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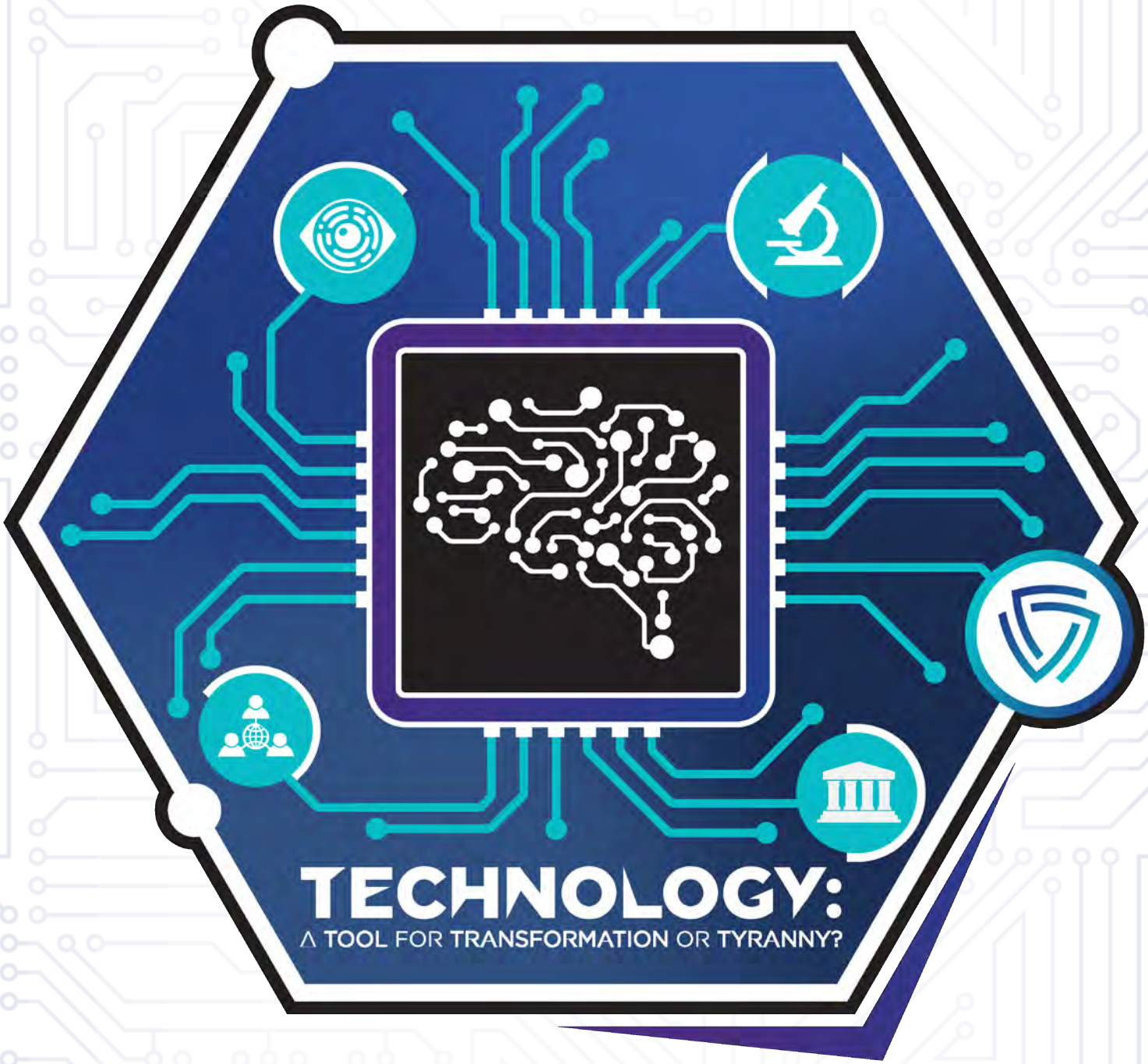

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PROCEEDINGS

of the American Academy of Forensic Sciences 77th Annual Scientific Conference

The *Proceedings* of the American Academy of Forensic Sciences is an official publication of the American Academy of Forensic Sciences (AAFS). It is devoted to the publication of the abstracts of technical oral papers and posters presented at the AAFS Annual Scientific Conference. These include various branches of the forensic sciences such as anthropology, criminalistics, digital evidence, forensic nursing, engineering, jurisprudence, odontology, pathology, psychiatry, questioned documents, and toxicology. Similar submissions dealing with forensic-oriented aspects of the social sciences are also included.

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PROCEEDINGS

of the American Academy of Forensic Sciences **February 2025**
Volume XXXI

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S1 Standards Around the World: How Is the World of Standards Influencing the Future of Forensics?

Teresa L. Ambrosius, BA, AAFS Standards Board, Colorado Springs, CO; Erin P. Forry, MS*, RTI International, Research Triangle Park, NC; Allison Getz, MS*, National Institute of Standards and Technology, Gaithersburg, MD; Christoph Hemme, DMD*, German Bundeswehr/NATO, Munich, Germany; Samiah Ibrahim, BA, BSc*, ForensInk, Ottawa, Ontario, Canada; Brian Milewski *, ASTM International, West Conshohocken, PA; Mark Mogle, BS*, U.S. Department of Justice, ICITAP, Washington, DC; Niamh Nic Daeid, PhD*, Leverhulme Research Centre for Forensic Science, University of Dundee, Dundee, Scotland; Antonel Olckers, PhD*, African Academy of Forensic Sciences & DNAbiotec, Pretoria, South Africa; Carolina Rojas, MFS*, Department of Forensic Sciences, Organismo de Investigación Judicial, Poder Judicial San Joaquín, Heredia, Costa Rica; Sheila Willis, DSc*, Leverhulme Research Centre for Forensic Science, University of Dundee, Dundee, Scotland; Agnes D. Winokur, PhD*, Drug Enforcement Administration, Miami, FL; Ivana Wolff, MA*, International Criminal Court, Mendoza, Argentina*

Educational Objectives: After attending this presentation, attendees will have a better understanding of how labs and organizations around the world are developing and implementing both US and International Standards. Attendees will also have a better understanding of how they can get involved in standards development around the world.

Impact on the Forensic Science Community: The presentation will impact the forensic science community by creating greater awareness of how standards are currently being drafted, used, and implemented internationally and the impact of standards in forensic sciences.

Program Description: Multiple forensic and standards organizations around the world are working with the forensic practitioners and partnering with respective stakeholders across the standards community to develop standards that can increase reliability on forensic analysis results and public trust in the forensic disciplines. These responsive programs and activities are designed to engage professionals across the world of standards and conformity assessment and ensure the strength and efficacy standardization.

There are numerous routes to the development of standards around the world. ISO, the International Organization for Standardization is a non-governmental organization that develops and publishes standards for products, processes, services, and systems. They also help facilitate global trade by providing common standards across different countries. In the US, the American National Standards Institute (ANSI) accredits standards developing organizations that develop discipline specific standards. National Standards Bodies (NSB) are organizations that prepare standards for global use. Each country or economy usually has one recognized NSB.

The morning will have presentations from non-US labs and how they are implementing and getting involved in standards development. An international Forensic Science Academy and a National Standards Body on their involvement with the development and implementation of Forensic Science Standards. In addition, information will be presented on how international standards have been addressing the emergence of machine learning and artificial intelligence, as well as the challenges when developing these standards.

The afternoon will focus on ISO and the TC on Forensics, NATO development of standards, a perspective on international standards from a university, and law enforcement development organization.

Standards; International; Implementation

S2 Young Forensic Scientists Forum—Back to Your Future: Forensic Opportunities in the AI Era

Sara C. Zapico, PhD, New Jersey Institute of Technology, Smithsonian Institution, Newark, NJ; Joe Adserias-Garriga, PhD*, Mercyhurst University, Erie, PA; Joanna Collins, MFS*, LINUS Investigations and Consulting, San Antonio, TX; Victoria M. Dominguez, PhD*, Lehman College, CUNY, Bronx, NY; Sarah Ellingham, PhD*, International Committee of the Red Cross, Schwarzenbek, Germany; Brandon Epstein, MS*, Magnet Forensics, Freehold, NJ; Joe Adserias-Garriga, PhD*, Mercyhurst University, Erie, PA; Maryann Hobbs, PhD*, SNA International, Honolulu, HI; Cheryl Hunter*, American Academy of Forensic Sciences, Colorado Springs, CO; Douglas S. Lacey, MS*, BEK TEK LLC, Stafford, VA; Steven B. Lee, PhD*, San Jose State University and FIU, Miami, FL; Christina A. Malone, MSFS*, USACIL, Atlanta, GA, Katherine N. Scafide, PhD, RN*, George Mason University, Fairfax, VA; Valerie Sgheiza, PhD*, SNA International, Joint Base Pearl Harbor-Hickam, HI; Christopher Thompson, MD*, David Geffen School of Medicine at UCLA Los Angeles, CA; Dirk M. Vastrick, BA*, Dirk Vastrick Presents, Spokane Valley, WA*

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 plays 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.

Program Description: The overall theme of the 2025 AAFS Conference is *Technology: A Tool for Transformation or Tyranny?* This year the YFSF Committee has chosen the theme *Back To Your Future: Forensic Opportunities In the AI Era* to showcase the various career paths young forensic scientists can choose, particularly focused on technological specialties, but also displaying how new technologies impact current forensic practices. 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 plays a unique role in advancing the field.

The speakers have a vast range of knowledge and experiences that will shed light on the ways forensic scientists use technology in the pursuit of justice. Specifically, our presenters will showcase various disciplines in forensic science, speak about performing research in the field, and offer professional development advice as well as 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 forensic science. Further, the session this year will offer a resume review workshop, so do not forget to bring your updated resume to the session! 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.

Professional Development; Students; Young Professionals

S3 Interdisciplinary Symposium—Technology: A Tool for Transformation or Tyranny?

Yong Guan, PhD, Iowa State University, Ames, IA; Katharine Pope, MA*, RTI International, Research Triangle Park, NC; Kenneth Aschheim, DDS*, NYC Office of the Chief Medical Examiner, New York, NY; JoAnn Buscaglia, PhD*, FBI Laboratory, Quantico, VA; Alicia Carriquiry, PhD*, Iowa State University, Ames, IA; Sarah Chu, PhD*, Perlmutter Center for Legal Justice at Cardozo Law, New York, NY; Nancy Downing, PhD, RN*, UNTHSC College of Nursing, Fort Worth, TX; Zeno Geradts, PhD*, Ministry of Security and Justice, Den Haag, Netherlands; Thomas P. Paonessa, Jr., MS*, The MITRE Corporation, McLean, VA; Katherine Scafide, PhD, RN*, George Mason University School of Nursing, Fairfax, VA; Hon. Donald Shelton, JD, PhD (ret.)*, University of Michigan-Dearborn. Saline, MI; Lucas Zarwell, MSFS*, National Institute of Justice, Washington, DC*

Educational Objectives: Participants will gain a comprehensive understanding of the transformative role of technology in forensic science, critically evaluating its dual capacity as a tool for innovation and a source of ethical and operational challenges. Attendees will learn about advancements in artificial intelligence and emerging technologies, their implications for forensic practices, and the evolving legal and societal standards shaping the future of forensic applications.

Impact on the Forensic Science Community: The 2025 IDS aims to provide attendees with an understanding of how emerging technologies are transforming forensic science and its applications in justice. Participants will gain insights into the dual role of artificial intelligence (AI) as both a tool for enhancing accuracy and a potential source of ethical challenges, such as systemic bias and privacy concerns. Presenters will also emphasize the importance of critical thinking and adherence to the scientific method in evaluating technological advancements, as well as the challenges of transitioning research into operational practice. By exploring real-world examples, legal implications, and case studies, attendees will learn how to responsibly adopt and integrate technologies such as probabilistic genotyping, forensic investigative genetic genealogy, and AI tools, while addressing ethical, legal, and practical concerns. Additionally, IDS aims to foster interdisciplinary dialogue on how technology can support victims of violence and uphold justice, ensuring that forensic innovations contribute positively to society.

Program Description: The 2025 AAFS Annual Scientific Conference's Interdisciplinary Symposium (IDS) spotlights the theme "Technology: A Tool for Transformation or Tyranny?" through a dynamic program of three keynote talks and two engaging panel discussions. The free event brings together leading experts representing all sections of forensic science in a mission to explore how technology shapes the field. IDS keynote speakers are Dr. Joanne Buscaglia from the FBI, Mr. Thomas P. Paonessa Jr. from MITRE, and Mr. Lucas Zarwell from the NIJ. Their presentations will address the integration of cutting-edge technology into forensic operations, the importance of adhering to the scientific method, and the challenges of transitioning innovative research into practical applications.

The first IDS panel, moderated by Dr. Yong Guan, focuses on "Artificial Intelligence (AI) in Forensics" and features insights from Zeno Geradts, JoAnne Buscaglia, Thomas P. Paonessa Jr., Kenneth Aschheim, and Alicia Carriquiry. This session explores the dual role of AI in forensic science—its potential to enhance accuracy and efficiency, alongside ethical concerns such as bias and privacy risks. Speakers detail real-world case studies and emerging AI tools to shed light on the question of whether AI serves as a transformative ally or a potential threat in the pursuit of justice.

The second IDS panel, moderated by Katharine Pope, delves into "Emerging Technologies and Legal Implications" and features insights from Alicia Carriquiry, Lucas Zarwell, Donald Shelton, Katherine Scafide, Nancy Downing, and Sarah Chu. This session explores how advancements in forensic tools transform investigations while raising critical legal and ethical questions. Topics range from probabilistic genotyping and forensic investigative genetic genealogy to policies safeguarding data collected during the criminal process. The entire IDS aims to provide forensic professionals with valuable insights into leveraging technology responsibly while addressing its challenges, fostering a thoughtful and collaborative approach to the future of forensic science across the sections.

Artificial Intelligence; Forensic Technology; Technology Ethics

CB1 "Excited Delirium": Abuse, Use, and Bias of This Term in the Courtroom

Stephanie Domitrovich, PhD, Administrative Office of PA Courts, Erie, PA; William M. Nuzum III, JD*, W. Milton Nuzum III, LLC, Vincent, OH*

Educational Objectives: Attendees will assume the role of the judge in each hypothetical case to determine the admissibility of expert testimony on the cause of death by applying the appropriate evidentiary standards for admissibility of the testimony. Underlying each scenario is the controversy of racial or ethnic bias affecting the diagnosis of cause of death. Attendees will understand how the diagnosis can impact the potential criminal or civil liability on the part of the responding law enforcement officers or first responders involved in each case. Is there qualified immunity for law enforcement? Attendees will understand the dilemmas that judges have in admitting testimony proffered by experts in these cases and how those dilemmas have been or are resolved.

Impact on the Forensic Science Community: This presentation will inform the forensic science community by presenting a series of hypothetical emergency scenarios based on actual court cases where law enforcement and first responders are dispatched to an emergency crime scene resulting in detention and treatment of an alleged perpetrator. Each scenario involves a tragic situation that results in the death of the perpetrator at the scene. Attendees will understand the dilemmas that judges have in admitting testimony proffered by experts in these cases and how those dilemmas have been or are resolved.

Program Description: After attending this presentation, attendees will understand legal principles for the qualification of expert witnesses and foundational requirements of admissibility expert testimony in civil and criminal cases specifically related to fatalities diagnosed as resulting from "Excited Delirium". While this diagnosis is recognized as a valid diagnosis in the medical community, not all embrace its validity. Attendees will understand the controversy of this diagnosis as a shield from liability for abusive injury and racial bias by law enforcement and first responders.

Each death is diagnosed by an expert as caused by excited delirium. At issue in each scenario is whether the death was from natural causes, self-inflicted, excessive force, or inappropriate medical treatment. Attendees will assume the role of the judge in each hypothetical case to determine the admissibility of expert testimony on the cause of death by applying the appropriate evidentiary standards for admissibility of the testimony. Is there qualified immunity for law enforcement?

Excited Delirium; Expert Witness; Bias

CB2 The Collapse of Surfside: A Multifaceted Approach to Disaster Victim Identification

Heather A. Walsh-Haney, PhD, Florida Gulf Coast University/Human Identity and Trauma Analysis Laboratory, Fort Myers, FL; Micki Besse, MS*, Florida Gulf Coast University/Human Identity & Trauma Analysis Laboratory, Fort Myers, Florida; Katherine Kenerson, MD*, Florida District 11 Office of the Medical Examiner, Miami, FL; Kenneth Hutchins, MD*, Florida District 11 Office of the Medical Examiner, Miami, FL; Benjamin Mathis, MD*, Florida District 11 Office of the Medical Examiner, Miami, FL*

Educational Objectives: After this presentation, attendees will understand the protocols and technologies implemented after a mass fatality event in Surfside, FL.

Impact on the Forensic Science Community: This presentation introduces the forensic science community to physical and digital decedent documentation following a mass fatality incident with a closed population from a forensic anthropological perspective as guided by the needs of the medical examiner and law enforcement personnel as well as the various community stakeholders.

Program Description: Forensic Anthropologists (FA) are called to assist in the discovery, recovery, inventory, and estimation of the Minimum Numbers of Individuals (MNI) represented by skeletal remains, irrespective of the decomposition state of the decedent. FAs also assist in the process of identification, trauma analysis, and reunification of human remains to their legal next of kin following mass fatality incidents.[1-3] On June 24, 2021, the South Champlain condominium collapsed, prompting the search, rescue, and recovery of a closed population comprised of 98 fatalities. Post-incident, the FAs were responsible for creating a skeletal inventory and MNI as requested by the medical examiner, law enforcement, families, and community leaders.

Though informed by prior mass fatality missions with open and closed populations (e.g., 9/11, ValuJet, hurricanes Wilma and Katrina) and published research, our protocols implemented post-incident allowed for the determination of MNI using a digital database. Using the medical examiner autopsy records, radiographs and photographs (total n = 4,348), and the FA analysis of the physical remains, 98 individuals were recovered with a minimum of 11,848 inventoried osseous elements. As large structural debris, thermal damage, water incursion, and other environmental influences obliterated the skeletal remains, this inventory focused upon MNI. Then these data were combined with medical examiner records, law enforcement information of associated physical evidence, and DNA information regarding sample processing success that corresponded to each decedent.

