele calls for sperm fractions processed in tubes and using DMF were identical for all loci, whether the STR analysis was performed using a conventional lab instrument or a rapid DNA analyzer.

In sum, DMF can be used to perform segments of a differential digestion protocol in an automated fashion. In the near future, we plan to process a wider array of sample types, including increased time intervals post-coitus. The outcome of this research project opens the door to sample-to-answer automated evaluation of sexual assault samples.

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Sexual Crimes; Forensic Science; STR

Y49   A Call for Community: Working Toward Humanitarian Solutions to Address Inadequate Indigenous Death Investigations in North America

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Learning Overview: After attending this presentation, attendees will understand the necessity of bringing humanitarian forensic action to North America to address the worsening crisis of Indigenous death and missing persons investigations. This foundational research will demonstrate how many North American investigations fail Indigenous peoples, how each forensic institution and professional can help create supportive solutions, and how related stakeholders can apply lessons from humanitarian forensic action to collaborate with and support differing cultural communities during investigations.

Impact Statement: This presentation will impact the forensic science community by uncovering how standard investigative practices have failed thousands of Indigenous peoples and providing next steps to ensure justice for past and future Indigenous cases. The results will demonstrate that guidance from within our community, such as humanitarian forensic scientists, and outside our community, such as Indigenous rights advocates, can create collaborative solutions toward equalized justice.

Humanitarian forensic action helps alleviate human suffering through the identification and recovery of human remains following mass deaths and disappearances. In the United States and Canada, the Indigenous community has endured an ongoing issue of mass death and disappearances (i.e., Missing and Murdered Indigenous People [MMIP]), yet the investigations into the majority of these cases are inadequate and have left many families entrapped in ongoing trauma.[1-4]

To find justice for Indigenous families, this research examined where North American investigations fail Indigenous cases and if humanitarian forensic action can guide future solutions. The research includes three parts: (1) in-depth examinations of Indigenous perspectives, with interviews and published testimonials, on investigations into the death and disappearance of their loved ones; (2) semi-structured interviews with investigative stakeholders (e.g., police, medicolegals, and forensic scientists) about the role of standard procedure and why it may contribute to inadequate investigations; and (3) n in-depth examination of humanitarian forensic action literature and case studies to uncover possible alternative investigative practices and collaboration tactics as prioritized in recommendations from Indigenous and investigative stakeholders. Inductive coding in the first two parts demonstrated conflict between cultural, scientific, and state investigative goals. It also uncovered broken communication between different investigative institutions and lacking trust between investigators and Indigenous peoples. The third part highlighted how the humanitarian forensic community regularly employs practices that could address such concerns by acknowledging the differences between institutional principles and cultural community needs. Their standards encourage open dialog between both sides to determine investigative practices and goals in each circumstance.

These outcomes suggest that not only is it possible for the forensic community to acknowledge where their traditional practices may not suit differing communities, but also that dedicated communication and collaboration are helpful tools in establishing such alternatives—an effort requested and advocated for by Indigenous people(s) for decades to address MMIP cases. For the forensic science community to achieve justice for Indigenous cases in North America, it is vital to examine the impact of existing standard practices on differing communities.

This research has begun to identify many Indigenous and non-Indigenous professionals dedicated to finding solutions for the future. In an ongoing effort to encourage "Justice for All" values in the forensic community, the researchers present an open call for investigative stakeholders to participate and collaborate with Indigenous partners to find solutions for MMIP cases.

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Death Investigation; Human Rights; Indigenous

Y50   A Probabilistic Genotyping Analysis of Trace DNA and Transfer Evidence

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Learning Overview: The goal of this presentation is to describe the performance of trace DNA in a defined transfer setting and analysis with probabilistic genotyping software. While allele counting is a common process among laboratories for determining the usability of low-level samples, probabilistic genotyping analysis can offer a more thorough understanding of the usefulness of low-level data.[1]

Impact Statement: This presentation will impact the forensic science community by informing forensic laboratories and other forensic personnel of the statistical power of low-level data.

The amount of trace DNA deposited on a surface is typically dependent on the individual's shedder status and the amount of pressure applied to the object.[2,3] This project was completed in two phases. The first phase of this project narrowed down deposition procedures, best shedders, and if a profile could be generated. The second phase focused on the transfer of trace DNA with two contributors.

Probabilistic Genotyping (PG) software, STRmix™, was utilized to examine the statistical confidence of potential contributor genotypes to better assess the usefulness of the data. For Phase II sample analysis, the numerator for all ratios included both contributors, while the denominators were designed to assess each contributor as a Person Of Interest (POI). Each POI deconvolution was run in triplicate. The PG analysis was evaluated for accuracy in the POI genotypes. Only likelihoods that met the 99% 1-sided Highest Posterior Density (HPD) were reported to communicate profile statistical strength, rather than only an allele count.[4]

As much of the data in this study was below potential stop at quants and calculated stochastic thresholds, laboratories without probabilistic genotyping would not define this data as usable. Additionally, when only focusing on allele counts, the same count does not always mean the presence of the same alleles, which adds to the complexity of evaluating the usefulness of touch/trace DNA evidence.

Touch DNA, while seemingly prominent, is not a good source of DNA to confidently decide on the presence of an individual. As seen through this experimentation and probabilistic genotyping analysis, it is unlikely a useful DNA profile is obtained only through transfer. This study also supports the need for validation and understanding of the performance of low-level data and transfers, which encourages the need for further research with current science and as science improves in the future with low-level data.

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Trace DNA; Transfer Evidence; Probabilistic Genotyping

Y51   A Semi-Automatic Tool for Footwear Impression Alignment

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Learning Overview: After attending this presentation, attendees will be informed about the alignment methodology implemented in our proposed tool and how to use it.

Impact Statement: Footwear impressions are crucial in crime scene investigations, but manual alignment, often done with software like Photoshop®, can be time-consuming and skill-dependent. This presentation will impact the forensic science community by explaining how our tool automates and expedites the alignment process, reducing the workload for examiners and enabling faster processing of multiple tasks. Thus, it can be a useful tool for enhancing the efficiency of footwear examinations.

We introduce a semi-automatic alignment tool tailored for two similar footwear impressions. The term "semi-automatic" is used because the alignment process is primarily automated, yet users have the flexibility to fine-tune the results by adjusting certain parameters. This presentation provides an in-depth explanation of the alignment methodology employed in our tool. Furthermore, we demonstrate the tool's capability to effectively align high-quality shoeprints with mock crime scene impressions made by bloody outsole.