The average quantity of skeletal remains recovered per individual was 124 (or approximately 60% of the individual). Of the 8,340 elements not recovered, 56% were hand and pedal elements, 19% were vertebrae or ossa coxae, 12% were ribs, 5% were skull and hyoid, 4% were lower extremity long bones or patella, 2% were upper extremity long bones, and 2% were clavicles, scapulae, or sternal elements. When parsed by cranial element, the left zygomatic (61%) was the least recovered with the occipital (70%) being the most recovered cranial element. Postcranially, the hyoid (43%) was least recovered, with the left humerus (73%) being the most recovered postcranial element. Twenty-six non-human elements were discovered as well as four non-osseous items. These findings helped to inform the subsequent searches of the debris field, guide bone extraction points for DNA, as well as plan for future training opportunities for first and last responders. No less important, this study helps to guide and inform future recovery missions as is the responsibility of forensic scientists.

References:

1. Gill, JR., Desire, M., Dickerson, T., and Adams, B. J. (2011). The 9/11 attacks: The medicolegal investigation of the World Trade Center fatalities. *Forensic Pathology Reviews*, 181-195.
2. Mundorff, A.Z. (2014). Anthropologist-directed triage: Three distinct mass fatality events involving fragmentation and commingling of human remains. In *Commingled Human Remains* (pp. 365-388). Academic Press.
3. Wiersema, J.M., and Woody, A. (2016). The forensic anthropologist in the mass fatality context. *Academic Forensic Pathology*, 6(3), 455-462.

Mass Fatality; MNI; Database

CB3 Forensic Identification of Synthetic Data: Identification and Isolation Techniques

Joseph C. Sremack, MS, Forensic Data Analytics, Marcum LLP, New York, NY*

Educational Objectives: Attendees will gain an understanding of what synthetic data is and how it is used, scenarios in which synthetic data needs to be identified in an investigation, and the results of techniques applied to the identification process. The presentation will focus on a case study, in which synthetic data was present, on how fraud analysis techniques were applied to identify the special characteristics of synthetic data and the analysis findings and results.

Impact on the Forensic Science Community: The presentation will provide the forensic science community with a better understanding of the emerging area of synthetic data, why it matters to a variety of cases requiring forensic analysis, and how such data can be identified and isolated vis-à-vis ground truth data.

Program Description: Synthetic data represents a new class of data that poses risks to large-scale forensic data analysis if not properly accounted for and isolated during an investigation. The use of synthetic data extends beyond artificial model training data, as it can be used to create falsified transactions or simply be indistinguishable from ground truth data. The presence of synthetic data increases risks to data reduction efforts and increases the likelihood of false positives.

The current academic literature is limited with respect to the analysis of synthetic data, and forensic analysis is not addressed. While published research on forensic identification methods is not available, literature on synthetic data generation is growing.[1] More importantly, research is available on assessing synthetic data to determine whether the source data code can be identified or otherwise deanonymized, which is important for identifying the presence of synthetic data.[2,3] These techniques are valuable for analyzing the integrity and identifiability of synthetic data, but, by themselves, they are insufficient for forensic analysis.

Fraud analytics is a more established field, with a corpus of defined and defensible techniques whose results can be measured and admitted in court. The techniques generally relate to the identification of anomalies. The anomalies can be identified through a variety of data tests to isolate patterns that do not conform to patterns found based on time-series analysis, univariate or multivariate distributions, or even the distribution of specific digits within numeric fields. The fraud analytics tests, however, are insufficient for identifying synthetic data because the data is created to be statistically representative of the ground truth data and are largely immune to statistical fraud analytics tests.

Several analysis techniques to identify and isolate synthetic data using a combination of current research and fraud analytics tests have yielded strong results in the context of commercial forensic investigations. Synthetic data identification analysis can be successfully performed based on reverse engineering the standard models that generate synthetic data and applying fraud analysis techniques. This presentation will present a case study of a commercial forensic data analysis involving synthetic data and the results and limitations of performing the identification analysis. The identification of synthetic data requires ongoing examination of the techniques to identify and isolate this class of data by researchers and practitioners.

References:

1. A. Kiran, S. Saravana Kumar, A Methodology and an Empirical Analysis to Determine the Most Suitable Synthetic Data Generator, *IEEE Access*, vol.12, pp.12209-12228, 2024.
2. A. Majeed, S. Oun Hwang, When AI Meets Information Privacy: The Adversarial Role of AI in Data Sharing Scenario, *IEEE Access*, vol.11, pp.76177-76195, 2023.
3. M. Hittmeir, A. Ekelhart, R. Mayer, Utility and Privacy Assessments of Synthetic Data for Regression Tasks, *2019 IEEE International Conference on Big Data*, 2019.

Forensic Investigation; Data; Statistics

CB4 Maximizing the Impact of Social Science Research and Evaluation on Forensic Science: The NIJ's Role in the Integration of Science

Danielle M. Crimmins, PhD, National Institute of Justice, Department of Justice, Washington, DC; Jonathan McGrath, PhD*, U.S. Customs and Border Protection, Houston, TX; Jillian Barnas, PhD*, AAAS/NIJ, Washington, DC*

Educational Objectives: After attending this presentation, attendees will understand: (1) how social science research and evaluation provides objective evidence to advance the fair and impartial administration of justice; (2) the application of social science research and evaluation methods to study the forensic science disciplines, including examples from the National Institute of Justice (NIJ's)'s Social Science and Forensic Research (SSRFS) portfolio; and (3) a discussion of the benefits, challenges, and future research directions to address the needs of the field.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by discussing the benefits and challenges of using social science research to formulate meaningful research questions and develop rigorous research designs to advance the impact and understanding of the forensic sciences. This presentation will also foster a discussion between researchers and forensic science practitioners on ways to assess and measure the impact of forensic science disciplines.

Program Description: The effective use of forensic science provides timely and impactful answers to the criminal justice system. The application of social science research methods and multidisciplinary partnerships can shed light on how the criminal justice system and forensic science practitioners can maximize the role and value of the forensic sciences. As forensic science innovations are implemented into practice, advancements such as the identification of emerging drug threats, 3D virtual microscopy for firearms evidence, digital and multimedia evidence, and rapid DNA and other field deployable technologies offer opportunities to empirically examine their impact on justice system outcomes. For instance, social science methods can aid in understanding how the implementation of current and new technologies, methods, or workflow processes impact backlog reduction, capacity enhancement, cost relative to benefit, workforce development, and case closure efficiency.

This presentation will provide an overview of 20 years of social science and forensic research funded by the NIJ.[1] For example, over the past two decades, researchers have sought to improve their understanding of: how the forensic sciences are communicated and understood in the courtroom; the use and impact of forensic science to inform law enforcement investigations, actionable intelligence, and case outcomes; how forensic toxicology and seized drug analysis can inform community drug surveillance; and the need to strengthen the resiliency of the forensic workforce that is faced with unique stressors.

In addition, researchers have identified benefits and challenges of engaging in social science research to study the forensic sciences. Criminal justice practitioners, forensic science practitioners, and policy makers benefit from the use of innovative methods examining the effective and efficient use of forensic science. However, collaborations between social science researchers and forensic science practitioners are rare. In addition, rigorous studies that examine the effective and efficient use and outcomes of forensic science are complex, and as such, strategies to overcome challenges are needed. For instance, building partnerships between social science researchers and forensic scientists is critical. This presentation will discuss lessons learned and address solutions to overcome these barriers.

Presenters will also discuss future research and evaluation opportunities in order to develop a better understanding of the applications of social science on the impact of forensic science operations. This presentation will discuss various areas for research and evaluation that are relevant to the criminal justice community, including the examination of the role, value, and impacts of forensic science in the criminal justice system; evaluating the implementation of new and innovative policies and practices; understanding the costs and benefits of forensic science services; and assessing the safety, wellness, health, and workplace needs of forensic practitioners.

Reference:

1. Browning, K. Social Science Research on Forensic Science: The Story Behind One of NIJ's Newest Research Portfolios, July 1, 2015, *nij.ojp.gov*: https://nij.ojp.gov/topics/articles/social-science-research-forensic-science-story-behind-one-nijs-newest-research.

Social Science Research; Forensic Science; Multidisciplinary

CB5 The Role of Y-STR Analysis in Forensic Genetic Genealogy (FGG)

Colleen M. Fitzpatrick, PhD, Identifinders International LLC, Fountain Valley, CA*

Educational Objectives: Upon attending this presentation, attendees will gain insight into the evolution of Forensic Investigative Genetic Genealogy (FGG) from Y-chromosomal Short Tandem Repeat (Y-STR) analysis through autosomal SNP testing. Case studies will be presented illustrating the use of Y-STR FGG to generate investigative leads on cases where the DNA has been exhausted or where a mixture is present. Attendees will also learn how Y-STR FGG can reduce the complex genealogical research often required by autosomal SNP testing by narrowing the possibilities within a family tree to only individuals with a given last name. The presentation will include statistics indicating the probability of successfully obtaining a last name using the technique.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the synergy that exists between Y-STR and autosomal SNP analysis techniques. The presentation will broaden the understanding of how FGG developed over the past few years to become a powerful tool that has brought resolution to over 1,100 cold cases as of December 2023.

Program Description: Y-STR testing was first introduced in 2000 by several Direct-to-Consumer (DTC) DNA testing companies as a means of complementing genealogical records with genetic data. The popularity of Y-STR analysis as a tool for genealogical research grew rapidly, especially among male adoptees, because of the patrilinear co-inheritance of the Y chromosome with the family surname. A male adoptee could take a Y-STR test; a match found in the FGG databases would indicate a possible name for his birth father.

The availability and increasing size of publicly available genetic genealogy Y-STR databases prompted their use by law enforcement as a source of forensic intelligence starting in 2011 with the 1991 Sarah Yarborough homicide.[1] Because of the overlap between loci included in the genetic genealogy Y-STR test panels and those included in Thermo Fisher Scientific's AmpFLSTR YFiler and Promega's PowerPlex Y23 PCR amplification kits, a forensic Y-STR profile could be compared to the genetic genealogy Y-STR databases without retesting the evidence. Genetic genealogy databases could be mined for matches to a Y-STR profile from a crime scene or from unidentified remains, to obtain a possible last name in the absence of a CODIS match or when a CODIS profile was unavailable. Even if a match was not found, the nationality or ethnicity of "near matches" could be informative. The first case solved using FGG, the Phoenix Canal Murders, was actually based on several matches to the surname Miller found in the genetic genealogy Y-STR databases to the YFiler profile from the crime scene. This led to the identification of Bryan Patrick Miller as a suspect.[2] Miller's DNA was found to be a CODIS match to the DNA profile developed from the victims in the original investigation.

More recently, DTC autosomal SNP testing has eclipsed genetic genealogy Y-STR testing in popularity for forensic casework because it is useful for discovering relationships along all branches of a family, both single- and mixed-gender lines. As of December 2024, over 1,100 publicly announced unidentified human remains and violent offender cases have been solved using autosomal SNP testing techniques similar to those developed by genetic genealogists for adoption searches.[3] Yet Y-STR FGG is holding its value for forensic casework in situations where SNP testing is not useful, that is, where a DNA sample has been exhausted or where a mixture is present. Y-STRs can also be used to reduce the complexity of the genealogical research that must often be put in to identify a suspect using autosomal SNP testing, by limiting the possible branches to be investigated to those bearing a surname that matches the corresponding Y-STR profile.

This talk will discuss the practical use of YSTR FGG for cold casework, including case studies where Y-STR FGG was the important factor in resolution. The presentation will compare various issues that arise for Y-STR FGG and autosomal SNP analysis, along with statistics on the probability of producing a last name.

References:

1. Green, Sara Jean. Man Found Guilty in 1991 Cold-Case Murder of 16-Year-Old Sarah Yarborough. *Seattle Times*, May 10, 2023. Accessed July 30, 2024. https://www.seattletimes.com/seattle-news/law-justice/man-found-guilty-in-1991-cold-casemurder-of-16-year-old-sarah-yarborough/.
2. Cassidy, Megan. How Forensic Genealogy Led to an Arrest in the Phoenix 'Canal Killer' Case. *The Arizona Republic*, reprinted in *AZ Central*. November 30, 2016. Accessed July 30, 2024. https://www.azcentral.com/story/news/local/phoenix/2016/11/30/how-forensicgenealogy-led-arrest-phoenix-canal-killer-case-bryan-patrick-miller-dna/94565410/.
3. Dowdeswell, Tracey (2024), "Forensic Genetic Genealogy Project version December 2023, *Mendeley Data*, V1, doi: 10.17632/cc5rh42mf9.1.

Y-STR; Forensic Genetic Genealogy; Phoenix Canal Murders

CB6 Fiction vs. Science: The Yara Gambirasio Case on Netflix

Luciano Garofano, PhD, Italian Academy of Forensic Science, Parma, Emilia-Romagna, Italy; Michele Vaira, JD*, Vaira Law Firm, Parma, Italy*

WITHDRAWN

CB7 Unveiling Forensic Signatures: Leveraging Soil Microbiome and Enzymatic Responses to Detect Cyanide-Induced Deaths

Aby Joseph, Amity University Dubai, Dubai, United Arab Emirates; Jannath Manaf, MASc*, Amity University Dubai, Dubai, United Arab Emirates; Muhammad Safar S.P.S, MASc*, Amity University Dubai, Dubai, United Arab Emirates*

Educational Objectives: By the end of the presentation, attendees will have a comprehensive understanding of how soil microbiome and enzymatic responses to cyanide can enhance forensic detection methods, along with practical knowledge on implementing these techniques in real-world forensic settings.

Impact on the Forensic Science Community: This presentation will have a profound impact on the forensic science community by introducing innovative methodologies to improve cyanide detection in postmortem investigations. The proposed integration of soil microbiome analysis and enzymatic activity profiling represents a significant advancement over conventional toxicological techniques, which often fall short in detecting cyanide in extensively decomposed remains.

Program Description: This study investigates the responses of soil microbiomes and enzymatic activities to cyanide exposure from decomposing rodent remains, aiming to improve forensic detection methods for cyanide-induced deaths. Cyanide is a potent poison that poses significant challenges in forensic investigations due to its rapid degradation and the complexity of detecting it in extensively decomposed bodies. This research combines microbial community analysis, enzymatic assays, and advanced analytical techniques to develop more reliable methods for identifying cyanide exposure postmortem.

Forensic Microbiome; Necrobiome; Microbiology

CB8 Forensic Examination of Environmental Crime and Its Solutions

Ashfaq Ahmed Kharal, Jr., PhD, Lahore, Punjab, Pakistan*

Educational Objectives: The attendees are interested in a green and clean environment and are using technology to trace the elements of crimes creating pollution and hazards.

Impact on the Forensic Science Community: No one can deny the impact of a clean environment as it is a need not only in this era but is very emphatic all the time without any discrimination and the use of technology for highlighting these crimes by forensic analysis is the most important impact.

Program Description: Environmental forensic examinations are an essential component of environmental crime investigations. Environmental forensic whizzes assist factfinders in reenacting the occasion timeline, assessing the size of the destruction and causality. Their sentiments are of ultimate germaneness to environmental criminal stuff. Thus, it is critical to confirm that expert examinations are steered following scientific canons providing reasonably more truthful and steadfast results. In the article, two different slants to the organization of environmental forensic examinations were equated. Forensic examination has been conducted in two states, especially in Russia; the consolidated and more rigid model has been practical concerning environmental forensic studies, while in Poland, such inspections are navigated by decentralized forensic laboratories and self-regulating consultants. The authors deliberated existing variances and parallels between those two methods.

Crime Prevention; Digital Evidence; Chain of Evidence

DD1 Leveraging Forensic Science Standards Development to Boost Your Professional Growth

Kris Cano, MA, Signature Science, LLC, Scottsdale, A; J. Keith Pinckard, MD, PhD*, Travis County Medical Examiner, Austin, TX*

Educational Objectives: After attending this presentation, attendees will learn about the importance of documentary standards in forensic sciences, how the standards development process works, and how participating in standards development can help develop skills and abilities that can advance one's career trajectory while also advancing the field.

Impact on the Forensic Science Community: Participating in standards development is more than just a task; it is an opportunity to shape the future of forensic science and advance your career. By embracing this work, you not only contribute to the quality and consistency of forensic practice but also enhance your own professional growth and leadership capabilities. This session aims to help you recognize and leverage these benefits, turning what may seem like a thankless task into a powerful career-building tool.

Program Description: The process of standards development and implementation in forensic science can indeed seem daunting and underappreciated. Engaging in standards development sharpens your skills and deepens your understanding of the forensic science field. It challenges you to think critically, solve complex problems, and engage with a wide range of perspectives from professionals with diverse expertise. This process enhances your ability to lead, communicate effectively, and navigate complex scenarios—skills that are invaluable as you advance in your career. By participating in the creation and implementation of standards, you position yourself as a leader within your organization and the broader forensic community. These experiences not only boost your leadership credentials, but also amplify your influence in shaping your field's direction. By contributing to the development of these standards, you help ensure that forensic practices are consistent, scientifically sound, and widely accepted. Being involved in standards development can set you apart from your peers and open doors to new opportunities. It demonstrates your commitment to the field and your willingness to tackle challenging and sometimes unglamorous tasks. This proactive approach can lead to recognition, career advancement, and greater professional satisfaction. The skills you develop in standards development are transferable to other areas of your career. Whether it's crafting policies, handling crucial conversations, or managing public perception, developing the analytical and strategic thinking involved in standards work will serve you well.

Standards; Forensic Professionals; Education

DD2 Legal Admissibility of Digital Evidence in the Era of AI-Generated/Synthetic Media

Brandon Epstein, MS, Magnet Forensics, Freehold, NJ; Chichi Lee, LLM*, Middlesex County District Attorney's Office, Massachusetts, Waltham, MA*

Educational Objectives: Attendees with a legal background will learn the authentication issues surrounding AI-generated or -modified synthetic media such as photos and videos, including several proposals to amend the authentication rules under Fed. R. Evid. 901(a). They will also learn how to admit expert testimony on synthetic media under Fed. R. Evid. 702, including possible challenges to the experts' qualifications, principles, and methods.

Attendees with a forensic background will learn the issues that may come up in court from pretrial motions in limine to qualify the expert under Fed. R. Evid. 702. Forensic expert attendees will learn how an attorney will present their testimony in court and what an opposing party might contest as to their qualifications, principles, methods. While going through these, the attendees will also learn the relevant technology surrounding synthetic media.

Impact on the Forensic Science Community: Because synthetic media authentication is new, this presentation will introduce the admissibility of synthetic media authentication in court to the forensic science community. This presentation will allow the forensic experts to: (1) understand if their qualifications and their training that they provide to future experts will pass the legal muster, and (2) allow the forensic experts to have a preview of their future testimony in court; whether they are understandable to non-experts and reliable under the law, with a portion of a mock direct examination. With this presentation, scientists will be able to work with legal practitioners to overcome the hurdles in court and further their trustworthiness in the legal world with this new, developing authentication method.

Program Description: Although synthetic media, such as Adobe Photoshop, is not new, generative AI has created a new era of synthetic media. Now, synthetic media is accessible to the general public without training, is easier to manipulate, and is easier to create on a large scale. When AI-generated or -altered photos or videos are knocking on the court's door, they will undoubtedly impact how lawyers and judges look at both civil and criminal cases.

This presentation will introduce the issues surrounding the unavoidable challenges to the authenticity of photos and videos introduced in court where attorneys and judges have to deal with authentication under Fed. R. Evid. 901. This presentation will also discuss and comment on several proposals to alter Rule 901 in response to the rise in AI-generated synthetic media.

But there's more. With AI-generated synthetic media, the ability of detecting synthetic media and authenticating photos and videos becomes paramount. This is especially true in the era of synthetic media where the jury may start questioning everything they see or hear. This presentation will introduce how a synthetic media authentication expert passes Daubert v. Merrell Dow Pharmaceuticals, Inc. and Fed. R. Evid. 702. With a portion of a mock direct examination, the presentation will walk you through the expert's qualifications, the underlying facts and data, and the reliable principles and methods the expert uses. In that vein, we will also see some of the challenges the opposing party may present.

Artificial Intelligence; Expert Testimony; Admissibility

DD3 Balancing the Scales: Supporting Mental Health in Forensic Science

Kevin Kulbacki, MSFS, KDX Forensic Consulting, Chicago, IL*

Educational Objectives: By the end of this session, attendees will be able to: (1) identify common mental and emotional health challenges faced by forensic practitioners and their root causes, (2) apply at least two evidence-based strategies for personal mental health resilience shared during the session, (3) evaluate organizational practices that foster supportive mental health environments and determine how these can be implemented in their own workplaces, and (4) engage in peer-sharing to adopt innovative tips and ideas for enhancing mental well-being in forensic science roles.

Impact on the Forensic Science Community: The mental and emotional well-being of forensic practitioners is critical to maintaining the integrity and effectiveness of their work. This session highlights the importance of addressing mental health at both personal and organizational levels, creating a platform for open dialogue and shared learning within the forensic science community. By equipping attendees with practical tools, fostering peer connections, and promoting awareness, the session aims to cultivate a healthier, more resilient workforce. The insights gained will not only benefit individual practitioners but also contribute to a more supportive culture across forensic agencies, enhancing the overall quality and sustainability of the field.

Program Description: Forensic practitioners often face unique mental and emotional challenges due to the nature of their work. This session brings together insights from diverse perspectives to address these challenges and explore solutions. Through presentations by a practitioner, an organizational leader, and a mental health professional, attendees will gain actionable strategies to enhance individual resilience, foster supportive workplace cultures, and access professional resources. The session concludes with an open discussion, inviting participants to share practices, tips, and ideas to collectively improve well-being in the forensic field.

Mental Health; Resilience; Well-Being

DD4 Determining the Viability of Breathwork and Meditation as Intervention Techniques for Stress in the Forensic Science Community

Prateek Shetty, BSc, University of Central Oklahoma, Edmond, OK; Keisha Jones, MS*, University of Central Oklahoma, Edmond, OK*

Educational Objectives: Attendees of the presentation will learn about the impact of breathwork (cyclic sighing) and meditation as methods to mitigate workplace stress in the forensic science field. Data gathered from analyzing variations in stress among students studying forensic science and professionals working in the field before and after implementing the interventions will be discussed along with the future implications and research opportunities. Session attendees can expect to learn the methods implemented in the study, practice them during this presentation, and join a discussion about how these methods could be implemented in their everyday lives.

Impact on the Forensic Science Community: Attending this session will allow participants to learn about stress mitigation techniques such as breathwork (cyclic sighing) and meditation, which can be implemented in their personal lives and help them combat workplace stressors that come with being a part of the forensic science community. Learning and practicing these exercises can contribute to the growth of a holistically healthy forensic science workspace in the present and future.

Program Description: Are you stressed out because of the things that happen at work? Do you want to improve how you deal with stress in the workplace? Are you a prospective or present member of the forensic science workforce? If your answer to one or all these questions was a resounding, "YES," this session is for you!

Join the discussion to share personal stories of stress in the field. Learn what research has to say about how practicing breathwork and meditation could impact the busy life of a forensic science professional. But don't worry, you won't be bored with just the numbers from a research study. You will also get to practice these exercises and take these tools with you to use in your everyday life as a forensic science professional or student.

Join us in this session as we explore the possibilities of using our breathing patterns to help build a holistically healthy forensic science workforce for the present and the future!

Mental Health; Breathwork; Forensic Science

DD5 Misconceptions and Limitations of Words Commonly Used in the Forensic Sciences—What Do They REALLY Mean?

Charlotte Word, PhD, Charlotte Word Consulting, Richmond, VA; John D. Schmid, JD*, Sixth District Public Defender's Office, Duluth, MN*

Educational Objectives: After attending this session, both forensic science practitioners and members of the legal community should have an increased awareness of terms and associated language with a high susceptibility of being misinterpreted during judicial proceedings. Attendees should gain an increased understanding and appreciation of the meaning and strengths of the four focus areas to be discussed, along with their limitations and common misinterpretations. Insights into the need for effective training and communications with robust safeguards to ensure the reliability of forensic science evidence presented in court should be gained by the attendees, along with knowledge regarding mechanisms of improvement and potential ways to overcome any apparent obstacles.

Impact on the Forensic Science Community: This presentation will impact the forensic science community through training and discussions aimed to improve stakeholder competence, performance, and outcomes by facilitating an accurate understanding of common laboratory quality assurance terms frequently misinterpreted in court proceedings. By increasing awareness of the strengths and limitations of scientific and legal safeguards, forensic science practitioners will be better equipped to avoid misstatements and errors in testing, reporting, and testimony.

Program Description: Quality crime/forensic science laboratories routinely participate in audits to achieve accreditation as well as training competency and proficiency testing, conduct validation studies prior to commencing casework testing with a new method or product, and provide discovery following jurisdictional requirements. These practices are in place to ensure reliability of the testing conducted and the results reported. However, the roles and meaning of these processes are often misunderstood both by laboratory personnel and the legal community. The goal of this session is to address these issues by providing training regarding these topic areas and their potential to collectively ensure reliability while addressing the misunderstandings among stakeholders about the limitations of each individual safeguard.

The four primary discussion areas will focus on: (1) laboratory accreditation and audits; (2) competency and proficiency testing; (3) validation studies; and (4) legal rules of evidence and discovery. The training and discussions will address: (1) what each of these terms actually mean in the context of the laboratory, including their strengths and limitations; (2) how they are often misunderstood in the legal context; (3) ways in which they are miscommunicated through conversations and during testimony in court; and (4) an exploration of ways to overcome identified obstacles and common misunderstandings. The attendees will be encouraged to engage in discussions regarding the proper use of the terms, their perceived misunderstandings and communicating their own ideas for improvement.

This session will be targeted to a mixed audience of legal and scientific practitioners with diverse experiences and backgrounds.backgrounds.

Accreditation; Validation; Quality Control

DD6 Using Forensic Investigative Genetic Genealogy (FIGG) Technology Ethically and Responsibly

Catherine Cupples Connon, PhD, Virginia Commonwealth University, Richmond, VA*

Educational Objectives: After attending this deep-dive session, attendees will be aware of key concerns regarding the use of FIGG and the need to use this technology in an ethical and responsible manner. We will discuss: (1) what types of cases this technology should be limited to, (2) privacy considerations, (3) use of approved genetic genealogy databases, (4) the intersection of law enforcement, forensic laboratories, and FIGG investigators, and (5) appropriate and necessary documentation. Last, we will also address the needs for legislation, research, and FIGG certification/training.

Impact on the Forensic Science Community: This session will impact the forensic science community by offering much-needed guidance surrounding appropriate practices of the emerging FIGG field and its related technologies.

Program Description: FIGG is a new and emerging field of forensic DNA analysis that serves as an investigative tool for law enforcement. Though powerful, it is not appropriate for use with all forensic cases containing DNA evidence. Currently, it is specifically reserved for violent crimes in which a traditional Short Tandem Repeat (STR) profile has not been fruitful from an investigative standpoint, including unidentified human remains cases. Furthermore, when we think about privacy concerns, we must consider the fact that individuals unassociated with the crime may be linked to it for no reason other than being genetically related to the individual in question; thus, necessary precautions must be taken to protect their identities. As an extension of this, only genetic genealogy databases that have been approved for law enforcement use may be utilized—with strict regulation and oversight to boot. Such databases include GEDmatch Pro and FamilyTreeDNA. Throughout this process, cooperation and collaboration of law enforcement, the forensic laboratory, and the FIGG investigator is crucial. These roles will be discussed, including whether overlap is acceptable or if complete separation is needed. Accurate documentation is essential; we will discuss finding a balance between thoroughness and timeliness to ensure quality documentation.

The session will conclude with discussions of what is still needed with respect to legislative regulations, research related to the technology itself, as well as what kind of formal FIGG certification/training is needed, if any.

Forensic Investigative Genetic Genealogy; DNA; Ethics

LS1 Empowering Forensic Science: How Technology, Building Intelligence, and Human-Centered Design Can Revolutionize the Forensic Workplace

Jinhee Lee, Crime Lab Design, Royal Oak, MI; Ken Mohr, BS*, Crime Lab Design, Royal Oak, MI; Scott Morgan, BS*, Crime Lab Design, Royal Oak, MI; Jon Howard*, Crime Lab Design, Royal Oak, MI; Patrick Slattery*, Crime Lab Design, Royal Oak, MI*

Program Description: A new generation of predominantly female forensic scientists is revolutionizing the field, demanding state-of-the-art facilities/systems that support their important work and minimize physical strain. The integration of advanced technologies and intelligent-building systems represents a crucial shift toward enhancing case load processing, minimizing work-related injuries, and improving security within forensic environments. This presentation will highlight how cutting-edge automated systems, such as real-time tracking technologies with RFID and intelligent sensors, play a vital role in monitoring evidence, managing critical supplies, and ensuring the safety of staff, visitors, and occupants.

Global case studies from Asia and the Middle East illustrate the successful application of automated waste management, delivery systems, and Automated Guided Vehicles (AGVs) in addressing staff shortages and reducing physical strain. Notably, 95% of newly built hospitals in the United States now use Tissue Sample Movement Pneumatic Tube Systems, which enhance sample transport efficiency. Medical Examiner's Offices (MEOs) benefit from automated racking systems that increase storage capacity while reducing physical strain on staff, and Automated Mobile Robots (AMRs) streamline the handling of hazardous samples, thereby optimizing case load processing.

By embracing these advancements, forensic facilities can significantly improve operational efficiency, minimize manual labor, and better support a predominantly female workforce.

These technological innovations offer strategic solutions to counteract the projected shortage of over 30,000 forensic analysts in the United States. This session will provide critical insights, using real world examples and data, into how these technologies enhance the quality of life for forensic staff and set new benchmarks for industry best practices.

Educational Objectives: This session will explore how advanced technologies and intelligent building systems can support staff and transform their forensic environments by optimizing case load processing and reducing physical strain, while ensuring a secure and healthy work environment. Attendees will learn to implement Automated Guided Vehicles (AGVs), automated gurneys, RFID Tracking Systems, and Environmental Controls to enhance security, chain of custody, and indoor air quality. Through a data-driven review of global case studies, participants will see how these technologies improve safety, functionality, and efficiency in forensic facilities. By the end, attendees will have a clear understanding of how these technologies can drive measurable improvements in forensic outcomes today and into the future.

Impact Statement: A new generation of predominantly female forensic scientists is revolutionizing the field, demanding state-of-the-art facilities and systems that support their important work and minimize physical strain. Attendees will learn that by incorporating cutting-edge technology and optimizing case load processing, forensic facilities can not only ensure a healthier and safer working environment by reducing workplace injuries but can directly contribute to significant percentage gains in productivity and case load management. Attendees will leave the presentation with the knowledge needed to catalyze change at their facilities, armed with data proving that labs that embrace innovative technology, automation, and human-centered design are better equipped to meet the challenges created by the global demand for forensic science while mitigating the projected shortage of 30,000 analysts in the United States.

Technology; Transformation, Diversity

W1 Pediatric Fracture Healing in Cases of Suspected Non-Accidental Trauma: Clinical, Radiographic, Anthropological, and Histological Perspectives and New Technologies

Diana Messer, PhD, West Tennessee Regional Forensic Center, Memphis, TN; Donna C. Boyd, PhD*, Radford University, Radford, VA; Bradley J. Adams Office of the Chief Medical Examiner, New York, NY; Andrew Baker, MD*, Hennepin County Medical Examiner's Office, Minnetonka, MN; Farah W. Brink, MD*, Nationwide Children's Hospital, Columbus, OH; Jered B. Cornelison, PhD*, Western Michigan University, Homer Stryker MD School of Medicine Kalamazoo, MI; Carolyn V. Isaac, PhD*, Michigan State University, East Lansing, MI; Andy Tsai, MD, PhD*, Boston Children's Hospital, Harvard Medical School, Boston, MA*

Learning Overview: After attending this workshop, participants will understand how antemortem skeletal fractures can be used to help interpret, document, and adjudicate medicolegal cases of Non-Accidental Injury (NAI) in pediatric deaths. Attendees will learn the anatomical basis for and variables affecting pediatric skeletal fracture, repair, and timing (i.e., Time Since Injury or TSI). Using multidisciplinary technology, including histology, light microscopy, radiology, and machine learning, they will learn to critically evaluate current methodological approaches for evaluating pediatric fracture healing and estimating TSI. Applications of pediatric antemortem fracture data toward public health (including advocacy for children's health) will be explored.

Impact Statement: This workshop will impact the forensic community by providing multidisciplinary perspectives and methodological training for critically evaluating and interpreting skeletal fracture and its timing in pediatric death investigations. This training will help the forensic community determine best practice approaches to pediatric fracture interpretations that are scientifically based, legally defensible, and applicable to public health efforts toward prevention of non-accidental child injury.

Program Description: Physical abuse in children results in >500 deaths in the United States each year.1 Patterned and repetitive skeletal fracture is often observed in these decedents, reflecting the typically serial nature of pediatric NAI. Accurate estimation of TSI for antemortem fractures is critical for identification, evaluation, and adjudication of fatal pediatric NAI.

However, TSI estimation has been described as an "inexact science," due to lack of recognition, understanding, and scientific validation of existing TSI methods.2.3These methods derive from a variety of inter-disciplinary sources with unknown accuracy, reliability, consistency, and limited understanding of variables that impact fracture healing. This has necessitated extreme caution in use of TSI estimations in the adjudication of fatal pediatric NAI.

Multidisciplinary approaches to understanding pediatric fracture healing that focus on scientifically validated methodologies are imperative for developing best practice recommendations and standards for forensic pediatric skeletal trauma analysis in pediatric death investigations.

This workshop provides a multidisciplinary approach for understanding and estimating pediatric fracture TSI. Best practice recommendations for analysis and interpretation of pediatric skeletal fracture and its timing are presented using interdisciplinary technologies involving imaging (Computed Tomography [CT], radiology), microscopy (light microscopy, histology), and machine learning. The scientific foundation for pediatric fracture repair and its timing is considered, as is application of these methods to the broader context of pediatric case adjudication and public health. Hands-on examination of case study examples allows participants to put topics covered into practice with a focus on interdisciplinary collaboration.

Antemortem; Child Abuse; Anthropology

W2 Implementing Recommendations From the NIST/NIJ Expert Working Group on Human Factors in Forensic DNA Interpretation Report

Angela Spessard, MSFS, Maryland State Police, Pikesville, MD; Jarrah R. Kennedy, MSFS*, Kansas City Police Crime Laboratory, Kansas City, MO; Niki Osborne, PhD*, Human Factors Training and Consultancy, Remuera, New Zealand; Melissa Taylor, BA*, National Institute of Standards and Technology, Gaithersburg, MD; Hope Zagaria, MSFS*, Bureau of Justice Assistance, Washington, DC*

Leaning Overview: In this workshop, we will guide participants through the process undertaken by the National Institute of Standards and Technology (NIST)/National Institute of Justice (NIJ) Expert Working Group (EWG) on Human Factors in Forensic DNA Interpretation. We will highlight the key findings and controversial topics and present the final recommendations from the report published in May 2024. This workshop will focus on providing examples of how forensic science service providers are already implementing these recommendations in their practice.

Upon completion of this workshop, participants will: (1) be familiar with the published recommendations from the NIST/NIJ EWG on Human Factors in Forensic DNA Interpretation report, (2) understand the implications of these recommendations for their practice, (3) recognize how these recommendations aim to improve DNA examination practices, (4) critique the recommendations and discuss their relevance and feasibility in participants' own practices and the wider forensic DNA community, and (5) have the tools to implement the recommendations in their own practice.

Impact Statement: The study of human factors in forensic science is crucial to understanding the interaction between humans and the systems they use. By addressing human factor issues, we can identify and mitigate the likelihood and consequence of errors and biases, improve laboratory culture, staff morale, and productivity. This workshop encapsulates four years of effort dedicated to understanding human factors in forensic DNA interpretation, providing valuable guidance for DNA analysts, laboratory management, and legal practitioners to enhance practice and achieve safer justice outcomes.

Program Description: In May 2024, the NIST/ NIJ EWG on Human Factors in Forensic DNA Interpretation published a comprehensive report containing 44 recommendations for how to reduce the likelihood and consequence of errors in forensic DNA interpretation.

In this workshop, we will guide participants through the EWG's process, highlight key findings, discuss controversial topics, and present the final recommendations. The workshop will focus on the following areas:

- Training and Education
- Quality Assurance
- Cognitive Bias and Error Reduction
- Interpretation
- Reporting and Testimony
- Management
- Work Environment
- Expressing Evidence Strength
- Research
- Technology
- The "How" and "When" Questions in DNA Analysis

This workshop is relevant to DNA and other forensic science practitioners, laboratory management, researchers, legal practitioners, and anyone interested in improving decision-making in forensic science. Participants will be expected to actively engage with the material and provide feedback on the relevance and feasibility of implementing the recommendations in their practice.

We will use an interactive approach to help participants develop a plan to implement any recommendations that will provide value in their practice. Join us for a comprehensive session that aims to enhance your understanding and application of human factors in forensic DNA interpretation, ultimately contributing to better practices and safer justice outcomes.