Our proposed tool is specifically designed to align two similar shoeprints, which could either originate from the same shoe or share common class characteristics despite originating from different shoes. The primary objective is to ensure the proper alignment of their outsole patterns when overlaid. When two similar shoeprints are provided as input, our tool generates aligned shoeprints as the output. The alignment process unfolds through three distinct steps, which we discuss below.

Step 1—Initial Downscale Alignment: We start by downscaling the two images, representing the Questioned footwear impression ([Q]) and the Reference impression ([R]) to facilitate faster processing. These downsampled images ([q] and [r]) are reduced to one-eighth of their original size. Subsequently, we employ cross-correlation, computed using Fast Fourier Transform for efficiency, to align [q] with [r].[1] The outcome of this step is denoted by (θ, x, y), representing the rotation angle and horizontal and vertical shifts necessary to align [q] with [r] within a ±30-degree range.

Step 2—Alignment in Original Scale: To align the original-sized [Q] with [R], we apply the rotation angle (θ) and shifts ($8x, 8y$) determined in the previous step.

Step 3—Refinement in Original Scale: This final step involves refining the alignment at the original scale. We compute the correlation between the aligned [Q] and [R] while considering a small range of translations and subsequently update the translation to maximize the correlation. By shifting the aligned [Q] according to the updated translation, we achieve the final alignment with [R].

Our tool then presents the aligned [Q], along with [R], to the user. Additionally, users have the option to manually adjust the rotation angle and translation as needed, providing a comprehensive alignment solution that combines automation with expert control to take advantage of forensic footwear examiners' knowledge and experience.

Reference:
1. Kaso, A. (2018). Computation of the normalized cross-correlation by fast Fourier transform. *PloS one*, *13*(9), e0203434.

Shoeprints; Footwear Evidence; YFSF

Y52 **An Interdisciplinary Approach for the Identification of Antique Skeletal Teaching Specimens: DNA Extraction, Forensic Bone Standards, and X-Ray Analysis**

Cynthia Nichols*, Cameron, NC

Learning Overview: By examining the morphological characteristics and growth patterns of juvenile bones, forensic anthropologists and archaeologists can refine their ability to ascertain sex and ethnicity from incomplete or fragmentary remains. The goal of this presentation is to shed light on the invaluable contributions of juvenile skeletal standards to the field of forensic anthropology, enhancing our understanding of past populations and aiding in the resolution of contemporary forensic cases.

Impact Statement: This presentation will impact the forensic science community by explaining how the outcomes of this research project will not only benefit the scientific community but also support museum curators, historians, and educators in contextualizing and preserving antique teaching specimens, ensuring their responsible display and interpretation for future generations.

The proposed research project aims to employ a multifaceted approach to identify the skeletal remains of an antique teaching specimen discovered to be the skeletal remains of a juvenile utilizing advanced techniques in DNA extraction, forensic bone standards, and X-ray analysis. This endeavor responds to the compelling need for accurate and ethically responsible identification of historical human remains, contributing to the preservation of cultural heritage and scientific knowledge.

The study will use DNA extraction from the skeletal remains, applying state-of-the-art protocols to obtain high-quality genetic material. This genetic information will then be compared to contemporary reference databases, allowing for the establishment of ancestry, sex, and potential familial relationships of the individual, thereby shedding light on their historical and geographic origins.

In parallel, forensic bone standards will be employed to assess age at death, stature, and other key biological attributes of the specimen. Utilizing established forensic anthropology methodologies, this analysis will provide valuable insights into the individual's life history, offering a deeper understanding of the era in which they lived.

Juvenile skeletal remains pose unique challenges due to their ongoing growth and development. To overcome these hurdles, this study explores established methodologies and emerging techniques for assessing skeletal elements that undergo significant changes during adolescence. By examining the morphological characteristics and growth patterns of juvenile bones, forensic anthropologists and archaeologists can refine their ability to ascertain sex and ethnicity from incomplete or fragmentary remains. This research sheds light on the invaluable contributions of juvenile skeletal standards to the field of forensic anthropology, enhancing our understanding of past populations and aiding in the resolution of contemporary forensic cases.

Additionally, X-ray analysis played a pivotal role in this research project. The integration of DNA analysis, forensic bone standards, and X-ray imaging will facilitate a comprehensive and multidisciplinary approach to the identification of the antique teaching specimen. This holistic method not only honors the dignity of the individual but also enhances our understanding of historical practices in the fields of medicine, education, and anthropology.

Anthropology; Subadult; DNA

Y53 Trend Identification in X-Ray Diffraction Peaks of Historic Cartridge Cases From Fort Ord, California

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Learning Overview: The goal of this presentation is to present information about changes in the chemical makeup of military ammunition cartridge cases ranging from the 1940s through the 1990s. After attending this presentation, attendees will have a better understanding of how X-ray analysis methods can uncover variations in the chemical compositions of remnant cases depending on factors such as the manufacturer, year of manufacture, and case type. Additionally, this research will reveal trends in the choice of cartridge manufacturer by the Fort Ord military training base in Monterey County, CA, possibly relating to consistency in chemical makeup.

Impact Statement: This presentation will impact the forensic science community in two ways. First, this research will advance historic-forensic analysis of military ammunition from the second half of the 20th century. The majority of ballistics research has focused on the bullets themselves, rather than the cases; furthermore, although some studies exist that focus on chemical makeup, case analysis has been primarily qualitative. Secondly, this project focuses on analyzing the chemical compositions of remnant cases and using information from the chemical make-up to raise awareness on the importance of proper clean-up following the closure of military training bases and on gun ranges.

Fort Ord, a former military training base located in Monterey County, is currently undergoing mass urbanization and restoration efforts. Cartridge cases from the early 1940s through the early 1990s can be found in many public-access areas of the former military training base. These cartridge cases provide a unique opportunity for historic-forensic analysis, which could potentially reveal trends in chemical makeup depending on factors such as manufacturer, year of manufacture, and cartridge case type. The chemical makeup of cartridge cases and differentiation thereof is largely understudied, and this project aims to remedy this.