Forensic DNA; Human Factors; Error

W3 A Military Criminal Investigative Organization and the Boon and Bane of Technology

Vanessa R. Neff, MFS, Department of the Army, Criminal Investigation Division, Woodbridge, VA; Matt Geniuk, MSc*, Department of the Army, Criminal Investigation Division, Fort Sam Houston, TX; Chris Adams, MFS*, Department of the Army, Criminal Investigation Division, Quantico, VA; Tim Bartman, BAS*, Department of the Army, Criminal Investigation Division, Fort Moore, GA; Ethan Dodson, MSFS*, Central Texas Field Office, Department of the Army, Criminal Investigation Division, Fort Cavazos, TX; Michael Allen Easter, Jr., MSFS*, Department of the Army, Criminal Investigation Division, Fredericksburg, VA; Pedro Hernandez, Jr., MFS*, Department of the Army, Criminal Investigation Division, El Paso, TX; Dale Sajdak, MSFS*, Department of the Army, Criminal Investigation Division, Stedman, NC; Jessica A. Veltri, MS*, Department of the Army, Criminal Investigation Division, Stafford, VA; Bryce Wolford, MA*, Department of the Army, Criminal Investigation Division, Quantico, VA*

WITHDRAWN

W4 Forensic Science Adaptation to Artificial Intelligence

Gina Londino-Smolar, EdD, IU Indianapolis, Indianapolis, IN; JCU Downs, MD*, forensX, LLC, Johns Island, SC; Carole E. Chaski, PhD*, Institute for Linguistic Evidence, Georgetown, DE; Brandon Epstein, MS*, Medex Forensics, Madison, WI; Ting-Yu Huang, PhD*, Central Police University, Taiwan; Michael A. Marciano, PhD*, Syracuse University, Syracuse, NY; David Pienkowski, MBA, PhD*, University of Kentucky, Lexington, KY; Kathryn C. Seigfried-Spellar, PhD*, Purdue University, West Lafayette, IN; Aaron M. Shaprio, PhD*, Provincial Health Services, Authority of British Columbia, Vancouver, BC, Canada; Henry Swofford, PhD*, National Institute of Standards and Technology, Gaithersburg, MD; Jorn Yu, PhD*, Sam Houston State University, Huntsville, TX*

Learning Overview: The proposed presentations are poised to significantly impact the forensic science community by integrating AI into various aspects of forensic analysis. They aim to enhance understanding of AI principles, propose strategies for implementing AI-enabled tools in pattern evidence analysis, and explore innovative biometrics solutions. They also seek to evaluate novel approaches to media authentication in the face of AI-generated content, apply AI in fire debris analysis and forensic DNA analysis, and use generative AI for rapid detection of novel drugs in toxicology samples. Last, they provide a historical perspective on the development of AI and discuss its challenges in forensic science. Collectively, these presentations could lead to more accurate, efficient, and objective forensic analyses, ultimately contributing to a more effective criminal justice system.

Impact Statement: This presentation will impact the forensic science community by providing examples of best practices with inter-agency partnerships, differing ways technology can be used to solve investigations, be a resource to assist with cold cases that may have a Department of Defense nexus, and how technology can be used in austere environments to solve investigations.

Program Description: This workshop was developed by the Forensic Science Foundation (FSF) Educational Committee. The workshop will explore the transformative potential of Artificial Intelligence (AI) in forensic science. It will delve into the basics of AI analyses, discuss strategies for implementing AI-enabled tools in pattern evidence, and present innovative biometrics solutions for digital forensics. The workshop will also evaluate novel approaches to media authentication in the age of AI-generated content, apply AI in fire debris analysis and forensic DNA analysis, and discuss the use of generative AI for rapid detection of novel drugs in toxicology samples. Last, it will provide a historical perspective on the development of AI and discuss its challenges in forensic science. Attendees will gain insights into the state of AI in forensic science, learn about current tools, and understand the considerations in the development and evaluation of AI-based techniques in the field.

Artificial Intelligence; Forensic Science; Education

W5 The Sherry Black Investigation — A Journey for Justice

Katherine M. Brown, PhD, Tarleton State University, College Station, TX; Kelsie Bryand, MS*, Sam Houston State University, Huntsville, TX; Heidi Miller*, Sherry Black Foundation, Sandy, UT; Erin BE Ryan, BS*, Sherry Black Foundation, Midway, UT; Patrick J. Zirpoli*, Sherry Black Foundation, Milanville, PA*

Learning Overview: After attending this presentation, attendees will gain a working knowledge of the process of violent crime investigation. Attendees will recognize: (1) the initial crime scene and evidence collection; (2) forensic analysis of the evidence; (3) crime acene assessment and behavior analysis; (4) Investigative Genetic Genealogy; (5) prosecutorial considerations and the impact on families.

Impact Statement: This presentation will impact the forensic science community by exploring a violent homicide from the initial investigation through the ten years of investigation, culminating in the identification and conviction of the offender. During this investigation, many forensics sciences disciplines were involved; this presentation discusses these practices and their application to homicide investigation.

Program Description: This program will take the attendees through the high-profile, 10-year investigation of the murder of Sherry Black. She was murdered in her small bookstore located on her property in South Salt Lake City, UT, on November 30, 2010. The program will start with the initial call to the South Salt Lake City Police Department and the processing of the crime scene by the Utah Bureau of Forensic Services Laboratory System. The evidence recovered at the scene and the impact the evidence had on the investigation will be discussed. The presentation will show the initial forensic testing and the results of the analysis. The Behavioral Analysis conducted on both the crime and offender will be reviewed with the attendees, and the evidence-based conclusions will be explained. Investigative Genetic Genealogy has become a critical tool in identifying unknown remains and unknown offenders in many violent crimes. We will discuss how this was used during the investigation, and how the offender was eventually identified. The attendees will learn the proper way to obtain reference samples to ensure that any results will not be questioned. This presentation will describe the prosecutorial process from the time of the offender's arrest through his sentencing. We will discuss considerations when prosecuting cold case homicides. Finally, the attendees will learn the steps that were taken to pass Utah SB156, also known as the "Sherry Black Bill." This legislative act introduces a more structured framework for law enforcement's utilization of optional genetic testing databases when investigating violent crimes.

Crime Scene Investigation; DNA; Genealogy

W6 NIST/MSP Hypergeometric Sampling Software for Seized-Drug Analysis

Sandra E. Rodriguez-Cruz, PhD, Drug Enforcement Administration, Dulles, VA; Catherine Savage, MS*, Maryland State Police, Forensic Sciences Division, Pikesville, MD; Laurel Bobka, MSFS*, Maryland State Police, Walkersville, MD; William Guthrie, MS*, National Institute of Standards and Technology, Gaithersburg, MD; Jack Prothero, PhD*, National Institute of Standards and Technology, Westminster, CO; Joshua Smith, BS*, Charles County Sheriff's Office, Windsor Mill, MD*

Learning Overview: After attending this workshop, the attendees will understand how different hypergeometric sampling tools can be used in a forensic laboratory to improve the information provided about the population of drugs submitted.

Impact Statement: This workshop will impact the forensic science community by providing an alternative to the sampling tools currently available on the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) website. The presenters will highlight the reasons why Maryland State Police (MSP) decided to provide statistical data for evidence submitted with a possession with intent to distribute charge. One of the current challenges of statistical sampling of seized-drug evidence is that the complex mixtures of street samples do not always have the same controlled substances reported for each specimen tested. This sampling app will allow a statistical statement to be more easily made in more situations than the hypothesis testing approach.

Program Description: This full-day workshop will present a new statistical sampling app developed by the National Institute of Standards and Technology (NIST) and the MSP to apply hypergeometric sampling to seized-drug evidence. The theory behind the calculations in the app will be thoroughly discussed, along with tools and techniques for both conceptualizing and deploying statistical sampling plans in seized-drug laboratories. Topics covered will include sample size selection, uncertainty quantification, generation of appropriate population inferences for net weight and identification purposes, and appropriate reporting language for net weight, unit count, and extrapolation scenarios.

Seized Drugs; Sampling; NIST

W7 DSM Demystified: Medical Disorders in the Courtroom

Corina Freitas, MD; Freitas and Associates LLC. Alexandria, VA; Donna Coleman, JD*, Office of the Public Defender, Mental Health Division, Towson, MD*

Learning Overview: The goals of this workshop are to: understand and articulate the role of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM*) in legal settings; analyze the limitations and challenges of relying on the *DSM* for legal determinations of mental health issues; identify and compare alternative diagnostic manuals and systems used in mental health assessments; differentiate between legal versus medical definitions of mental disorders in the assessment and testimony of mental health conditions; acquire knowledge to effectively challenge the testimony and findings of opposing experts in court. learn to identify potential weaknesses or biases in expert evaluations and testimonies; evaluate the credentials, experience, and methodology of potential expert witnesses; and review landmark cases that have significantly influenced mental health law and discuss their impact on current legal standards and practices.

Impact Statement: By achieving these learning objectives, participants will gain a comprehensive understanding of the interplay between mental health and legal systems, particularly the role and authority of the *DSM* in court. They will be equipped with the knowledge to critically evaluate and challenge forensic limitations, alternative diagnostic options, and the legal versus medical definitions of disorders. Through case examples and practical strategies, attendees will enhance their ability to select and prepare expert witnesses, ensuring robust and credible testimonies. This training will empower legal professionals to effectively navigate the complexities of forensic mental health evaluations, ultimately improving the quality and fairness of judicial outcomes.

Program Description: This presentation is designed to equip legal professionals with the knowledge to confidently discuss the mental health in court proceedings, explore alternative diagnostic options, and understand the crucial distinctions between legal and medical definitions of disorders. Participants will delve into real case examples to see theory in action and gain practical insights into challenging opposing experts and selecting the right expert witnesses.

Interactive elements will include engaging group discussions and role-playing scenarios to enhance learning and retention. Role-playing in this session will involve analyzing various case scenarios from jurisdictions with different legal standards and tests. Participants will engage in mini mock trials with forensic evaluations where they will apply their understanding of the *DSM*'s authority, forensic limitations, and the distinction between legal and medical definitions of disorders. By attending this session, you will not only enhance your ability to navigate forensic evaluations but also ensure ethical and accurate representations of mental health in legal contexts. Join us to transform your courtroom strategies and become a more effective advocate for your clients.

Forensic Psychiatry; Law; Attorneys

W8 Forensic Postmortem Radiology and Medicolegal Death Investigations

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Learning Overview: After attending this workshop, attendees will: (1) appreciate the evolution of forensic imaging: (2) develop an understanding the fundamentals of Postmortem Computed Tomography (PMCT), including interpretation; (3) gain insights regarding the applications of Postmortem Computed Tomography (PMCT), with a particular focus on its role during the opioid epidemic; (4) identify unique considerations for pediatric forensic imaging, and (5) discuss 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.

Program Description: Forensic postmortem radiology is an essential element of a comprehensive medicolegal death investigation. State-of-the-art techniques and procedures will be discussed through case presentations and interactive interpretations of radiology images.

Forensic Imaging; Computer Tomography; Forensic Pathology

W9 Statistical Thinking for Forensic Science: From Probability to Black Box Studies to Likelihood Ratios

Michael J. Salyards, PhD, CSAFE, Tucson, AZ; Linton Mohammed, PhD*, Forensic Science Consultants, Inc., Poway, CA; Hal S. Stern, PhD*, University of California, Irvine, Irvine, CA*

Learning Overview: The workshop is intended to review basic concepts from probability and statistical inference and demonstrate their relevance in the current discussions regarding the assessment and interpretation of impression, pattern, and trace evidence. Participants will: (1) become 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; (2) become 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. The workshop is intended to leave participants 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.

Program Description: This workshop provides an introduction to key concepts in probability and statistics by linking them to key topics that are currently impacting the forensic science community. The target audience is anyone (e.g., practitioners, lawyers, judges, educators, and stakeholders) with a desire to better understand ongoing conversations about validation studies, error rates, inconclusive findings, likelihood ratios, and general concepts in statistics and probability.

Probability; Statistics; Validation

W10 Alcohol Calculations and Expert Testimony: How ANSI/ASB BPR 122 and 037 Can Be Applied in Forensic Toxicology Cases

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Learning Overview: Attendees will be able to use the alcohol calculation guidelines in a wide variety of forensic case types. They will learn how different toxicologists apply those calculations to expert opinion testimony.

Impact Statement: This workshop will impact the forensic toxicology and criminal justice community by educating stakeholders in how the principles of American National Standards Institute/Academy Standards Board Best Practice Recommendation (ANSI/ASB BPR) 122 and ANSI/ASB BPR 037 can be applied to a variety of forensic case types involving alcohol calculations.

Program Description: Forensic toxicologists are frequently asked to perform calculations related to alcohol in a wide variety of situations. ANSI/ASB BPR 122 Best Practice Recommendation for Performing Alcohol Calculations in Forensic Toxicology, First Edition, 2024, outlines a scientific approach to improve the quality and consistency of this type of work. Those calculations can then serve as the basis for expert opinion in matters related to motor vehicle investigations, drug-facilitated crimes, workplace injury inquiries, and other criminal and civil cases. Performing the calculations in a standardized manner can also assist experts in meeting the recommendations of ANSI/ASB BPR 037 Guidelines for Opinions and Testimony in Forensic Toxicology, First Edition, 2019.

This workshop will review the alcohol calculation best practices and expert opinion guidelines, then demonstrate how the calculations can be applied to a variety of real-world case scenarios. Experts will also share how those calculations are then presented in court as part of their expert opinion testimony.

Alcohol Calculations; Retrograde Extrapolation; Expert Testimony

W11 Sequencing 101

Rachel H. Oefelein, MSc, DNA Labs international, Deerfield Beach, FL; Marybeth Sciarretta, MS*, DNA Labs International, Deerfield Beach, FL; Laurence Devesse, PhD*, Qiagen, Zurich, Switzerland; Kevin Lord*, Astrea, Austin, TX; Mandi S. Van Buren, MS*, DNA Labs International, Deerfield Beach, FL*

Learning Overview: The goal of this workshop is for attendees to walk away with a better baseline knowledge of sequencing and to provide a foundation for informed decision-making for those considering deploying sequencing in their laboratory.

Impact Statement: Sequencing can be intimidating for those that are used to the existing DNA technologies that have been available over the past several decades. This workshop will broaden that knowledge.

Program Description: What is a library? If it is whole genome sequencing, why don't I get the whole genome? This workshop is designed to be a crash course in all things sequencing. Existing commercial and custom panels for autosomal Short Tandem Repeat (STR), X-STR, Y-STR, mitochondrial, and Single Nucleotide Polymorphism (SNP) testing will be discussed. Additionally, the differences between targeted sequencing, shotgun sequencing, amplicon versus ligation-based libraries, and additional sequencing methods will be compared. Whether or not bioinformatics is required based on the sequencing method will be explored as well as the types of bioinformatic processes that may be employed, including imputation. With any new technology comes the introduction of this procedure in the courtroom as well as the inevitable court challenges. Successfully presented expert witness testimony will be previewed as well as a look at ongoing court challenges. Finally, cases where sequencing has been utilized will be highlighted. The presenters of this workshop will span industry experts, a bioinformatician, and active casework users of sequencing technologies.

Forensic DNA; Sequencing; Next Generation Sequencing

W12 Can Human Remains Detection Canine Teams Detect Residual Odor? Results of Odor Recognition Tests and Chemical Analysis

Dawnie W. Steadman, PhD, University of Tennessee, Knoxville, TN; Mary E. Cablk, PhD*, University of Tennessee Knoxville, Reno, NV; Shawn R. Campagna, PhD*, University of Tennessee, Knoxville, TN; Mary Davis, MSc*, University of Tennessee, Knoxville, TN; James C. Ha, PhD*, University of Tennessee. Knoxville, TN*

Learning Overview: Residual odor is an odor that persists after the target is removed. This workshop addresses canine detection of residual odor of humans, both deceased and alive. After attending this workshop, attendees will understand: (1) if Human Remains Detection (HRD) canine teams reliably detect residual odor of human remains; (2) if these canines differentiate between residual odors of deceased human remains and live human scent; (3) if analytical chemistry methods detect and differentiate residual odor of living and deceased individuals; and (4) how the integrated results of chemistry and canine behavior analysis help us understand if and how residual odor training can be improved.

Impact Statement: The legal threshold for admitting canine evidence relies on the experience of the handler and their description of the canine's training, neither of which provide the errors associated with the technique required by standard rules of evidence. This workshop will assist attorneys and judges in assessing the probative value of canine alerts that may be detection of residual odor and thus considered evidence by providing results of a coupled canine-analytical chemistry analysis of intact decedent residual odor study. In addition, the standardized Odor Recognition Test (ORT) design will establish guidelines for canine training and testing to improve Human Remains Detection (HRD) certification efforts.

Program Description: Dogs have keen olfactory senses and are deployed for a number of forensic purposes in criminal investigations. HRD canines (a.k.a. cadaver dogs) are trained to detect the odor of human remains, which is comprised of Volatile Organic Compounds (VOCs) that emanate from a deceased body. Despite their widespread use, the reliability of HRD canines is complicated and understudied.

Here, residual odor refers to the odor from a deceased human that remains after it is removed from its resting place, including transient places or objects a body touched. Little is known about deceased residual odor except that its VOCs degrade over time. However, canine alerts are being treated as scientifically valid indicators that an intact decedent was present at one time, even when no physical or corroborating (e.g., chemical) evidence exists, and without regard for the level of training, certification, or proficiency of the canine.

A study of 35 HRD canine teams (handler and dog) tested on residual odor from both deceased and living individuals provides the data and results presented in this workshop. The presenters will describe the development of a standardized, double-blind ORT for canine teams and discuss the accuracy of the canine teams in detecting deceased human residual odor. The presenters will explain the chemical characterization of the VOCs from the odor samples used in the ORTs. Finally, the presenters will utilize select video and audio recordings of the canine team participants from the ORT to contextualize the results and discuss implications of the study.

Cadaver Dog; Accuracy; Multidisciplinary

W13 The Body as the Crime Scene: Traces, Signs, and Scientific Methods

Michelle D. Miranda, PhD, Farmingdale State College, SUNY, Farmingdale, NY; Claude Roux, PhD*, University of Technology Sydney, Sydney, Australia; Patrick Buzzini, PhD*, Sam Houston State University, Huntsville, TX; Yanko G. Kolev, MD, PhD*, Medical University - Pleven/District Hospital MBAL, Gabrovo, Bulgaria; Antonel Olckers, PhD*, African Academy of Forensic Sciences (AFSA) & DNAbiotec, Pretoria, South Africa; Patricia M. Speck, DNSc*, Hoover, AL; Jessica Volz, DNP*, Adventist HealthCare, Shady Grove Medical Center, Rockville, MD*

Learning Overview: This workshop addresses the multidisciplinary approach to forensic investigations, drawing attention to the factors that connect forensic scientists, regardless of discipline or specialization. Specifically, forensic scientists rely on imagination, curiosity, observation, and reasoning in an effort to detect and assign meaning to traces in order to understand events of interest. After attending this workshop, attendees will: (1) understand what connects forensic scientists at our core, notably the scientific approach to problem-solving through observation, reasoning, and the detection and evaluation of traces; (2) consider the types of traces encountered across several disciplines (e.g., AAFS sections, including Criminalistics, Forensic Nursing Science, Pathology/Biology, Toxicology, General, and others); (3) apply what they have learned by considering traces in context and comparing and contrasting discipline-specific approaches to assign meaning to traces of significance; and (4) understand that the Sydney Declaration was developed with the goal of promoting a shared understanding of traces across forensic science.

Impact Statement: This presentation will impact the forensic science community by fostering the understanding that scientific problem-solving in forensic science extends beyond one's specific-area discipline and should be the foundation on which forensic science endeavors are built.

Program Description: This workshop highlights the multidisciplinary approach to forensic investigations, drawing attention to the factors that connect forensic scientists, regardless of discipline or specialization. After learning fundamentals common to forensic science as a whole, attendees will delve into knowledge specific to disciplines (criminalistics, nursing, pathology, toxicology, and scene investigation). Each discipline will focus on the observation, detection, and interpretation of traces to aid in understanding complex events. With a focus on sexual and gender-based violence, attendees will apply the knowledge gained from this workshop interactively via a case study that requires insight from and collaboration across various disciplines.

Sydney Declaration; Traces; Scientific Methods

W14 The Longest Journey Starts With a Single Step: Evaluating Biological Results Given Activity-Level Propositions—The Problem, the Theory, the Solution, and Strategies for Implementation

Tim Kalafut, PhD, Sam Houston State University, Huntsville, TX; Sheila Willis, DSc*, Leverhulme Research for Forensic Science, Dublin, Ireland; Simone Gittelson, PhD*, DC Department of Forensic Sciences and The George Washington University, Washington, DC*

Learning Overview: After attending this workshop, attendees will have a better appreciation of how to evaluate DNA results given activity level propositions. They will practice how to correctly formulate activity-level propositions, assign likelihood ratios for biological results given activity-level propositions, apply the Case Assessment and Interpretation (CAI) framework, and see how to use a probabilistic model (e.g., a Bayesian network). With this knowledge, attendees will be able to start to better address hypothetical questions commonly asked in court by recognizing poorly worded questions and providing scientifically correct answers to these questions.

Impact Statement: This presentation will impact the forensic science community by providing guidance on how to answer activity-level questions on biological results in a way that is useful to the court based on a sound logical framework and following international guidelines.

Program Description: It is clear that the court is interested in "how-the-DNA-got-there" questions. However, answering hypothetical activity-level questions with "it's possible" is not a scientifically justifiable, useful, nor recommended practice. The refusal answer of "I can't answer questions related to how DNA got there" is perhaps even more problematic. This workshop will engage participants in how to prepare and answer activity-level questions with answers that: (1) are not misleading, and therefore minimize the risk of a wrongful conviction; (2) are based on a sound, logical framework; and (3) follow international guidelines. A combination of lectures, demonstrations, and hands-on group activities will be used to show how DNA results may be evaluated with regard to activity-level propositions as well as how to communicate this information. This workshop will cover Case Assessment and Interpretation, existing guidelines, probabilities, likelihood ratios, likelihood ratio equations given activity level propositions, Bayesian networks and sensitivity analyses. In addition, this workshop will focus on how to assign probabilities and likelihood ratios using scientific, peer-reviewed publications. Since case-relevant publications do not always exist, we will also explore how to assign probabilities and likelihood ratios in the presence of limited information. This workshop will cover challenges in answering commonly phrased questions at court and communicating the value of the evidence. Finally, strategies to help with implementation via a series of achievable goals will be presented to equip and empower experts to give answers to these questions of importance to the court.

Activity Level; Evidence Evaluation; Forensic DNA

W15 Artificial Intelligence (AI) in Forensic Education: The Good, the Great, and the Truth

Gina Londino-Smolar, EdD, IU Indianapolis, Indianapolis, IN; Kelly M. Elkins, PhD*, Towson University, Towson, MD; Theresa M. DeAngelo, MSFS*, Maryland State Police, Forensic Sciences Division, Pikesville, MD; Amber L. Fortney, MS*, University of Central Oklahoma, Perry, OK; Meagan Raddatz, MS*, University of Central Oklahoma, Perry, OK; Claire E. Shepard, MS*, LA Delta Community College, Monroe, LA; Ying Zhang, MS*, University of Florida, Gainesville, FL*

Learning Overview: This presentation aims to examine and apply the use of AI in forensic science education, create learning opportunities for students using AI tools, and evaluate the ethics and challenges of using of AI in education.

Impact Statement: This workshop will have a profound impact on the forensic science community by highlighting the transformative potential of AI in education. The workshop targets all people in the forensic science community, especially educators, as the use of AI enters our disciplines. It will provide insights into the ethical implications of AI, explore the application of supercomputers, demonstrate how students can leverage AI for efficient exam preparation, and address complex issues of victimization. The workshop will delve into the ethical considerations of AI use within forensic education, discussing concerns such as privacy, bias, transparency, and academic integrity. The workshop will equip attendees with the skills to craft precise, context-rich prompts for AI-powered tools, facilitating the creation of engaging, adaptive, and industry-relevant learning experiences for forensic science students. This workshop promises to be a game-changer in harnessing the power of AI for the advancement of forensic education.

Program Description: This workshop offers a comprehensive exploration of AI's role in forensic education. It begins with an introduction to the ethical considerations of AI use in academia, highlighting the intersection of AI and plagiarism. The session then delves into the application of AI systems in higher education, including an overview of the HiPerGator supercomputer. Attendees will learn how students can leverage AI for exam preparation, utilizing AI-powered platforms, chatbots, virtual tutors, and flashcard apps. The workshop also addresses the application of AI in solving complex societal issues, specifically the Missing and Murdered Indigenous Women crisis. Ethical considerations of AI use within forensic education are discussed, with a focus on privacy, bias, transparency, and academic integrity. The workshop concludes with a hands-on session on mastering AI prompt generation, equipping attendees with the skills to create engaging, adaptive, and industry-relevant learning experiences for forensic science students. This workshop promises a deep dive into the transformative potential of AI in forensic education.

Education; Artificial Intelligence; Forensic Science

W16 How to Be a Long-Term Unidentified and Missing Persons Investigator: The Investigators' Toolkit

Lara Newell, MA, Virginia Office of the Chief Medical Examiner, Richmond, VA; Katharine C. Pope, MA*, RTI International, Fruitland, MD; Hannah Barcus, MS*, Bureau of Justice Assistance, Washington, DC; Mark A. Bush, BAS*, Loudoun County Sheriff's Office, Leesburg, VA; Bryan T. Johnson, MSFS*, FBI, Quantico, VA; Carol Schweitzer, MA*, National Center for Missing & Exploited Children, Alexandria, VA; Jennifer Randolph, MSc*, DNA Doe Project, Hanover, DE; Nancy Rissi, BS*, Riverside County Sheriff's Department, Riverside, CA*

Learning Overview: During this presentation, attendees will receive information and training on various resources used to investigate long-term unidentified human remains cases, as well as long-term missing persons cases. Attendees will learn how juvenile cases differ from adult unidentified human remains and missing persons cases. Information will be shared on best practices for investigation and will cover a case from start to finish.

After attending the workshop, attendees will: (1) have a better understanding of how to investigate unidentified human remains and long-term missing persons cases; (2) know how to start an unidentified human remains and/or long-term missing persons investigation; (3) understand the resources and support agencies available; (4) be able to determine the best resources that can be utilized for their own cold cases; (5) be able to discuss their own cases and investigations and receive real-time support from the speakers on how best to move their investigations forward.

Impact Statement: The workshop will impact the forensic science community by providing a toolkit for investigators to use during unidentified human remains and missing persons investigations. While there are some agencies who have a dedicated cold-case unit who is responsible for long-term unidentified human remains cases and long-term missing persons cases, the reality is that most agencies across the nation are too small, do not have the budget, and/or do not have the manpower to have a dedicated unit. This workshop will provide attendees with information how to begin a cold unidentified human remains case or missing persons case, and how to keep the cases active. It will also provide attendees with a knowledge base on investigations and a point of contact for resources on cold cases nationwide.

Program Description: The idea for the workshop came from the Long-Term Unidentified Coordinator at the Virginia Office of the Chief Medical Examiner (VA OCME). This position is relatively new with the VA OCME and is rare nation-wide. This is a dedicated position created in 2021 to provide designated care and coordination to the Commonwealth's long-term unidentified cases. The value of a long-term unidentified coordinator is immeasurable. The increase in violent and drug deaths leaves medical examiner offices across the nation overwhelmed with current cases. Without a dedicated individual who has defined duties and responsibilities to coordinate long-term unidentified investigations with law enforcement, these cases fall by the wayside and remain unidentified in perpetuity.

It has been called the nation's "Silent Mass Disaster:" in the United States there are in excess of 14,000 long-term unidentified human remains and 25,000 long-term missing persons. Often, cases have had a cause and manner of death assigned, and occasionally some homicides have been prosecuted, without knowing the identity of the decedent. Even if the case is resolve from a criminal and/or medicolegal standpoint, without resolution of the identity of the person, the case is still incomplete and deserves attention. Resources and tools needed to investigate each of these cases vary on a case-by-case basis. This workshop will give an overview on the resources from NamUs/RTI, NCMEC, the FBI, non-profit DNA labs, and local law enforcement.

One of the challenges with investigating unidentified human remains and missing persons cases is the lack of assistance, support, and resources. The speakers for this workshop are all well-established in their respective fields and are valuable resources for investigating unidentified human remains cases and long-term missing persons cases. At the end of the workshop, there will be a discussion panel where attendees can ask questions about the process of investigation, as well as receive individual support and assistance for their own investigations. At the end of the workshop, attendees will have a better understanding of the resources available to them and will hopefully be able to implement new techniques in their own agencies.

This workshop was designed to assist investigators from law enforcement agencies and medical examiners/coroners in investigating their long-term unidentified human remains cases as well as long-term missing persons cases. Representatives from the National Missing and Unidentified Persons System (NamUs), National Center for Missing and Exploited Children (NCMEC), the DNA Doe Project, the Federal Bureau of Investigation (FBI), and a cold case detective from Virginia will discuss how their agencies can assist and provide support to those investigating unidentified human remains and long-term missing persons cases.

The presentation on NamUs will include updated information on staffing, system services, and training. The Program Manager for the Forensic Services Unit at NCMEC will share resources and case examples on how NCMEC can assist and support juvenile investigations for both unidentified human remains and missing persons investigations. The Bureau of Justice Assistance (BJA) will discuss federal resources and grant opportunities for those investigating missing and unidentified persons cases.

The second block includes presentations that are more hands-on. The executive director from the DNA Doe Project will discuss Forensic Investigation Genetic Genealogy and the resources that the DNA Doe Project can provide. The FBI's Major Incident Program Manager will give an overview on the Latent Print Unit in the FBI and their support services, as well as a chemical demo on how to retrieve fingerprints on degraded human remains. The last presentation is from a local cold case detective who will present their case of an unidentified human remains case from start to finish.

Unidentified Persons; Missing Persons; Casework

W17 Practical Neuropathology for Coroners and Medical Examiners: Basic Concepts and Advanced Principles

Lorenzo Gitto, MD, Cook County Medical Examiner's Office, Chicago, IL; Ponni Arunkumar, MBBS*, Cook County Medical Examiner's Office, Chicago, IL; Jared Ahrendsen, MD, PhD*, Northwestern University, Chicago, IL; Anfisa Baiandurova, MD*, West Tennessee Regional Forensic Center, Memphis, TN; Rudolph J. Castellani, MD*, Northwestern University, Feinberg School of Medicine, Chicago, IL; Samantha N. Champion, MD*, Cook County Medical Examiner, Chicago, IL; Michelle A. Jorden, MD*, Santa Clara County Medical Examiner/Coroner, San Jose, CA*

Learning Overview: After attending this workshop, participants will have an enhanced knowledge base of neuropathology as seen in a busy forensic pathology office. Emphasis will be placed on natural diseases, trauma, microscopic sampling and examination, and pediatric non-accidental head trauma. Attendees will develop skills necessary for comprehensive brain assessments at autopsy and techniques to address both common and unusual pathologies. They will be exposed to various specimens and techniques through didactic presentations, and case-based discussions.

Impact Statement: This workshop will emphasize the multifaceted nature of brain pathology in the context of forensic autopsy. Didactic lectures and practical instruction using detailed anatomical images will be provided to enhance autopsy performance and competency, with an additional focus on the workup and evaluation of pediatric non-accidental head injury.

Program Description: Assessing Central Nervous System (CNS) trauma is critical in neuropathology and forensic pathology and encompasses closed- or open-head injuries, multiple types of forms of parenchymal brain injury, and secondary complications such as edema and increased intracranial pressure. Establishing a link between trauma and death is crucial for determining the cause and manner of death.

Child abuse, particularly abusive head trauma, is a complex and vital focus in forensic neuropathology. A comprehensive workup is essential to document the findings and determine their relevance in terms of evidentiary significance and to rule out potential confounders to pediatric head trauma and child abuse.

CNS disorders such as epilepsy may present significant challenges when associated with sudden and unexplained death (Sudden Unexplained Death in Epilepsy [SUDEP]), as they often lack morphological indicators. Other natural disease processes, such as undiagnosed brain tumors, aneurysms, infections, neurodegenerative diseases, and cerebrovascular accidents, can further complicate forensic assessments and may have public health implications.

Different brain pathologies necessitate varied approaches, including detailed external examinations, precise sampling for microscopic analysis, and specialized dissection techniques tailored to highlight specific findings.

This workshop will explore fundamental and advanced aspects of brain pathology in forensic autopsies, including but not limited to CNS anatomy, head trauma, neurovascular diseases, infections, specialized autopsy dissection techniques, and microscopic examination of rare or uncommon conditions. Attendees will participate in didactic sessions, case studies, step-by-step dissection guides, and evidence-based discussions led by forensic and neuropathologists, facilitating interactive learning and skill enhancement.

Forensic Pathology; Neuropathology; Central Nervous System Trauma

W18 A New Frontier — Federal Agencies Paving a Way for Forensic Drug Analysis With AI

Agnes D. Winokur, PhD, Drug Enforcement Administration, Washington, DC; Kimberly Westberry, PhD*, USACIL, Forest Park, GA; Sandra Ferreira, MSc*, Canada Border Service Agency, Ottowa, Ontario, Canada; Scott R. Oulton, BS*, Drug Enforcement Administration, Arlington, VA; Henry Swofford, PhD*, National Institute of Standards and Technology, Gaithersburg, MD; Melissa Taytlor, BA*, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Overview: After attending this workshop, attendees will: (1) be able to better understand how federal agencies in the United States and internationally are using machine learning tools and other technological advances in seized drug analysis; (2) be able to evaluate the challenges with robotics and algorithms to improve laboratory analytical capabilities and seized drug workflow strategies; and (3) be able to explain how federal agencies are addressing the new frontier of machine-learning and artificial intelligence in support of forensic science investigations.

Impact Statement: This workshop will benefit the forensic science community by highlighting how federal agencies are considering the benefits, risks, and limitations that machine learning capabilities and technological advances have brought to forensic science seized drug analysis, such as collaborations on investigations, epidemiology, drug intelligence, and canine training, and operations. It will also highlight how embracing these opportunities can strengthen the efficiency of laboratory operations, utility of the results, and timeliness of testing.

Program Description: Federal agencies around the world are embracing the new frontier of machine learning and advanced technology and considering how to AI within the current systems. This workshop will provide insight into various examples of how federal agencies are applying machine learning capabilities and other technological advances to forensic science and drug analysis. The National Institute of Standards and Technology (NIST) researchers will be discussing their efforts in creating a catalog of AI-enabled tools currently being used by forensic science service providers and researchers for forensic science applications, to include available or near-market tools. Other examples include Drug Enforcement Administration's (DEA's) implementation of state-of-the-art automation for sample preparation related to screening and quantitative analysis. Using advanced robotics, samples are prepared for general screening of controlled substances and quantitative analysis. The Canada Border Services Agency will present on the transformative capabilities of existing software in conjunction with the portable mass spectrometers for narcotics detection at the Canadian Border. Participants will gain insights into the sophisticated software algorithm, which significantly enhances confidence in direct inject Mass Spectrometry (MS) spectral matching, providing rapid and precise results.

In addition, attendees will participate in an interactive exercise in which they will identify areas in seized drug processes where AI-enabled tools have the potential to be transformative (e.g., evidence analysis, quality control, reducing the likelihood and consequence of error and cognitive bias, and research activities. The workshop will finish with information on the progress toward establishing a road map for the responsible development, testing, evaluation, and adoption of trustworthy machine-learning and AI systems in forensic science.

Machine Learning; Algorithms; Forensic Analysis

W19 Unmasking the Evidence: How Defense Experts Prevented Wrongful Convictions

Anjali Ranadive, JD, SciLawForensics, Ltd, Phoenix, AZ; Hon. Roderick T. Kennedy, JD*, Retired Appellate Judge/Attorney, Los Ranchos, NM*

Learning Overview: By attending this workshop, attendees will: (1) understand the adversarial nature of the legal system and its implications for forensic evidence; (2) recognize the challenges faced by attorneys and judges in evaluating scientific evidence without a background in science; (3) explore the impact of prestigious institutions like the Federal Bureau of Investigation (FBI) on perceptions of evidence credibility; (4) analyze the role of language in forensic reports and testimony, including potential inaccuracies and biases; (5) enhance skills in scrutinizing evidence collection, chain-of-custody procedures, and testing methodologies; (6) develop awareness of confirmation bias and the dangers of tunnel vision in legal proceedings; (7) recognize the importance of competent expert testimony in challenging misleading evidence presentations; (8) examine real-life cases to illustrate systemic issues in handling evidence and presenting cases in court; (9) enhance critical thinking skills in evaluating laboratory procedures, interpretation methods, and statistical analyses; and (10) promote ethical practices in legal and forensic professions to ensure the pursuit of justice.

Impact Statement: The training program aims to empower legal professionals working in the criminal justice field with the knowledge and skills necessary to navigate the complexities of forensic evidence. By shedding light on systemic issues, promoting critical thinking, and advocating for ethical practices in evidence presentation, this program seeks to enhance the pursuit of justice in the legal system. Participants will be equipped to challenge misleading evidence, recognize biases in language, and ultimately contribute to a fairer and more transparent judicial process. Through real-case examples and expert guidance, this program strives to make a lasting impact on how forensic evidence is handled, ensuring that the quest for truth and justice remains paramount in legal proceedings.