Previous studies conducted on metal pollution resulting from military activity revealed significant levels of soil contamination with Pb, Cu, Cd, Sb, Cr, Ni, and Zn.[1-3] Ballistic remnants such as bullets can weather down over time, causing toxic elements to leach out into surrounding soils and potentially contaminate groundwater. Military ammunition manufactured during or prior to World War II contained corrosive primers, and many of the cartridge cases found on Fort Ord were manufactured in that time period. Analyzing the chemical compositions of remnant military cartridge cases could potentially reveal whether corrosive residue left behind on remnant cartridge cases can leach toxic metals into the surrounding environment.

For this project, a sample of 1,228 cartridge cases were collected from the former Fort Ord military training base and are undergoing X-Ray Diffraction (XRD) analysis. Preliminary data analysis of diffraction peaks reveals statistically significant differences between cartridge case types and point to additional trends dependent on manufacturer and year of manufacture. As this analysis continues, we aim to determine whether these initial trends become more robust as our sample size increases to all 1,228 cartridge cases. Future directions include complementary analysis with X-Ray Fluorescence (XRF) to determine precise chemical compositions for each sample to further elucidate the trends observed during XRD analysis.

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Cartridge Casings; Environment; Evidence Evaluation

Y54   A Qualitative Analysis of Xylazine, Nitazenes, and Common Opioids/Analogs by Liquid Chromatography/Tandem Mass Spectroscopy (LC/MS/MS) Following Collection With the M-Vac® Wet-Vacuum System

Madeline I.L. Robinson, BS*, Marshall University, Huntington, WV; Lauren L. Richards-Waugh, PhD, Marshall University, Huntington, WV; Catherine Rushton, MSFS, EdD, Marshall University, Jacksonville, FL

Learning Overview: This presentation will emphasize the need for testing methods concerning the rise of xylazine and nitazene compounds in the United States drug supply. It will also provide a discussion point for employing the M-Vac® Wet-Vacuum System for use in recovering trace drugs from packaging materials in addition to touch DNA recovery.

Impact Statement: This presentation will impact the forensic science community by describing a method to detect drug residues in the solution collected during the wet-vacuuming of porous evidence for DNA collection. The developed method demonstrates the potential evidentiary value for a sample already collected but discarded after filtering to collect DNA evidence.

Clandestinely produced drugs and adulterants are constantly being introduced to the drug market by suppliers seeking to avoid detection and penalties. The introduction of synthetic opioids like fentanyl and nitazene compounds along with the non-opioid sedative, xylazine, to the illicit drug supply has made it increasingly difficult to determine the true contents of already dangerous drugs. Fentanyl boasts a potency that is 100-fold greater than that of heroin, while nitazene compounds are 10-fold more potent than fentanyl.[1,2] Even more unsettling, xylazine, also known as "Tranq" and "Zombie Drug," is known to worsen the life-threatening effects caused by opioids while also causing severe necrotic skin ulcerations.[3] Although naloxone is effective in reversing the effects of opioids, like fentanyl, it proves inadequate in regard to the potency of nitazene compounds and the pharmacological effects of xylazine.[2,3] The combination of xylazine with fentanyl has been seen in 48 states and appears to be following the same East-to-West progression as fentanyl.[3,4] In April 2023, the use of Fentanyl Adulterated or Associated with Xylazine (FAAX) was declared an "emerging drug threat" by the United States government.[3] These drugs/adulterants are likely entering the United States drug supply at a rapid rate due to a lack of detection at the postal inspection level as there is currently no method for detecting these drugs in the increasingly small amount required to exhibit their effects. The Marshall University Forensic Science Center sought to devise a new method for qualitative analysis of these compounds along with commonly encountered opioids found on packaging materials. The inside of packaging material was investigated for trace amounts of drugs using an M-Vac® Wet-Vacuum System. This instrument has been successful in recovering trace amounts of touch DNA and holds potential when applied to trace drug recovery.[5] The phosphate-buffered solution used during M-Vac® recovery is typically discarded following filtration to isolate DNA. The discarded solution can then be analyzed for the presence of drugs.

Thirty-four drugs/adulterants/standards were included in this study: lidocaine, quinine, dextromethorphan, diphenhydramine, protonitazene, N-desethyl isotonitazene, N-pyrrolidino protonitazene, nitazene, xylazine, and the scheduled drugs/adulterants included codeine, morphine, fentanyl, cocaine, heroin, oxycodone, hydrocodone, methamphetamine, methadone, buprenorphine, ketamine, isotonitazene, metonitazene, carfentanil, valeryl fentanyl, U-47700, AH-7921, 6-monoacetylmorphine (6-MAM), acetylcodeine, isotonitazene-d7, metonitazene-d3, fentanyl-d5, heroin-d9, acteylmorphine-d3 and acetylcodeine-d3. The M-Vac® was used to recover a 5mg homogenized sample containing these 34 compounds from the inside of a Tyvek® envelope. A 5mL aliquot of the phosphate-buffered solution from the M-Vac® collection underwent solid phase extraction followed by qualitative analysis. All 34 compounds were successfully identified using LC/MS/MS to analyze the sample extract. The results from this initial study demonstrate the potential for the identification of drug residues from porous substrates (such as packaging materials) and from a sample previously discarded following DNA collection.

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Drug Analysis; Opioids; Liquid Chromatography

Y55   **Can the Use of Sharpies® in Forensic Analysis Be a Source of DNA Transfer and Contamination When Examining Difference Fabric Types?**

Haley Elizabeth Murphy, BS*, Duquesne University, Leesburg, VA; Pamela Marshall, PhD, Duquesne University, Pittsburgh, PA

Learning Overview: This presentation will investigate the possibility of DNA transfer from forensic casework Sharpies® by analyzing two case scenarios: human error and pre-existing DNA. After attending this presentation, attendees will have a better understanding of DNA contamination during evidence collection when Sharpie® markers are being used to outline dried body fluid stains.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of investigating forms of potential DNA contamination in a crime laboratory. DNA analysts will take greater precautions when handling DNA evidence with a Sharpie® marker. This will minimize the chances of DNA transfer and contamination throughout the evidence analysis process.