Program Description: The program will highlight the challenges faced by legal professionals who may lack the scientific background needed to assess forensic evidence accurately. It emphasizes the critical role that defense experts play in preventing wrongful convictions by scrutinizing evidence, reports, testimony, and the actions of forensic analysts.

Anjali Ranadive and Tiffany Roy, both attorneys and forensic scientists with significant experience, aim to shed light on systemic issues within the legal system through real case examples. They will provide insights into various problems such as evidence mishandling, credibility of testing procedures, misleading language in reports, and biases in case theories. By bringing attention to these issues, they will emphasize the importance of having competent experts for the defense to challenge flawed evidence, interpretations, and statistical analyses that could sway court decisions unjustly.

The workshop underscores the notion that without the input of knowledge of experts on the defense side, there is a risk of overlooking crucial opportunities to question the validity of evidence presented by the prosecution. This can ultimately impact the fairness of legal outcomes and the dispensation of justice. By advocating for a more balanced and informed approach to evaluate forensic evidence in court, the speakers aim to promote a greater sense of integrity and equity within the criminal justice system.

Expert Testimony; Criminalistics; Language

W20 Signed by Hand or by Machine?

Linton Mohammed, PhD, Forensic Science Consultants, Inc., Poway, CA; Zain Bhaloo, MSc*, Canada Border Services Agency, Ottowa, Ontario, Canada; Jonathan Heckeroth, MSc*, Zurich Forensic Science Institute, Zurich, Switzerland*

Learning Overview: This workshop will introduce attendees to new methods of creating machine-generated signatures. Features of these types of signatures in comparison with handwritten signatures will be discussed and demonstrated.

Impact Statement: This workshop will provide Forensic Document Examiners (FDEs) with information on how to deal with the examinations of signatures that are possibly machine-generated. The examiners' knowledge should enhance the quality and reliability of opinions given as to signature authenticity.

Program Description: This workshop will introduce FDEs to new types of machine-generated signatures. There will be a significant hands-on session where attendees will examine and compare original handwritten signatures and original machine-generated signatures. There will be information given as to how the signatures were produced. Discriminating features of the signature types will be discussed by the attendees.

Forensic Analysis; Signature Identification; Machine Learning

W21 All Rise 2.0: Navigating the Judicial Process as an Expert Witness

Laura C Fulginiti, PhD, MCOME, Phoenix, AZ; Daniel W. Martin, JD*, State of Arizona, Phoenix, AZ; Katelyn L. Bolhofner, PhD*, Arizona State University, Phoenix, AZ; Betty Layne DesPortes, JD*, Benjamin & DesPortes PC, Richmond, VA; Kristen Hartnett-McCann, PhD*, CT Office of the Chief Medical Examiner, Farmington, CT; Gary C. McDonald, Jr., JD*, Dallas County Criminal District Attorney's Office. Dallas, TX; Andrew C. Seidel, PhD*, King County Medical Examiner's Office, Seattle, WA*

Learning Overview: After attending this workshop, attendees will be well-versed in the decorum, procedures, expectations, and environment of a modern United States courtroom through active participation in courtroom proceedings.

Impact Statement: This presentation will impact the forensic science community by offering real-world experience in expert testimony in an environment conducive to productive learning without the stress of an actual trial.

Program Description: Forensic scientists are trained in the techniques, theory, history, and standards associated with their particular discipline. Often lacking, however, is training in the application of those skills to the courtroom setting and, in particular, the presentation of expert testimony. A forensic expert's ability to effectively communicate in a courtroom setting is vital if that expert is going to make meaningful contributions to the justice system. This workshop seeks to improve courtroom communication skills by providing a combination of lectures and hands-on learning that will introduce the basic foundations of the United States court system, the roles of the participants, the types of pre-trial and trial processes that occur, and the opportunity for attendees to practice testifying in a true-to-life courtroom environment with case-based scenarios.

Expert Testimony; Court; Mock Trial

W22 Chasing the Dragon: Navigating Death Investigations in the Era of Novel Psychoactive Substances

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Learning Overview: Upon completion of this workshop, the participant should be able to: (1) interpret key differences between the Novel Psychoactive Substance (NPS) classes and how they impact cause and manner of death; (2) discuss the difficulties encountered in investigating NPS in the context of forensic toxicology, seized drug analysis, death scene investigation, toxicological interpretation, and accurate certification of cause and manner of death and; (3) appreciate the importance of disseminating timely information regarding emerging NPS trends and the impact on public health and harm reduction.

Impact Statement: These presentations will impact the forensic science community by emphasizing the importance of collaboration and dissemination of relevant and accurate information across forensic science disciplines regarding the identification and interpretation of NPS. Additionally, these presentations will increase understanding of the different classes of NPS, how they are marketed and often mislabeled, types of products to look for on the scene, and the role these drugs play in death investigation and certification.

Program Description: This half-day workshop is a collaboration between the AAFS ad-hoc Opioid and Emerging Drugs Committee and the Society of Forensic Toxicologists (SOFT) NPS Committee. From the scene of a suspected overdose to the final determination of cause and manner of death, this workshop will demonstrate how information from a range of forensic scientists can be applied to a death investigation and subsequently further the understanding of NPS for both forensic practitioners and the public.

There are hundreds of NPS on the market today with a wide range of psychoactive effects; however, most of these substances have unknown pharmacological properties (e.g., potency, metabolism, toxicity), complicating the interpretation of their impact on public health and safety. In addition, keeping up with the drug market becomes a challenge, and forensic practitioners and researchers are left chasing information on NPS that quickly becomes outdated due to the volatility of the drug supply. Whether it is crime scene investigation, pathological findings, pharmacological and toxicological interpretations, or chemical analysis, when trying to understand NPS, the work of one forensic discipline is regularly influenced by that of another. By working together, forensic scientists can increase their chances of providing timely information that can aid in drug-related death investigation, updating drug trends, and maintaining public policy and drug prevention outreach in the community.

Novel Psychoactive Substances; Postmortem; Death Investigation

W23 A Skeletal Atlas of Elder Abuse: Beta Testing a Digital Diagnostic and Educational Tool

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Learning Overview: After attending this workshop, attendees should be able to: (1) describe methods used to characterize/assess skeletal fracture patterns in elderly individuals and the ways in which aging affects fracture and recovery; (2) understand there are different patterns of trauma associated with abuse/neglect and with accidental falls, (3) understand how to navigate the presented digital atlas of elder abuse, both for educational and diagnostic purposes; and (4) participate in beta testing the atlas and contribute usability data toward its final construction.

Impact Statement: Ten percent of adults aged 65+ will experience some form of abuse annually. Physical abuse and caregiver neglect account for most cases, yet they remain difficult to prove. 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 (NIJ), our research aims to produce a standard for the diagnosis of elder abuse in the skeletal system. This presentation will impact the forensic science community by offering the first iteration of a digital tool for diagnosis and education and for collaboration and learning so that we may improve our understanding of fractures and their contexts in elderly individuals.

Program Description: Increasing rates of elder abuse in the United States over the past few decades have produced an urgent need for improved diagnostic criteria to better design intervention procedures, to implement recognition criteria, and to achieve justice for victims. Physical abuse of elders represents the most severe manifestation of this trend. Yet physical abuse is difficult to prove and may be masked by attribution of injury to medication or accident. In this workshop, attendees will learn how aging impacts skeletal trauma incidence and patterning. The presenters will provide an overview of differences in the skeletal patterns of injury between accidental falls and cases of abuse/neglect uncovered in our NIJ-funded research, as well as the impact of bone density loss on these patterns. Attendees will be introduced to the first complete iteration of the Skeletal Atlas of Elder Abuse — A Digital Tool for Diagnosis and Education. The presenters will discuss the development of the online platform, its aims and future direction, and will request attendee feedback through the beta testing of the site. Participants will have the opportunity to hear from the developers of the machine learning and AI models driving the diagnostics of the site as well as from the software development team responsible for the creation of the atlas and its many interactive features. This workshop probes the opportunities (and challenges) associated with the development of such a tool, asking attendees for participation, suggestions, direct interaction, and user experience measures.

Skeletal Fracture; Elder Abuse; Digital Atlas

W24 ANSI/ASB Standard 175 — Standard for Interpreting, Comparing, and Reporting DNA Test Results Associated With Failed Controls and Contamination Events: Understanding, Implementation, and Legal Issues

Charlotte Word, PhD, North Chesterfield, VA; Virginia Barron, JD*, Birkholz Law, Westbrook, MN; Tabitha Bandy, MS*, BODE Technology, Old Hickory, KY*

Learning Overview: Attendees at this workshop will learn about each of the requirements in the newly published Standard in relationship to requirements in other standards (e.g., Federal Bureau of Investigation Quality Assurance Standards (FBI QAS), International Organization for Standardization (ISO) 17025, American National Standards Institute/Academy Standards Board (ANSI/ASB) published, Organization of Scientific Area Committees (OSAC) Registry) while gaining insights for their implementation. Approaches to consider for evaluating, decision-making, and reporting data without re-testing will be presented along with hands-on problems and through interactive discussions. In addition, the attendees will learn about the legal necessity for implementing this standard when the DNA results are exculpatory to avoid contributing to a Brady violation.

Impact Statement: This presentation will impact the forensic science community by providing laboratory personnel, attorneys, and judges critical information regarding the responsibility of evaluating DNA data when a control has failed or a contamination event has been detected, especially when the DNA results may be exculpatory for an individual. The specific requirements in this Standard for: (1) the laboratory protocols for evaluating, interpreting and comparing data associated with failed controls and contamination events; (2) assessment of the risks of re-testing vs. not re-testing; (3) documentation; and (4) the reporting of the event, and the associated data and conclusions will be discussed along with considerations for successful implementation in the laboratory. Additional discussions will include the effective communication of the data, conclusions, and events in the criminal justice system through conversations, trainings, and testimony.

Program Description: Incidents of contamination of controls and evidence in laboratories conducting Polymerase Chain Reaction (PCR) -based Short Tandem Repeat (STR) testing have been increasingly detected as the sensitivity of the assays has improved over the past decade through improved technological advances and with the implementation of elimination databases to detect the DNA profiles of laboratory staff and other individuals involved in the collection and handling of evidence, along with the use of advanced software for evaluating data. Positive control failure may occur when the DNA is not added correctly or may be due to a technology failure.

Historically, when these events occurred, re-testing was conducted on a portion of the remaining evidence, and the new test results were reported. However, when the evidence and DNA extract have been consumed, re-testing is not an option. It is also not always prudent to consume more evidence with re-testing. Situations where this may be relevant and how this pertains to the requirements in the Standard for Interpreting, Comparing, and Reporting DNA Test Results Associated with Failed Controls and Contamination Events will be discussed. This Standard has recently been published as an ANSI/ASB Standard but has been listed as a Proposed Standard on the OSAC Registry (OSAC 2020-S-00040) and has been available for implementation in DNA testing laboratories for several years.

This workshop may be beneficial to DNA analysts/technical leaders/supervisors, QA/QC managers, trainers, and laboratory directors overseeing any type of testing where controls may fail or contamination can occur and to attorneys and judges encountering DNA tests results from these situations.

Forensic DNA; Contamination; Quality Control

W25 Death of an Expert Witness: Discrediting Document Examiners Who Violate Acknowledged Standards or Binding Laboratory Policies or Who Express Handwriting Opinions With Low Levels of Certitude

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Learning Overview: After attending this workshop, the attendees will have a better understanding of how lawyers can discredit an expert witness who failed to adhere to an acknowledged industry standard or binding laboratory policy. Attendees will also learn why handwriting opinions expressed with low levels of certitude such as "indications" or "probable" can easily be impeached and discredited and why it would be better if such opinions were excluded by gatekeepers.

Impact Statement: This presentation will impact the forensic science community by providing document examiners and forensic scientists in various forensic pattern-matching disciplines with a clear understanding of the basic unreliability of expert opinions expressed with a low level of certitude.

Program Description: A distinguished faculty comprising two highly credentialed forensic document examiners, one of the nation's leading evidence scholars, and a recently retired appellate court judge will provide attendees with a clear understanding of the problems facing document examiners who violate acknowledged industry standards or binding laboratory policies and the reliability concerns associated with handwriting opinions expressed with a low level of certitude. Attendees will be provided with actual case histories illustrating how handwriting opinions expressed as "indications" or "probable" are challenged and discredited. Attendees will also learn about the reliability issues gatekeepers must address when deciding whether or not to admit handwriting opinions expressed with a low level of certitude.

Discrediting Handwriting Opinions; Reliability; Standards

W26 Expanding the Forensic Professional's Toolbox: New Technological Resources for the Evaluation of Evidence

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Learning Overview: Individuals who participate in the workshop will learn about new computational tools developed by the Center for Statistics and Applications in Forensic Evidence (CSAFE) researchers in collaboration with the National Institute of Standards and Technology (NIST) scientists. The tools are designed to answer forensic questions of interest in several pattern comparison disciplines and enable practitioners to provide a probabilistic assessment of the evidence. At least in the near future, the new resources are meant to be used alongside traditional examination methods and serve to either confirm categorical conclusions or call attention to potential issues with those conclusions.

Impact Statement: Imaging, computational, and other tools are already used by forensic practitioners to analyze some types of evidence (e.g., DNA), but their introduction has been slower in the pattern comparison disciplines. Once they are more extensively tested and validated, the tools developed by CSAFE will empower practitioners to carry out quantitative analysis and interpretation of evidence and reinforce reporting and testimony with data-based results. The tools we will introduce at the workshop are free and placed in the public domain to maximize access and transparency.

Program Description: Forensic practice changes at a pace that varies by discipline. Sophisticated instrumentation and computational tools have been used by forensic DNA examiners for decades, but introduction of new methods has been slower in pattern comparison disciplines, probably because comparing images is complex. Yet, examiners in these disciplines are facing frequent challenges when relying on expert opinion alone. Research groups, including CSAFE, have, in the past decade, tried to address the lack of data-based methods for the forensic analysis of patterns. These groups have demonstrated that it is possible to quantify similarity between two images, even when one is degraded. While several new methods have been proposed in the scholarly literature, the gap between research and practice has not been breached, except by a few private companies that develop proprietary tools. To encourage adoption of new methodology, CSAFE researchers have created tools that enable forensic practitioners to implement quantitative methods for the evaluation of firearm, footwear, and handwriting evidence. During the workshop, participants will learn about and use three applications that are (or soon will be) ready for testing on real case work. These applications are all in the public domain and can be used at no cost. Participants will be asked to bring their own laptop and will get access to software and example data before the meeting. In addition to hands-on learning, the workshop will include lecture-style presentations to ensure that users of the applications have a reasonable understanding of the science and data that underpin the apps.

Artificial Intelligence; Error Rate; Statistics

W27 Mind Games and Machines: Navigating Cognitive Biases in Forensic Technologies

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Learning Overview: After attending this workshop, participants will be able to: describe Cognitive Biases: Participants will be able to recognize various cognitive biases, such as confirmation bias and anchoring bias, and understand how these biases influence decision-making processes within forensic science and related fields; analyze the Impact of Technology on Biases: Participants will gain insights into how forensic technologies like Artificial Intelligence (AI) and machine learning interact with and potentially exacerbate cognitive biases. They will learn to evaluate the strengths and limitations of these technologies in mitigating or amplifying biases in forensic investigations, and explain Strategies to Mitigate Bias: Participants will acquire practical strategies and best practices for addressing and reducing cognitive biases in forensic settings. This includes applying lessons learned from case studies and expert presentations to improve decision-making accuracy and fairness in forensic technology applications.

Impact Statement: This workshop will enable participants to effectively navigate cognitive biases within forensic technologies, offering strategies to enhance the accuracy and fairness of technological applications. By understanding the interplay between human biases and tech innovations, attendees will be better equipped to leverage technology while mitigating its potential pitfalls.

Program Description: Explore the critical intersection of cognitive bias and forensic technology in this engaging and interactive presentation. We'll start by illustrating cognitive biases through everyday life examples, helping you recognize these common mental shortcuts and their impact on our daily decisions.

Cognitive biases, while often beneficial for quick decision-making, can introduce significant errors, especially in high-stakes environments like forensic science. We'll review a range of biases — such as the halo effect, confirmation bias, and anchoring bias — and examine their effects on various stakeholders, including forensic professionals, law enforcement, jurors, and the public.

Next, we'll dive into the world of forensic technology, specifically exploring the fundamentals of AI and machine learning. We'll clarify what these technologies are, how they have evolved in recent decades, address misconceptions about their capabilities, and discuss challenges associated with their misuse.

Interactive segments will include case studies from past high-profile forensic cases, where we'll identify and analyze biases at play. This hands-on approach will help you see how biases can influence forensic outcomes and public perception.

Experts will present strategies to counteract cognitive biases related to forensic technology, offering practical insights into improving accuracy and fairness. We'll conclude with a Q&A session using live polls to engage with audience thoughts on the implications of AI in forensic science and beyond.

Attendees will gain a deeper understanding of how cognitive biases interact with forensic technology and discover actionable strategies to enhance justice and accuracy in forensic practices.

Forensic Science; Bias; Technology

A1 Epi and Histo: Partners in Improving Age-At-Death Estimation

Victoria M Dominguez, PhD, Lehman College, City University of New York, Bronx, NY; Sara C. Zapico, PhD*, New Jersey Institute of Technology/Smithsonian Institution, Newark, NJ*

Learning Objectives: After attending this presentation, attendees will understand the importance of utilizing interdisciplinary techniques to improve aspects of the biological profile.

Impact Statement: This presentation will impact the forensic science community by demonstrating for the first time that the combination of epigenetic and histological methods has the potential to increase the accuracy of age-at-death estimation in skeletal remains and merits further research.

Abstract Text: In the identification of unknown remains, reliable age estimates are essential for the creation of the biological profile that could match with a missing person. However, current methods of age estimation using gross morphology for adults are often less precise than estimates for juveniles. While multifactorial models that incorporate age estimates from various locations in the skeleton exist and demonstrate improved estimates, at present these rely solely on macroscopic assessments. Other techniques for the estimation of age, such as histological and epigenetic approaches, have individually demonstrated promise for accurately and more precisely estimating age, particularly in middle-age and elderly adults. These techniques have been around for a while; however, there are currently no studies focused on combining these techniques, as with macroscopic approaches. Based on this premise, the goal of this study was to use a combination of histological and epigenetic techniques in a sample of known age as a first step for developing a new method to improve age-at-death estimation.