Body fluids on fabric items are a commonly encountered type of evidence at a crime scene. During the processing of evidence, the fluid is outlined using a Sharpie® marker to assist the DNA analyst to sample the correct area. After the marker has been used, it is capped, cleaned, and put away for its next use. The felt tip of the Sharpie®, which has direct contact with the fabric, does not get cleaned between uses. Additionally, the felt fibers of a Sharpie® tip are similar to the natural fibers of a cotton swab, which are utilized in crime labs to collect sources of DNA. The similarities between swab and Sharpie® fibers as well as the repeated direct contact with body fluid-stained evidence increases the potential for DNA transfer. The ability of a Sharpie® to collect DNA was compared using a human error scenario and pre-existing DNA scenario. Furthermore, the amount of DNA transferred was analyzed based on the number of uses, fabric type, and what fluid was being outlined.

The process began with each Sharpie® being swabbed using a cotton swab moistened with sterile water. The extraction method involved the QIAGEN® QIAamp® Quantifiler® kit with the cotton swab extraction protocol. Then, QuantiFiler® HP was used to quantify the amount of DNA extracted from each sharpie. Afterward, the GlobalFiler® amplification kit in tandem with capillary electrophoresis on the SeqStudio™ genetic analyzer was utilized to generate DNA profiles.

Preliminary testing completed by Danielle Guckin confirmed the possibility of DNA transfer from Sharpie® tips; therefore, the testing in this study is expected to produce similar results.[1] It is hypothesized that Sharpie® markers have the ability to collect DNA and that the concentration of DNA in the Sharpie® tip increases with more uses. The importance of this research is to prevent future contamination of DNA samples through the continuous use of Sharpie® markers.

Reference:

[1]   Guckin, D. DNA Contamination From Handled Sharpie® Markers Used to Outline Bodily Fluids in a Forensic Laboratory. *University, D., Ed.*; 2020; p 189

Sharpie®; Forensic DNA Contamination; DNA Transfer

Y56　　The Validation of a Manual Selective Degradation Method of Differential Extraction

Katelyn Peyton, BS*, Marshall University Forensic Science Graduate Program, West Liberty, KY; Kelly J. Beatty, MSFS, Marshall University, Huntington, WV; Amy K. Smith, MS, Kentucky State Police Central Forensic Lab, Frankfort, KY

Learning Overview: This presentation demonstrates the importance of an efficient separation of DNA contained on sexual assault evidence into non-sperm cell and sperm cell fractions to better facilitate genetic Short Tandem Repeat (STR) analysis and interpretation of the evidence. This presentation recognizes differential extraction as the gold standard of separation; however, it highlights the risks associated with this method and introduces selective degradation as an alternative that includes all of the benefits of a differential extraction and minimizes the risks. Attendees of this presentation will grasp the importance of having a separation technique for sexual assault evidence that is efficient, will better understand the selective degradation method of a manual differential extraction, and will recognize the benefits of preparing selective degradation reagents in-house.

Impact Statement: This presentation will impact the forensic science community by showing that the implementation of the selective degradation method of a manual differential extraction, while requiring an abundance of testing, troubleshooting, and resources for implementation, can provide a faster manual method of separation that decreases the risk of sample contamination and produces more pure sperm cell fractions generating STR profiles that are readily interpretable. This presentation will be an effort to inform forensic biology laboratories on how the efficiency of a manual differential extraction can be increased through the use of manufactured and/or in-house selective degradation reagents and how, in turn, laboratories can save time, money, and resources to increase throughput in a field that faces an increasing demand for the processing of sexual assault evidence.

Differential extraction is a tried-and-true method for the separation of sexual assault samples into non-sperm cell (female) and sperm cell (male) fractions, which allows for a more efficient analysis of sexual assault evidence.[1] This method of separation, however, can issue many challenges due to the multiple pipetting steps involved.[2] Selective degradation is a promising technique used for differential extraction that makes separation a quicker process by eliminating pipetting and centrifugation steps through the addition of a nuclease and makes interpretation easier and more efficient by producing a more pure male fraction.[3] Such qualities of selective degradation led the Kentucky State Police Central Laboratory to pursue validation of the automation and manual application of this technique using the Erase Sperm Isolation Kit and similarly made in-house DNase reagents.

For this study, a comparison of the Erase reagents and in-house DNase reagents was made using serial dilution swabs consisting of semen on buccal swabs to mimic real casework swabs. Using either Erase or DNase reagents, these swabs were run once in triplicate with both reagents to ensure that similar or same results would be obtained for each sample and run once more to be able to ensure that the reagents gave consistent results. One mock casework study was performed using either Erase or DNase reagents with ten mock casework swabs already processed using KSP's current method of differential extraction, on the QIAcube®, to test reproducibility. With the mock casework study, both Erase and DNase methods were more efficient at male fraction male DNA recovery and elimination of female DNA from the male fraction for most mock casework samples. While Erase reagents recovered a comparable amount of female total human DNA to the QIAcube® with most mock casework samples, the QIAcube® was much more efficient at female total human DNA recovery than the DNase reagents. With the sensitivity study, the QIAcube® was able to outperform both the Erase and DNase methods with male fraction male DNA recovery. However, the Erase and DNase methods both outperformed the QIAcube® with removal of female DNA from the male fraction and female total human DNA recovery. When the sensitivity study was reproduced, both Erase and DNase reagents gave results that were consistent when the methods were performed by the same analyst. However, Erase reagents consistently outperformed DNase reagents in male fraction male DNA recovery. All three methods produced electropherograms that were sufficient for interpretation.

The results obtained from this study are promising and support justification for the implementation of the Erase Sperm Isolation Kit for the method of manual differential extraction at the Kentucky State Police Central Laboratory. Further research could be conducted to ensure that Erase consistently performs as well as or better than the QIAcube® at male fraction male DNA recovery by processing a larger variety of mock casework samples. Before the DNase method can be implemented, additional research is needed to increase the male fraction male DNA recovery in comparison to the Erase method, as well as to ensure that the expected amount of female total human DNA is recovered through a repetition of the mock casework study. Implementation of either Erase or DNase methods for a manual differential extraction would provide the Kentucky State Police Central Laboratory with a method of separation that is time, resource, and cost efficient and that produces STR typing results that are readily interpretable.

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Method Validation; Semen; Mixture Analysis

Y57   Getting Down and Dirty: Analyzing Changes in Soil Microbiota During Three Stages of Mouse Decomposition

Madeline Robinson*, Curry College, Marshfield, MA; Emily Nowicki, PhD, Curry College, Milton, MA; Samantha J. Sawyer, PhD, Curry College, Milton, MA

Learning Overview: After attending this presentation, attendees will have a better understanding of how soil microbes present during distinct stages of mammalian decay could be applied to more accurately determine the postmortem interval.

Impact Statement: This presentation will impact the forensic science community by discussing the changes within soil microbiota within three different stages of decomposition using DNA sequencing and the subsequent influence of microbial communities on soil nutrient content.

Understanding the ecological process of decomposition improves the forensic science community. Research has identified substantial changes within the microbial community composition during decomposition of mammals in soil from various locations. It remains uncertain, however, how the unique microbiota present in different soil types influences decomposition at distinct taphonomic stages of decay.

The aim of this research is to analyze changes in microbial community composition and soil chemistry during the decomposition of mice on sandy loam soil collected from eastern Massachusetts. Deceased weaned mice were placed in soil microcosms and their decay in a controlled indoor environment was monitored. Microcosms were destructively sampled at distinct taphonomic stages of decay (fresh, active, and advanced) by collecting soil from beneath the mouse or from the center of soil-only controls as well as swabbing mouse noses and bellies. Soil pH and nutrient levels were measured, and DNA was extracted from each sample collected. Mice reached active decay by day 6 and advanced decay by seven weeks. Data from our soil analysis revealed a significant increase in the pH within the soil from the microcosms with mice relative to control soil as decomposition progressed throughout our experiment. A standard error of the mean was used to calculate the average across all microcosms at each decomposition stage and sample type. Nitrogen, phosphorus, and potassium levels all showed an increase during decomposition with significant differences relative to controls as well as shifts in microbiota during the decomposition process as determined through community amplicon sequencing of the 16S rRNA gene. This presentation will highlight the importance of expanding our understanding of microbial decomposition ecology. Knowledge of soil microbes present during distinct stages of mammalian decay could be applied to forensic investigations to more accurately determine the postmortem interval as well as provide more information about microbial ecology as it relates to decomposition.

Microbiology; Decomposition; DNA

Y58   A Commotio Cordis Case After a Fall From Height

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Learning Overview: After attending this presentation, attendees will have learned about a different variety of commotio cordis.

Impact Statement: This presentation will impact the forensic science community by increasing forensic pathologists' awareness of commotio cordis.

Background and Aim: Commotio cordis is an increasingly recognized cause of sudden cardiac death. Although commonly linked with athletes, commotio cordis occurs across a spectrum of non-sport-related settings, including assault, motor vehicle accidents, and daily activities. Both categories affected a younger and male-predominant demographic. Mortality is higher in non-sport-related commotio cordis due to lower rates of cardiopulmonary resuscitation, defibrillation, automated external defibrillator availability, and extended time to resuscitation. The diagnosis is complex because it is based on witnesses' statements and on the exclusion, through negative autopsy findings, of other causes of death. Maron and colleagues described the following inclusion criteria: (1) witnessed occurrence of a blunt, non-penetrating blow to the chest immediately preceding cardiovascular collapse; (2) detailed documentation of the circumstances surrounding the blow from available newspaper articles, police reports, and telephone interviews with family members or other witnesses; (3) absence of structural damage to the sternum, ribs, or the heart itself; and (4) absence of any underlying cardiovascular abnormalities.[1] Although there are cases with a diagnosis of commotio cordis in the literature in which rib fractures occur, we did not see that commotio cordis was discussed in the case of falling from a height.

The Case: This is a case of falling from the fifth floor at the age of 22. External Examination: Height 172cm; weight 55kg, numerous millimetric abrasions and ecchymosis on the left upper eyelids, linear abrasions with the largest 1cm in the left cheek zygomatic and lower jaw regions, 5cm long and 0.5cm deep incisions in the front of the left chin, around the arcus costarum above. Linear and millimetric abrasions on the upper right side of the chest and abdomen, t-shaped abrasions extending from the lower left chest region to the lumbar region, numerous linear and point abrasions on the left lower forearm and ulnar side of the hand. Internal Examination: subepicardial hemorrhage at the level of the septum on the anterior and posterior sides of the heart, no macroscopic pathology was observed in the heart valves and heart cavities, fracture in the body of the mandible, epidural hemorrhage at the level of the spinal cord, fracture of left orbital lateral wall, sternum fracture and multiple rib fractures, focal hemorrhage in both temporal muscles, fracture of left wrist and right proximal radius, histopathology findings: fresh bleeding areas in the dura and adjacent to the dura in the medulla spinalis, myocardial congestion, focal interstitial hemorrhage, contraction band necrosis areas, on heart microscopy: 1cm dense hyperemic areas on the posterior wall near the apex on myocardial sections. Chemical analysis: in blood, urine and nasal swab, there is no alcohol (ethanol, methanol), no volatile substances in the system, no drugs or stimulants and drug active substances in the system, and we could not find any reason to explain the cause of death.

Results: In our case, commotio cordis was observed, although internal and external examinations were not negative. In cases of death involving more than one mechanism, justice systems should also consider the commotio cordis when identifying the perpetrator of the person who caused the commotio cordis or other findings. Increased awareness of non-sport-related commotio cordis is essential for a better justice system. In addition, because of the complexity of the sudden death topic, we need a multidisciplinary evaluation to achieve a correct diagnosis. Furthermore, in commotio cordis lethal cases, the execution of the autopsy represents a possible diagnostic tool.

Reference:
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Autopsy; Cause of Death; Diagnosis

Y59   Comparing 3D Photogrammetry Models Created Using Either Routinely Acquired Autopsy Photographs or Those Taken Specifically for Photogrammetry

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Learning Overview: After attending this presentation, attendees will understand how taking photographs in a specific way during autopsy can increase the quality of Three-Dimensional (3D) documentation of injuries.

Impact Statement: Both written and pictorial documentation of injuries are important in criminal investigations. Although digital 3D imaging techniques are being used by some forensic medicine institutes worldwide, the additional cost in equipment, personnel, and time often limit a broader implementation in daily forensic medicine practice. This presentation will impact the forensic science community by showing how an adequate implementation of 3D methods in forensic medicine practice, with minimal additional costs and efforts through photogrammetry, can lead to an increase in the quality of injury documentation.

Background: Both written and pictorial documentation of injuries are important in criminal investigations. Some forensic medicine institutes worldwide have started to implement 3D imaging techniques that allow for superior documentation, interpretation, and presentation of injuries in forensic cases. Standard Operating Procedures (SOP) for 3D documentation of cadavers have already been presented and discussed in scientific literature. However, these are not used routinely worldwide since additional cost in personnel, equipment, and time can be a limitation for their implementation. Photogrammetry represents one of the most cost-effective methods for producing 3D models of documented injuries.