The sample consists of 12 individuals of known age from whom both a femoral bone sample and a canine tooth were available. Samples were drawn from a donated cadaveric collection that allows destructive sampling in the United States. Samples ranged from 16 to 88 years of age, with an average age of 58 years (±26 years), with four females and eight males, though biological sex was not considered in these analyses. For histological analysis, a single midshaft section of the femur was prepared and data were collected following Crowder and Dominguez.[1] For the epigenetics DNA methylation analysis, DNA from dentin and pulp from a single canine were extracted following previous protocol from Zapico and Ubelaker.[2] DNA methylation of previously assessed genes (ELOVL2, FHL2, KLF14, SCGN, NPTX2) was analyzed by pyrosequencing on bisulfite converted DNA according to Zapico et al.[3] To assess the relationship of the variables with age, linear regressions were applied.

For the histological data, Osteon Population Density (OPD), a measure that combines the count of intact and fragmentary osteons over a given area, demonstrated the strongest relationship with age ($R^2$0.793), while fragmentary OPD had the second strongest relationship (R^2 =0.719). For the epigenetic data, FHL2, and particularly CpG4, show the strongest correlation with age in pulp (R^2=0.818) and a lower correlation in dentin (R^2 =0.686). These results align with previously published data supporting the utility of both these methods independently for estimating age. Combining these data, adding OPD to FHL2 at CpG4 in pulp increased the strength of the correlation (R^2 =0.940). The correlation with age increases further when combining OPD with FHL2 at CpG4 and CpG1 to R^2 =0.993. In dentin, the combination of OPD and FHL2 at CpG1 displays the highest correlation (R^2 =0.932), increasing when adding SCGN CpG9 (R^2 =0.968).

Thus, it is clear that the FHL2, a transcriptional co-factor involved in bone formation, plays a key role in the aging process and when combined with the histological variable of OPD, which quantifies bone remodeling, has the potential to improve age estimates. This is a preliminary study to establish the utility of these techniques to contribute and improve on existing aging methods, particularly in cases of limited or fragmented remains. Future research will expand these results, increasing the number of samples, skeletal elements, and genes analyzed and developing a new method for estimating age based on both techniques.

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Age-at-Death; Bone Remodeling; DNA Methylation

A2 Age Estimation Through Osteon Histomorphometry: An Analysis of Autopsy Samples

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Learning Objectives: Age estimation has a crucial role in forensic science. It is often necessary to estimate the age of skeletal remains. After this presentation, participants will get a broader overview of a proposed model for age estimation based on histological femur specimens and gather information on the conduct of research.

Impact Statement: Forensic age estimation is usually based on morphometric analysis of skeletal remains. The forensic pathologist and anthropologist also possess histological skills, being able to combine forensic expertise with the study of histological preparations of analyzed bone tissue. This presentation will have an impact on the forensic community by showing the importance and value of histomorphometry in the forensic and archaeological context, as well as an insight into variables that are significantly related to age.

Abstract Text: Age estimation in the forensic field is of fundamental importance. Different are the fields of application, where the traditional biological profile is based on gross bone morphology and morphometric analysis.[1] However, that is not always possible; therefore, the forensic expert must resort to a more multidisciplinary approach. Histomorphometry has proven over the years to be efficient and reliable mainly in older individuals and is quite useful in cases of highly fragmented or incomplete skeletal remains.[2,3] The main difficulty is to consider all the variables available to the forensic pathologist to achieve the expected result. The objectives of this study are thus to evaluate the possible correlation of some of the microstructures present in cross sections of autopsy samples with age and sex and to furthermore evaluate whether these variables can be used to derive a reliable model to estimate the age of the individual.

Osteons are functional and trophic units, almost autonomous, and they are made up of lamellar tissue arranged around the Havers' canal.[4] Bone tissue undergoes continuous processes of reabsorption and deposition of bone tissue, regulated by hormonal stimuli as metabolically active.[5] This remodeling, therefore, influences variables such as the number of osteons, their density, and their area.

The sample used for the analysis was composed of 95 cross sections of autopsy samples from the previous cases. The age at death of the individuals ranged between 18 and 92 years, of which 54 were females and 41 were males.

Following a thorough literature review, the number and densities of primary osteons, intact and fragmentary secondary osteons, and drifting osteons, were selected as variables to investigate their relation in expressing the age-related variations during the growth and aging process. Further variables included the mean osteoanal area and circularity. The primary objective of the research was to establish the correlation and significance of the variables considered in relation to the age maturation of the bone tissue. This was achieved by taking pictures from cross sections of the femoral diaphysis and analyzing them using the digital image processing tool ImageJ. The secondary goal was to establish a model to estimate age at death, computing the variables previously analyzed.

The statistical analysis showed that while there are no significant differences for each variable between males and females, the Number of Secondary Osteons and the Intact Population Density increased with age while the Mean Osteoanal Area decreased with age, confirming what has been previously observed in the literature.[6,7] Although these results align with previous research, the development of the model was limited due to the nature of the sample. Future research will be conducted using the same protocol analysis and statistical model proposed in this presentation that will include a broader sample size and a more uniform distribution of juveniles and older individuals.

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Age-at-Death Estimation; Compact Bone Histomorphometry; Forensic Age Estimation

A4 Age Estimation of Young Adults in the Italian Population: An Application of Schmeling's Method Using Conventional Radiography

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Learning Objectives: Age estimation for forensic purposes is a crucial topic of international interest, especially in relation to the increasing number of migrants and asylum requests from citizens of war-torn countries. Numerous techniques have been proposed in recent decades, categorized into skeletal and dental methods. Therefore, our study aimed to assess the method of evaluating the degree of ossification of the clavicle for age estimation.

Impact Statement: Our study, which is the result of a joint effort between the Department of Forensic Medicine and the Department of Radiology of the University of Bari, provides a basis for developing a mathematical model capable of predicting the age of undocumented minors with the highest possible accuracy. This is essential for safeguarding the rights of minor asylum seekers and for various applications requiring chronological age estimation.

Abstract Text: A total of 366 chest radiographs taken in 2023 from Italian-born patients aged 10 to 30 years were collected at the AOUC Policlinico and the Paediatric Hospital "Giovanni XXIII" in Bari, Italy. Among these, 26 were excluded due to the presence of fractures at the medial third of the clavicle. The study population thus included 340 radiographs (M: 234, F: 106). The experiment was conducted in a blind way: patient demographic data were obscured, no clinical information was provided to the observers, and each image was assigned an identifying alphanumeric code. The images were analyzed separately by two observers. The degree of ossification of the Medial Clavicular Epiphysis (MCE) was assessed by each observer according to Schmeling's classification (stage 1-5), using the atlas by Yoon et al. (2016).[1,2] Interobserver Agreement (IOA) was then evaluated. A final score (1-5) was assigned subsequently. The results were compared with the chronological age of the patients: descriptive statistics of the results (mean, median, SD, minimum-maximum age) were performed. Receiver Operator Curve (ROC) analysis was used to assess the diagnostic accuracy of the method in determining whether the patient was over or under 18 years of age. The agreement between Observer A and Observer B, evaluated using Cohen's kappa, was found to be satisfactory (k = 0.652, 69%). The mean age, divided by sex, observed in each stage was as follows: Stage I (M: 14.26, SD: 3.10, Total: 42 – F: 12.82, SD: 4.26, Total: 11); Stage II (M: 17.72, SD: 3.59, Total: 50 – F: 17.09, SD: 4.57, Total: 22); Stage III (M: 20.76, SD: 3.65, Total: 55 – F: 21.18, SD: 3.55, Total: 34); Stage IV (M: 25.20, SD: 3.97, Total: 44 – F: 29.95, SD: 3.05, Total: 19); Stage V (M: 26.98, SD: 3.15, Total: 43 – F: 27.15, SD: 2.78, Total: 20). The ROC demonstrated a high diagnostic accuracy of the test (cutoff: 3, PPV: 89.77%, sensitivity: 82.13%, specificity: 79.05%). Furthermore, a linear regression analysis has been performed, which showed a positive, strong correlation between age and stages. The mean age, stratified by sex, observed in each stage was comparable to the results reported in previous studies.[3] The mean age for each category obtained were comparable to those of previous studies.

Although several skeletal and dental methods for estimating age have been developed, their main limitation is that they rely on specific reference populations. Researchers have yet to reach a consensus on whether these methods are suitable for all groups, particularly migrants, who may exhibit delayed bone development due to malnutrition or other medical conditions, such as growth disorders. Our study aimed to assess the reliability of the Schmeling method using a sample of Italian youths aged between 10 and 30 years as a reference. Consequently, our results are applicable to a Caucasoid population in generally good physical health. Hence, our study represents an additional step in the overall validation of the MCE method for age estimation. Future studies should examine different reference populations and incorporate various factors (e.g., malnutrition, developmental disorders) to assess the global reliability of the method. Our findings on inter-observer agreement (k = 0.652, 69%) underscore the importance of this method being performed by experienced and well-trained professionals in the field of forensic age estimation. Finally, to reduce the degree of overlap in stages, it would always be useful to combine this method with radiographic examinations of other districts (e.g., hand-wrist, dental), while awaiting the development and validation of future deep learning-based AI methods.

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Age Estimation; Forensic Biometrics; Radiology

A5 Estimating Age-at-Death in Adults Using the Calce Method

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Learning Objectives: After attending this presentation, attendees will gain knowledge regarding the utility of using morphological changes to the acetabulum for age estimation. Specifically, they will learn about the accuracy and reliability of the Calce method, which uses the acetabulum of the os coxa to estimate adult age-at-death.[1]

Impact Statement: This presentation will impact the forensic science community by providing data that could influence a practitioner's decision to apply the Calce method in their casework.

Abstract Text: For forensic anthropologists, age estimation is an integral part of producing a biological profile to aid law enforcement in identifying human remains. The Calce method suggests using morphological characteristics of the acetabulum of the os coxa to estimate age at death. This method uses observable changes to osteophyte development, the acetabular groove, and apex growth to provide an age-at-death estimation of young adult (17–39 years), middle adult (40–64 years), or old adult (65+ years). Digital renderings along with morphological descriptions of the traits in their different stages are provided to guide the individual employing the method. The Calce method was originally found to have 81% accuracy when estimating age at death for both males and females. Additionally, intra-observer and inter-observer tests found the method to display moderate to substantial agreement.

The present study sought to evaluate if the accuracy, inter-observer, and intra-observer results changed when applying the method to a different population sample. To achieve this, the Calce method was applied to a sample of 476 individuals of known sex, age, and ancestry from the Hamann-Todd Human Osteological Collection at the Cleveland Museum of Natural History. This sample included males and females ages 17–105 of Black and White ancestry. The study was conducted blindly to prevent any potential bias. After the method was applied to the entire sample, intra-observer and inter-observer tests were conducted on a random sample of 50 and 47 individuals, respectively. When applied to this sample, the method exhibited an accuracy of 56.51% with the intra-observer test showing substantial agreement and the inter-observer test showing fair agreement.[2] The results show a deviation from the findings in the original study.[1] Possible reasons for this discrepancy could be variation across different populations. Additionally, the use of digital renderings, rather than photographs of os coxae, could have caused confusion when categorizing each trait as they are not direct representations.

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Age Estimation; Accuracy; Method Validation

A6 Exploring the Role of Early Life Stress in the Accuracy of Age-at-Death Estimation Among Adults

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Learning Objectives: After attending this presentation, attendees will better understand how early life stress impacts the accuracy of different age-at-death estimation methods commonly used by forensic anthropologists to report a final age range estimation to law enforcement agencies.

Impact Statement: This presentation will provide insights regarding the accuracy of common age estimation methods for unidentified human remains for individuals and communities who experience early life stress and structural inequities. Practitioners aware of these methodological and theoretical limitations will increase their competency in understanding the biocultural dimensions of health that impact skeletal senescence and, thus, affect the accuracy of forensic anthropological methods.

Abstract Text: Age-at-death estimation is a component of the biological profile created by forensic anthropologists to assist medicolegal practitioners with identifying decedents. Yet, our ability to accurately estimate chronological age from skeletal remains can be impaired when adverse lived experiences become embodied physiologically and impact the pace of biological aging. Previous research has shown that the long-term physical embodiment of chronic stress associated with structural inequities such as poverty can lead to premature biological aging and consequently decrease the accuracy of age estimations.[1] This process of premature aging, termed "weathering," originated from the discipline of social epidemiology and has been evidenced by widening health disparities throughout the life course of living United States Black and White adults.[2,3] The extent to which early life stress experienced during growth and development affects the accuracy of age-at-death estimation remains unclear.

This research examines the association between early life stress, as inferred from skeletal stress markers, and the accuracy of age estimation methods that rely on degenerative changes to the pubic symphysis and sternal rib ends. Skeletal stress markers were recorded and age at death was estimated for 83 adult donors from the University of Tennessee Donated Skeletal Collection. Vertebral Neural Canal (VNC) Anteroposterior (AP) and Transverse (TR) diameters (T1-L5) were measured, and the presence of porotic lesions of the skull, including porotic hyperostosis and cribra orbitalia, were scored. VNC AP and TR diameters mature in early childhood and adolescence, respectively, with small diameters suggesting disrupted development.[4] Similarly, porotic lesions may be caused by genetic or acquired chronic anemias that disrupt skeletal remodeling, reflecting early life health and nutritional status.[5] Age was estimated using the Suchey-Brooks, Işçan et al., and Hartnett methods, and accuracy was determined by whether the known age fell within the estimated age range.[6-9]

Fisher's Exact Tests failed to detect associations between the presence of porotic lesions and the accuracy of each aging method at the $p < 0.05$ level. A series of independent-samples t-tests were used to compare VNC diameter (in mm) between individuals based on method accuracy (correct vs. incorrect). Significant differences at the $p < 0.05$ level were detected for both VNC AP and TR diameters with positive findings frequently associated with the Suchey-Brooks and Hartnett methods in the former (AP) and Suchey-Brooks only in the latter (TR). In contrast, significant differences in VNC diameter were not associated with accuracy in either sternal rib method. These findings suggest that the accuracy of aging methods that rely on the pubic symphysis may be affected by experiences of early life stress. Moreover, the lack of positive findings associated with porotic hyperostosis and cribra orbitalia suggests that different skeletal stress markers may variably impact age-at-death estimates depending on the timing, frequency, or duration of early life stress experiences. Thus, it is recommended that forensic anthropologists acquire a deep understanding of the social contexts and communities in which they work so they may consider how experiences of physiological stress might impact their ability to accurately estimate age at death in their decedent population.

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Age Estimation; Stress; Biological Profile

A7 A Validation Study of Bergs' Method for Aging the Pubic Symphysis

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Learning Objectives: The attendees will gain a better understanding of aging for the pubic symphysis among older-aged adults. Attendees will also understand when Berg's seven-phase method for aging the pubic symphysis should be used on males and the method performs, compared to females, as it was initially developed to be used on females.

Impact Statement: Proving the reliability of diverse methods for both older-aged males and females has a significant impact on unidentified person cases. Currently, most methods predict age to about 60 years old. Through this study, it is demonstrated that males and females can be accurately aged well into advanced age, 70+ years.

Abstract Text: There have been numerous studies testing Berg's method compared to standard methods such as Suchey and Brooks and others; however, none have focused on the accuracy and reliability of the method itself, nor have they expanded its use to male samples.[2-4] This study tests Berg's seven-phase system. The specific research questions test the accuracy, reliability, and repeatability of Bergs' method of aging older adults. This study also tests the method's applicability for males.

The data was collected from the University of South Florida Donated Collection, housed at the Florida Institute of Forensic Anthropology and Applied Sciences, University of South Florida. Data were collected for 55 males and 48 females, with ages ranging from 41 to 101 years old. To examine the research questions, this study will age 103 individuals based on a blind study. Repeated data were collected from three independent observers to test inter-observer variation. Intra- and inter-observer errors are used in this study to determine reliability and repeatability. One master's student and one Ph.D. candidate will perform a repeatability test for the study, analyze the data using statistics, and put them into charts to compare the accuracy, repeatability, and reliability of both males and females.

There were many statistical methods used within this study. One of those methods used for this study was the chi-square analysis to compare the author's observations with those of the other graduate students who aged some of the pubic symphyses. The study also used the Pearson correlation analyses to compare variations among my observations with the other graduate students who are aging the pubic symphyses. The Bayesian theorem was used as well to help explain the results from the study. The results show that Berg method for aging the pubic symphysis is statistically reliable and consistent for both males and females. This is especially true when it comes to older aged females that are 60+ years old. The data shows that there is not a statistically significant inter-observer or intra-observer error within the method.

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Age Estimation; Bone; Anthropology

A8 Age Estimation of the Pubic Symphysis From 3D-Printed Proxies

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Learning Objectives: After attending this presentation, attendees will understand the accuracy, precision, and suitability of using 3D scanners and printers for creating reproductions of skeletal human remains for analysis. This presentation shows whether established anthropological techniques (Suchey-Brooks) still apply on 3D reproductions with comparative reliability, and whether adjustments are required for these samples.[1]

Impact Statement: This presentation will impact the forensic science community by showing the suitability of 3D reproductions for anthropological techniques, thereby breaking down barriers and improving accessibility of education resources capturing worldwide skeletal variation.

Abstract Text: 3D scanning and printing have received much attention through many areas of forensic science, including evidence reproduction, geometric analysis, crime scene reconstruction, and courtroom presentation. Across studies, 3D models are shown to be topographically accurate, without significant differences in metric measurements between the original and the reproduced object. However, less attention is paid toward whether 3D prints can be used for tactile analysis in omission of the original object.

A selection of pubic symphyses of different ages and preservation conditions were digitized using a 3D surface scanner. 3D digital models were processed for 3D printing in plastic and resin. Each of the real bone, plastic print, and resin print counterparts were randomly arranged for participants to view and assign a Suchey-Brooks phase for age estimation.[1] The printed and real bones were within 1.0mm accuracy, with surface features presented well. However, the plastic prints maintained more topographic definition, whereas the surfaces of resin prints were smoothed out, resulting in resin prints being assigned the same age phase as the real bone less frequently than with plastic prints. Furthermore, bones within early age phases were assigned the same phases more frequently than bones within later age phases, partly due to the key features being more susceptible to damage.

Overall, this research shows that 3D printed reproductions of bone are suitable for tactile and morphological assessments, but care must be required when selecting suitable preserved bones and the printing medium of choice. The success of this helps to break down barriers to access; there is prospect for establishing a printable skeletal collection to capture and educate on skeletal variation in populations from across the world in the localized classroom. Attendees will be able to view the 3D prints and submit their own phase scores at the accompanying poster session.

Reference:

1. Brooks, S., and Suchey, J.M. (1990). Skeletal age determination based on the os pubis: A comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. *Human Evolution*, 5(3), 227–238. https://doi.org/10.1007/BF02437238.