Aim: The standards for photogrammetry require taking a high number of pictures in well-controlled environments in order to gain satisfying 3D models. The aim of this study was to compare 3D models gained from pictures taken routinely at autopsy ("autopsy models") with those taken following the existing SOP for photogrammetry ("standard models") (a slightly modified version of Gitto et al, 2020), in order to evaluate if satisfactory 3D model could be gained just on the basis of routine autopsy pictures.[1] Pros and cons of the two techniques and ways to optimize routine autopsy photographing for photogrammetry will be presented.

Methods: The models evaluated in this study have been gained from a single forensic medicine case: a man with multiple injuries to the head and trunk. During the autopsy, routine photographs of the injuries were taken (47 photographs of the head and 10 photographs of the upper trunk). The same injuries have been photographed after the autopsy according to photogrammetry standards (64 photographs of the head, 29 photographs of the trunk), following the rules of overlap and angles, but without changing the surroundings (light, background). Photogrammetric 3D-models from both photoshoots were created using Agisoft® Metashape 2.0.3 software.

Results: The two models differed significantly. The "standard models" were of good quality and had a homogeneous structure. Their accuracy was high, and the proportions preserved, thus enabling measurements and the assessment of correlations between injuries. The photographs have been taken with minimal resource costs (a total of seven minutes for photographing the head and two minutes for the trunk), as no adjustment to the surroundings/background has been made.

The "autopsy models" had a significantly lower quality. The biggest issue with these models was the occurrence of artifacts, which can cause misinterpretation of the findings (e.g., the presence of artifacts that resemble injuries or the absence of injuries). These artifacts are not present/do not occur to a much lesser extent in the "standard models."

In addition, the "standard models" showed a more pronounced three-dimensionality by picturing a larger body area than the "autopsy models". The latter depicted just small and focal areas of injuries, disregarding the peripheral parts of the injuries.

Conclusions: With minimal additional time investment, by using the standard equipment present in an autopsy room (digital camera), one can create more extensive and detailed 3D models that may be used in medicolegal and police investigations, as evidence in court, but also as valuable teaching material. Thus, we still recommend extended photographing according to existing photogrammetry standards, at least for cases where documentation of injuries is vital for the investigation.

Even if our study showed that the "autopsy models" cannot give satisfactory results, we argue that by implementing small changes in how the routine autopsy photos are taken, a better model quality could be achieved. These include, but are not limited to, photographing peripheral parts of injuries, using the same ruler and camera throughout the photographing procedure, placing the ruler in the same place, avoiding the zoom, and holding the camera closer to the body for close-up photographs

Reference:
2.   Gitto S, Cuocolo R, Emili I, et al. Effects of Interobserver Variability on 2D and 3D CT- and MRI-Based Texture Feature Reproducibility of Cartilaginous Bone Tumors. *Journal of Digital Imaging*. 2021 Aug;34(4):820-832. DOI: 10.1007/s10278-021-00498-3. PMID: 34405298; PMCID: PMC8455795.

3D Technology; Autopsy; Forensic Imaging

Y60   **Fun Run the Bowel: Two Cases of Fatal Gastrointestinal Hemorrhage From Duodenal Ulcers**

Jessicia Schmitt, MD*, University of Utah, Holladay, UT; Neil G. Haycocks, MD, PhD, Utah Office of the Medical Examiner, Taylorsville, UT

Learning Overview: After attending this presentation, attendees will recognize the potential for duodenal ulcers to cause a subacute gastrointestinal hemorrhage that is ultimately fatal.

Impact Statement: This presentation will impact the forensic science community by documenting two cases of fatal gastrointestinal hemorrhage that occurred as a result of cryptic duodenal ulcers. Forensic pathologists should be aware of the many diagnostic possibilities for gastrointestinal hemorrhage and use investigative information to contextualize the spatial and temporal relationships between the hemorrhage itself and possible bleeding sites.

Blood in the gastrointestinal tract has a wide differential diagnosis, with causes ranging from innocuous to acutely life-threatening.[1,2] Forensic pathologists often encounter fatal gastrointestinal hemorrhage as a consequence of ruptured esophageal varices, but other possibilities must be kept in mind.[3] In this report, two patients were admitted to the hospital with significantly different clinical pictures, yet both succumbed to complications related gastrointestinal hemorrhage from unrecognized duodenal ulcers.

The first was an 86-year-old female in generally poor health who was admitted with weakness and frank blood from her rectum. She was pancytopenic, and despite multiple transfusions, she declined and expired within 8 hours. The second was a 76-year-old man who had a lengthy hospitalization following an auto-pedestrian accident. He expired on day 17 after frank blood was observed in his undergarments. Autopsies performed on both individuals had similar findings, with gross hemorrhage in the distal ileum and large intestine and erosive ulceration(s) of the proximal duodenum. Histologic sections of the ulcers showed necrotic beds extending into the submucosal tissues with proximity to sizable blood vessels. The intestines were opened longitudinally but no other possible sources of bleeding were found.

The etiology of the ulcer in the first case is unclear. Possibilities include chronic use of Nonsteroid Anti-Inflammatory Drugs (NSAIDS), *Helicobacter pylori*, and/or vascular insufficiency. There were reported accusations of neglect made against the woman's caretakers, which may have potentiated her disease processes and delayed significant medical intervention. In the second case, the ulcers were likely secondary to the stress of trauma and subsequent hospitalization (so-called Cushing ulcers). It is noteworthy that the decedent in the second case, while an inpatient, reportedly made numerous inquiries regarding physician-assisted suicide, and at one point attempted to asphyxiate himself with oxygen tubing while in the intensive care unit.

In both cases, the ulcers were not actively bleeding, and gross blood was not evident until the distal ileum. This suggests the bleeding was controlled by natural hemostatic mechanisms, but the resulting blood loss initiated an irreversible cascade of events that culminated in hypovolemic shock and death. These cases underscore the fact that a diligent search for a site of gastrointestinal hemorrhage should encompass the entire proximal gastrointestinal tract and that patients with terminal blood loss may remain alive for some time, particularly if they are receiving supportive care.

References:
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2. Felix WR Jr, Stahlgren LH. Death by undiagnosed perforated peptic ulcer: analysis of 31 cases. *Ann Surg*. 1973 Mar;177(3):344-51. doi: 10.1097/00000658-197303000-00017. PMID: 4540287; PMCID: PMC1355538.
3. Dolinak D, Matshes E, Lew E. *Forensic Pathology: Principles and Practice*. (Listewnik M, Chester P, eds.). Elsevier Inc; 2005.

Death Investigation; Decision Making; Cause of Death

Y61   Impacts of Desiccants on DNA Quantity and Quality in Sexual Assault Kits Over a One-Year Period

Catherine F. Hull, BA*, Duquesne University, Lancaster, PA; Randy Nagy, BSc, Gentueri Inc., Verona, WI; Dana Voris, MS, Independent Forensics, Lombard, IL; Lisa R. Ludvico, PhD, Duquesne University, Pittsburgh, PA; Pamela Marshall, MS, Duquesne University, Pittsburgh, PA

Learning Overview: This presentation will be used to emphasize the importance of the storage of sexual assault kits on DNA quantity and quality. After attending this presentation, attendees will better understand the possible solutions to DNA contamination or the mix-up of samples due to the drying process and storage of sexual assault kits.

Impact Statement: This presentation will impact the forensic science community by helping determine if there are more efficient ways to store sexual assault kits in Pennsylvania. Based on the results of this study, new storage techniques can be tested across the United States to establish a standardized procedure for the storage and collection of sexual assault kits.

The way sexual assault samples are collected in hospitals requires a drying process before storage. The drying process could allow for cross-contamination with other samples or contamination from personnel. Contamination could result in poor DNA quantity and quality and inconclusive results. The drying step could also cause a mix-up of samples to occur in the hospitals. If a desiccant were to be used, the drying process could be eliminated to reduce contamination, limit mistakes, and reduce storage space. A desiccant is a substance that absorbs water to allow for dry conditions in storage. A desiccant can be made of silica beads. The silica beads absorb the water from the air as the air circulates through the desiccant. Since the water in the air is absorbed, the air coming out of the desiccant is dry, allowing for dry conditions. Finding the ideal collection and storage process is crucial to maintaining the quality and quantity of DNA.

A desiccant-based collection kit, manufactured by Gentueri, and a non-desiccant-based collection kit, used by the Pennsylvania State Police, will be used to make contrived sexual assault samples. The sexual assault samples will be stored for up to 12 months to determine which kit will better preserve the quantity and quality of DNA in the sample. While previous research has shown that both sexual assault kits perform fairly equally over 30, 60, and 90 days, it also showed that the Gentueri kit offered a more robust, standardized process. This allows for a controlled design, reducing the number of variables such as temperature, weather, and state of collection. The results will show which collection kit would be ideal for sexual assault samples. The results can be applied to forensic crime labs across Pennsylvania to preserve DNA quantity and quality. The study could then be expanded to different states with different climates to achieve a more standardized procedure for collecting and storing sexual assault kits across the United States.

Desiccant; Forensic DNA; Sexual Assault Kits

Y62 The Identification of Biomarkers Associated With Prolonged Starvation in Cat (*Felis Catus*) Bones

Annagrace J. Radocaj, BS*, Duquesne University, Greensburg, PA; Lisa R. Ludvico, PhD, Duquesne University, Pittsburgh, PA; Becky Morrow, DVM, MS, Penn State University/Frankie's Friends, New Kensington, PA

Learning Overview: The goal of this presentation is to highlight the need for a development of new methodology for animal abuse victims who have undergone starvation. Attendees will better understand the importance of identifying a biomarker for starvation due to the gap of current research within the field of veterinary forensics.

Impact Statement: This presentation will impact the forensic science community by identifying a biomarker for starvation within the decomposed remains of felines. Currently, there are no procedures in place for identifying prolonged starvation in cases where bone is in advanced stages of decomposition. The results of this study may assist forensic veterinarians in developing techniques for determining whether an animal has been subjected to nutritional neglect.

According to the *Animal Legal Defense Fund*, around 250,000 animals each year are victims of hoarding, with the number of cases steadily increasing. Hoarding can be defined as the human desire that is satisfied through the collection or control of animals that supersedes the animals' needs. In many incidents of hoarding animals, starvation or emaciation of the animal is not uncommon. In the late stages of decomposition, little to no tissue is present, and only depleted bone samples can be collected from the victim. During starvation, Bone Turnover biological Markers (BTMs) are expressed due to increased bone resorption. BTMs are degradation products of type I collagen and include the C and N-terminal cross-linking telopeptides of type I collagen that reside at the ends of collagen chains, and which are cleaved off into the bloodstream during the metabolic breakdown of bone. Collagen proteins found within the bone will be analyzed to determine if a BTM can be identified to determine starvation for that individual.

Immunoassay testing for N-terminal telopeptides of type I collagen was done before bone protein extraction with the BioTang N-telopeptide Feline specific immunoassay kit to confirm that these selected biomarkers can be identified in both well-nourished and malnourished felines. Three trials have been completed with both well-nourished and malnourished feline serum samples. A calibration curve was created to quantify the concentrations of BTMs in the serum samples. It was concluded that based on the calibration curve, malnourished feline samples displayed significantly larger concentrations of N-telopeptides in comparison to the concentrations quantified for nourished samples.

Once the bone samples have been collected the extraction of proteins from the bones will be done without any demineralization. More confirmatory testing will be completed with an immunoassay and western blot using a respective antibody to determine the identity of said biomarkers. Once data has been collected, the amounts and concentrations of the identified BTMs will be measured and compared to those found in the control sample from a feline that has not undergone starvation.

Bone Turnover Marker; Starvation; Feline

Y63   A Low-Cost Microfluidic Biochip Integrated With LAMP for the Rapid Detection of *Streptococcus Pneumoniae* to Aid in Forensic Pathology

Angel Gabriel Gutierrez Ruiz, El Paso, TX*

Learning Overview: After attending this presentation, attendees will better understand microfluidic devices and how they can be applied to forensics to reduce the amount of time spent trying to detect pathogens postmortem; specifically, attendees will learn about a new approach that our laboratory has developed to identify *Streptococcus pneumoniae* (*S. pneumoniae*).

Impact Statement: Forensic pathologists often encounter challenges when determining the cause of death of a patient. One of these challenges is the identification of pathogens causing the death in patients. Therefore, developing technologies to improve the detection of pathogens that may have caused the patient's decease is essential to aid forensic pathologists in reaching a proper diagnosis. Our lab has been working on the development of an innovative device that will help the forensic pathologist reach this goal. Our microfluidic devices can be used to detect pathogens faster and more accurately, providing more reliable results than other detection methods. This presentation will impact the forensic science community by presenting information that can reduce the number of resources needed to investigate a criminal case and provide better results.

S. pneumoniae is a common human pathogen that can lead to different diseases ranging from rhinosinusitis and otitis media to the more severe conditions of meningitis and pneumonia. In 2000, 14.5 million new cases and 826,000 *S. pneumoniae*-related deaths were reported worldwide.[1] Due to the life-threatening consequences of some of these diseases, it is crucial to quickly identify the specific bacteria causing the disease to determine the appropriate line of treatment. Moreover, rapid identification of death-causative pathogens during autopsy is important for forensic pathologists' postmortem diagnosis. Therefore, we have developed a polymer/paper hybrid microfluidic biochip integrated with Loop-Mediated Isothermal Amplification (LAMP) to aid in the rapid detection of *S. pneumoniae*. The results of this innovative approach can be easily observed by the naked eye in less than 40min without the use of specialized laboratory equipment.

Compared with other detection methods like bacterial culture and serology, which may take days to achieve results, or Polymerase Chain Reaction (PCR), which requires costly equipment, our innovative microfluidic biochip provides lower cost, faster, and sensitive detection of *S. pneumoniae*. It is expected that the use of this device will aid in the rapid identification of *S. pneumoniae* and thus allow physicians to accurately treat diseases such as meningitis and pneumonia, as well as help forensic pathologists determine a patient's cause of death on crime science. This biochip will not only help forensic scientists but also physicians, as it can also be used in the clinic to diagnose patients. Its simple yet effective design makes it appropriate for use by health care and forensic professionals with varying levels of expertise. Due to its efficient design, materials, and size, this detection device can be used in well-equipped laboratories as well as places with limited resources. This device will be an advancement in the detection methods being used nowadays for detecting pathogens.

Reference:

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Microfluidic Biochip; LAMP (Loop-Mediated Isothermal Amplification); *Streptococcus pneumoniae*

Y64   An Autopsy Case of an Unusual Association Between the Chiari Network and the Patent Foramen Ovale: An Anatomical Variation or a Risk Factor? A Case Report

Hind Abouzahir, MD, PhD*, Medico-Legal Institute, Casablanca, Morocco

Learning Overview: The goal of this presentation is to cover the following key points:

- Attendees will gain an understanding of what the Chiari Network is, including its anatomical characteristics and its usual role in the human body.

- The presentation will introduce the Patent Foramen Ovale (PFO), explaining what it is and its typical physiological implications.

- The presenter will delve into the specifics of the autopsy case, providing details about the individual involved, the clinical context, and the observations made during the autopsy.

- Attendees will learn about the unusual association between the Chiari Network and PFO in this particular case. This may include the anatomical details of how these two features interacted or coexisted within the patient's heart.

- The presentation will likely include a discussion on whether this association is a mere anatomical variation or if it holds any significance as a potential risk factor for health issues or clinical conditions.

- Attendees will gain insights into why this case is noteworthy and what implications it may have for the medical field. This may include considerations for future research, clinical pracice, or diagnostic criteria.

- The presentation will conclude by summarizing the key takeaways and potential areas for further study or investigation.

Impact Statement: This presentation will impact the forensic science community as follows:

- By presenting a rare and unusual anatomical association discovered during an autopsy, this presentation raises awareness within the forensic science community about the diverse range of anatomical variations and anomalies that can be encountered during postmortem examinations.

- Forensic pathologists and investigators may gain insights into the importance of meticulous examination and documentation. This case study can serve as a reminder to be thorough in assessing anatomical features, even those considered rare, to avoid overlooking potentially important factors in forensic investigations.

- The presentation, if documented and published, can contribute to the medical literature and forensic science knowledge base. This can be valuable for future reference and research, enabling other forensic professionals to access and learn from the case.

- The findings from this case may prompt discussions within the forensic science community about the need to incorporate additional examinations or considerations when performing autopsies. It may lead to updates in autopsy protocols to ensure that such rare anatomical associations are not missed in future cases.

- The presentation's focus on both anatomy and cardiology could foster greater interdisciplinary collaboration between forensic pathologists and medical specialists. This collaboration can lead to a deeper understanding of the potential medical significance of anatomical findings, which could impact the accuracy of cause-of-death determinations.

- This case study can serve as an educational resource for forensic science training programs. It can be used to teach aspiring forensic pathologists and investigators about the importance of thorough examinations and the possibility of encountering unusual anatomical variations.

Background: It is a highly unusual occurrence for a patient to have both a Chiari network and a PFO at the same time. This case report illustrates the coexistence of these two anatomic anomalies, raising questions about the clinical importance of each of them as well as whether or not they could offer possible risks contributing to death.

Case Presentation: This case involves a 26-year-old female with a history of diabetes who had been taking insulin throughout the course of the disease for ten years. She reported feeling uneasy and then fainted before being transported to the emergency room, where she was pronounced dead prior to any attempts at resuscitation being made. During the autopsy, it was discovered that the patient had both a PFO and

a Chiari network. There were no other findings that were especially significant. Hyperglycemia was diagnosed based on the biochemical markers found in the blood sample, which showed glycemia levels of 3g and glycated hemoglobin levels of 10.

Discussion: It is extremely rare for a single person to have both a PFO and a Chiari network at the same time. The Chiari network has been linked to a few heart diseases, including atrial fibrillation, paradoxical embolism, and infective endocarditis; however, its relevance to clinical practice is still up for debate. On the other hand, a PFO has been implicated in the development of cryptogenic stroke, migraines associated with aura, and decompression sickness. The presence of these two defects at the same time raises questions about the possible additive or synergistic impact on clinical risk that may contribute to death in the absence of underlying pathology.

Conclusion: The findings of this case study provide evidence of the extremely uncommon coexistence of a Chiari network with a PFO in a single patient. To determine if the coexistence of these anatomic anomalies could be deemed an anatomical variation or a risk factor for specific clinical diseases, additional research is required. Clinicians need to be aware of this possible association because it may have repercussions for the treatment and prognosis of patients whose findings are comparable to those described in the study.

Patent Foramen Ovale; Chiari Network; Diabetes

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