Anthropology; 3D Technology; Age Estimation

A9 Observer Confidence Versus Accuracy: Analysis of 3D-Printed Pubic Symphyses

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Learning Objectives: After attending this presentation, attendees will understand the disparity between observer confidence and assessment accuracy when analyzing 3D printed reproductions of skeletal material.

Impact Statement: This presentation will impact the forensic science community by showing the need for training and experience in analyzing 3D printed replicas in addition to experience handling real human remains.

Abstract Text: 3D scanning and printing have received much attention through many areas of forensic science, including evidence reproduction, geometric analysis, crime scene reconstruction, and courtroom presentation. However, there are concerns around whether 3D prints are suitable for morphological analysis and whether methods need tailoring for the reproduced surface to improve observer accuracy and confidence.

3D digital models of a selection of pubic symphyses of different ages and preservation conditions were processed for 3D printing in plastic and resin (39 samples total). Each of the real bone, plastic print, and resin print were randomly arranged for seven participants to view. Participants had varying levels of experience, from students up to active practitioners with years of experience. Participants assigned age phases to the real and reproduced samples using the Suchey-Brooks method and scored their confidence in each assessment on an ordinal scale of 1-5.[1] Estimation accuracy was determined by whether the assigned score matched the known age phase. Data were analyzed in R for data assumptions, Mann-Whitney U, and Chi-Square testing as appropriate.

The early age phases saw both the highest confidence scores and the most consistent phase assignments. Participants with more experience provided higher confidence scores and were correct more frequently, whereas less experienced participants showed wide variation in their assigned scores. Participants were most confident with real bones because some discreet features were visually less distinct in the reproductions. However, participants assigned age phases more quickly with prints than with real bones. Participants were more confident and accurate with plastic prints than resin prints because the resin surface was smoother and consequently less distinct, particularly at later age phases that require recognizing more discreet evidence.

Overall, this research shows that 3D printed reproductions of bone are an effective resource but require experience, consistency, and integrity as with established anthropological techniques. The success of this research helps break down barriers to access; there is prospect for establishing a printable skeletal collection to capture and educate on skeletal variation in populations from across the world in the localized classroom. The 3D prints will be available for viewing at this session, and attendees can submit their own sample phase scores to contribute toward this research.

Reference:

1. Brooks, S., and Suchey, J.M. (1990). Skeletal age determination based on the os pubis: A comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods. *Human Evolution*, 5(3), 227–238. https://doi.org/10.1007/BF02437238.

3D Technology; Anthropology; Age Estimation

A10 Sex Estimation Using Metrics of the Innominate: A Validation of the DSP2 Method

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Learning Objectives: The goal of this presentation is to inform attendees about the reliability and accuracy of the DSP2 method, which uses up to ten measurements of the innominate to estimate sex.

Impact Statement: This presentation will impact the forensic science community by presenting data that demonstrates high classification accuracy and low intra- and inter-observer error for the DSP2 method of sex estimation in a modern United States sample.

Abstract Text: Sex estimation is a critical component of the biological profile, and forensic anthropologists may use a variety of sex estimation methods depending upon the degree of completeness and state of preservation of the skeletal remains being analyzed. Most skeletal elements have been evaluated for their use in sex estimation; however, the innominate is widely accepted to be the most sexually dimorphic skeletal element. Methods for estimating sex from the innominate focus on either morphological or metric data.

The *Diagnose Sexuelle Probabiliste* (DSP) method, which uses ten measurements of the innominate, was introduced in 2005 and updated as DSP2 in 2017.[1,2] Both DSP and DSP2 are based on Fisher's linear discriminant analysis and require a posterior probability of > 0.95 in order for sex to be predicted. While DSP2 has been reported to have high classification accuracy rates in studies of South American and European populations, the method has not been widely tested in United States samples, and few United States practitioners incorporate this method into their casework.

The goal of this study was to test the reliability and validity of DSP2 using a large, modern sample from the United States (n = 174; 92M/82F). Two observers blindly collected the ten innominate metrics included in DSP2. Two-sample t-tests were used to test for significant differences between males and females for each of the innominate measurements. Observer error rates were calculated using the Technical Error of Measurement (TEM), relative Technical Error of Measurement (rTEM), and Intraclass Correlation Coefficient (ICC) with a 95% Confidence Interval (CI). Accuracy rates were calculated based on the proportion of individuals for whom sex was correctly classified by DSP2 with a posterior probability that exceeded 0.95. The inclusivity rate, or the percent predicted, was calculated based on the proportion of individuals that reached the posterior probability threshold of 0.95 and had sex predicted by DSP2 (either correctly or incorrectly).

Significant differences were found between males and females for all measurements except SA (p = 0.1954) and PUM (p = 0.0547). Intra- and inter-observer error analyses showed acceptable levels of agreement for all measurements (< 1.5% rTEM for intra- and < 2.0% rTEM for inter-observer error), except for IIMT (6.56% rTEM for interobserver error) and SPU (2.83% rTEM for interobserver error). Classification accuracies exceeded 95% for females and reached 100% for males for both observers and using various measurement combinations; however, an inclusivity sex bias occurred with approximately 15-20% more males than females reaching the 0.95 posterior probability threshold required by the DSP2 program to provide a sex classification estimate. Based on the high reliability and validity of DSP2, forensic anthropologists in the United States are encouraged to incorporate this method for sex estimation into their casework, although additional methods relying on morphological traits of the innominate will continue to be needed when the posterior probability threshold of DSP2 is not reached.

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Sex Estimation; DSP2 Method; Validity and Reliability

A11 The Effect of Mental Eminence Trait Scores on Sex Estimation Accuracy Rates Using MorphoPASSE

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Learning Objectives: The goal of this presentation is to inform attendees about the effect of mental eminence trait scores and inter-observer error on sex estimation accuracy rates using MorphoPASSE.

Impact Statement: This presentation will impact the forensic science community by improving the reliability of the MorphoPASSE sex estimation program. Practitioners will be provided with data demonstrating that the mental eminence trait does not provide significant information contributing to sex estimation accuracy.

Abstract Text: Forensic anthropologists often assist in medicolegal death investigations involving unidentified human skeletal remains. To aid decedent identification, an anthropologist will develop a "biological profile," including estimations of the individual's biological sex, age, stature, and population affinity (ancestry). There are five sexually dimorphic traits of the skull that are commonly used for sex estimation: nuchal crest, mastoid process, supraorbital margin, glabella, and Mental Eminence (ME). MorphoPASSE is a free, online program that uses a random forest statistical model to analyze these traits.[1] Each morphological trait is scored on an ordinal scale from 1 (more gracile) to 5 (more robust), based on descriptions and exemplar images. Using these trait scores, MorphoPASSE will provide an estimate of the unknown individual's sex based on the calculated posterior probability, with a threshold of 0.50. Previous research has found high inter-observer error rates for ME scoring.[2-4] As a result, MorphoPASSE now recommends against using ME; however, no research has examined the effect of inter-observer error for ME on sex estimation accuracy rates. The project aims to test the effect of simulated inter-observer error for ME and exclusion of ME on accuracy rates using MorphoPASSE.

One observer blindly scored each of the five skull traits in a sample of 323 individuals (155 F; 168 M) from five modern United States skeletal collections. To examine the effect of ME scores on accuracy rates, several datasets were tested. The initial dataset included scores for all five morphological traits of the skull as a baseline for comparison. To simulate the effect of systematic inter-observer error, a dataset was created with ME scores increasing by 1 (except for original scores of 5), and a dataset was created with ME scores decreasing by 1 (except for original scores of 1), with both datasets leaving the other four trait scores as originally recorded. Ten additional datasets were created that simulated random inter-observer error with weighted, squared Cohen's kappa (k) values ranging from 0.0 (agreement no better than chance) to 0.9 (excellent agreement). Finally, a dataset was created that excluded the ME score entirely, such that sex estimation was based solely on the other four traits.

Trait scores from each dataset were entered into MorphoPASSE, with the estimated sex for each individual recorded as male or female, based on which posterior probability exceeded the 0.50 threshold. Accuracy rates were calculated for each of the datasets as the percentage of individuals for whom sex was correctly estimated. Chi-square tests of independence were used to determine if there were significant differences in accuracy rates between the datasets. Pooled accuracy rates ranged from 86.4% (original dataset) to 84.5% (simulated $k = 0.0$). While female accuracy rates generally decreased, male accuracy rates actually increased with decreasing k values, resulting in larger sex biases. Excluding ME resulted in a slight decrease in the female accuracy rate (85.8% to 83.9%) and an increase in the male accuracy rate (86.9% to 88.7%). However, there was no significant difference in accuracy rates between the original dataset and any of the simulated datasets or the dataset that excluded ME (all $p > 0.05$).

Based on these results, ME should not be used for sex estimation in the MorphoPASSE program not simply because of the risk of high inter-observer error but also because ME scores do not contribute significant information as part of the random forest statistical model. Even with a weighted, squared k of 0.0, there was no significant difference in accuracy rate or distribution of posterior probabilities.

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Forensic Anthropology; Sex Estimation; MorphoPASSE

A12 An Assessment of MorphoPASSE Software for Undergraduate Forensic Anthropology Education: Integrating Sex Estimation Technology Into the Classroom

Elauna R. Hicks, BS, Middle Tennessee State University, Murfreesboro, TN; Shannon C. Hodge, PhD, Middle Tennessee State University, Murfreesboro, TN*

Learning Objectives: After attending this presentation, attendees will assess how the program MorphoPASSE, developed in 2018, can provide accurate results for sex estimation of skeletal remains at the undergraduate level of study in forensic anthropology.

Impact Statement: This presentation will help inform the forensic science community on how MorphoPASSE can assist with identification at all levels of education, which will further the scope of learning for sex estimation in forensic anthropology. This will be demonstrated by a case study where a student earning their undergraduate degree utilized MorphoPASSE[1].[1]

Abstract Text: Forensic anthropology, an evolving discipline within forensic science, gained significant attention following the 2009 National Academy of Sciences (NAS) Report, which highlighted deficiencies in forensic methodologies.[2] The report emphasized the need for reforms and standardization across forensic disciplines. In response, the Organization of Scientific Area Committees (OSAC) for Forensic Sciences was established in 2014 under the National Institute of Standards and Technology (NIST) to drive these necessary changes through the development of standards and funding of new research initiatives. Among the initiatives supported by OSAC is the "MorphoPASSE[1]: Morphological Pelvis and Skull Sex Estimation" program, developed by Dr. Alexandra Klales with National Institute of Standards and Technology (NIST) funding. Launched in 2018, MorphoPASSE[1] aims to standardize non-metric methods for estimating sex from osteological remains, thereby reducing potential biases. The program utilizes a set of eight morphological traits—five from the skull and three from the pelvis—scored on a scale from one to five. Scores of one and two are typically associated with female remains, while scores of four and five are associated with male remains. A score of three represents an ambiguous classification. Each trait has its own drawing, photographs, and verbal description that goes into detail on how the trait will be scored. After all the necessary information is provided, the program will generate a report that includes the probability of the individual's sex. The report also provides relevant statistical data, including percentages based on how accurate the program is when given a similar case.

This presentation evaluates the efficacy of the MorphoPASSE[1] program from an undergraduate perspective, focusing on the reliability of data generated by a non-professional student. Data for this study was collected during the summer and fall academic semesters from the Middle Tennessee State University (MTSU) osteological collection and the University of South Florida (USF) Donated Skeletal Collection. The MTSU collection is located at Middle Tennessee State University in Murfreesboro Tennessee and consists of six individuals who had limited known life histories. The USF Donated Skeletal Collection is located at the University of South Florida in Tampa and has 135 donated individuals. A total of 76 individuals were scored in the osteological labs using the MorphoPASSE[1] program by a student currently earning a bachelor's degree in anthropology. Each individual's MorphoPASSE[1] assessment was then compared to their known sex, and preliminary results indicate that 83% of the estimations matched.

The information gained from this case study was then utilized to create a curriculum integrating MorphoPASSE1 for the undergraduate's level of understanding of forensic anthropology. By integrating MorphoPASSE[1] at various educational levels, the curriculum aims to enhance students' proficiency in sex estimation and foster greater standardization within the discipline. This approach seeks to improve educational outcomes and promote consistency in forensic anthropological practices.

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Anthropology; Sex Assessment; Morphopasse

A13 Assigned-Sex-at-Birth Estimation Using a 3D-Printed Replica of the Os Coxa: A Case Study of a Transgender Woman Who Used Gender-Affirming Hormone Therapy During Life

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Learning Objectives: Attendees will learn the outcomes of a study conducted at the American Academy of Forensic Sciences (AAFS) conference in 2024, where forensic anthropologists estimated the Assigned Sex At Birth (ASAB) of an individual whom participants did not know was transgender using a 3D-printed replica of the decedent's left os coxa. This presentation will help attendees understand the reliability of commonly used methods for estimating ASAB and how the anthropologist's training, experiences, and demographics impacted their overall estimates.[1,2] Potential impacts of gender-affirming care on sexually dimorphic regions of the os coxa will also be discussed as part of the ongoing conversation surrounding sex, gender, and forensic anthropology.

Impact Statement: This presentation will encourage members of the forensic science community to better understand the nuances surrounding sex, gender, and their human skeletal embodiment as well as how practitioner experience and implicit bias may impact ASAB estimates in forensic anthropology. The forensic science community will feel called to action following this presentation as our results suggest that biases and forensic methodologies may inhibit the accurate identification of decedents and further perpetuate necro-violence toward gender-diverse populations.

Abstract Text: Non-metric skeletal traits impacted by ASAB are often used in forensic anthropology training and research due to their reported efficiency and effectiveness; non-metric methods are also preferred by forensic anthropologists in casework.[3] The os coxa is also generally favored when estimating ASAB in forensic anthropology cases because of reproductive differences between those assigned female and male at birth.[3] While these differences certainly impact skeletal presentation, the characteristics are not dimorphic. Rather, there is a spectrum of skeletal presentation influenced by biology, culture, epigenetics, and the sociopolitical medicalization of the body.[1,2]

To understand where transgender individuals fall on the spectrum of ASAB characteristics, this study explored the reliability of forensic anthropologists' ASAB assessments using a 3D-printed replica of the left os coxa from the only transgender woman in the New Mexico Decedent Image Database confirmed to use Gender-Affirming Hormone Therapy (GAHT) during life. Over 60 forensic anthropologists examined the 3D replica and analyzed four non-metric traits.[1,2] Each participant reported a final ASAB estimate, a rating of the replica's quality, their confidence, their academic background, and demographics.

We had three key hypotheses: (1) the anthropologist's demographics and background would impact their assessments and the classifications provided by each method, as well as their confidence; (2) the reported quality of the 3D replica would correlate with final estimates; and (3) there would not be strong agreement between participants due to group differences (i.e., trainees compared to American Board of Forensic Anthropology [ABFA] Diplomates). Additionally, we aimed to better understand if non-metric results correlate more closely with an individual's ASAB or lived gender when they used GAHT during life.

Surprisingly, our results showed only a few instances where participant experiences or demographics impacted trait scores or final ASAB assessments. For example, the participant's ASAB significantly ($p = 0.055$) impacted the scores attributed to the ventral arc, with those assigned female at birth interpreting this trait as more pronounced (mean = 2.84) than those assigned male at birth (mean = 3.42). Historically, a pronounced ventral arc is associated with those assigned female at birth, suggesting possible GAHT-induced alterations or that the participants subconsciously projected their own presumed skeletal characteristics onto the sample due to difficulty in assessing this trait. Participant's prior experience with MorphoPASSE also significantly impacted their assessment of the ventral arc ($p = 0.002$) and subpubic contour ($p = 0.022$), with chi-squared tests showing a significant ($\chi 2 = 8.80$, $p = 0.003$, $df = 1$) relationship between MorphoPASSE experience and the ASAB outcome reported by MorphoPASSE. An Intra-Class Correlation showed over 90% agreement between all participants, indicating consistent assessments of the 3D replica despite a few group differences (i.e., participant ASAB). Finally, participants gave an intermediate average rating of the 3D replica, but this did not correlate with their interpretation of the skeletal replica or their confidence levels.

A male bias was also observed, though not always statistically significant. Many participants provided intermediate scores for each of the four traits, yet the estimates provided by MorphoPASSE, Walker, and the participants were overwhelmingly "Male" (77.1% in MorphoPASSE, 96.7% using Walker, 60.7% of final participant estimates) or "Probable Male" (29.5% of final participant estimates).[1,2]

This study highlights the reliability of 3D-printed replicas of the os coxa among forensic anthropologists, but reveals a potential subconscious male bias that led to the transgender woman being overwhelmingly classified as "Male" or "Probable Male." This case study supports the use of a biocultural profile and continued research focusing on marginalized groups in forensic anthropology to improve identification efforts.

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3D Technology; Sex Assessment; Gender

A14 A Geometric Morphometric Examination of Possible Patterns of Sexual Trait Expression Across Five Skeletal Elements

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Learning Objectives: Attendees will learn how the degree of morphological sexual trait expression co-varies between sexually diagnostic regions of the innominate, skull, sacrum, and scapula. How these potential patterns may relate to intrinsic and extrinsic factors as well as the impact this may have on sex estimation within biological anthropology is also discussed.

Impact Statement: Sex estimation is an integral part of forensic anthropology. Understanding how sexual trait expression varies within the individual is important and is understudied within forensic anthropology. This presentation will examine the concordance of the levels of sexual trait expression throughout the individual and what these patterns may suggest in regard to possible contributing factors to sexual trait expression in human remains.

Abstract Text: Although sex estimation classification accuracy of some element-specific methods can exceed 90%, the relationship between morphological traits within the individual and how they may respond to different intrinsic and extrinsic factors remains poorly understood.[1-10] Previous research, while scant, suggests variable sexual trait expression between different skeletal regions, with a lack of concordance between regions in the level of expression.[11-16] Understanding these relationships are vital for forensic anthropologists, as subsequent analyses rely on correct classification of biological sex. Further, instances of commingling, fragmentary and partial remains, and inconsistent sex estimation also rely on accurate understanding of human variation in terms of morphological expression. Attendees of this presentation will examine the complex interrelation of sexual trait expression between five skeletal elements to better understand what, if any, pattern is present in skeletal trait expression and what factors may be contributing to these patterns.

Utilizing 3D surface scans of the cranium, mandible, innominate, sacrum, and scapula collected from 235 individuals from the University of Tennessee (UTK) Donated Skeletal Collection and the Robert J. Terry Osteological Collection, this research uses geometric morphometric techniques to evaluate how sexual trait expression is correlated across skeletal regions. The following hypotheses were evaluated: (1) human sexual trait expression is not correlated between all skeletal regions, (2) skeletal sexual traits withing a localized anatomical region are influenced by the same factors, but these factors differ by anatomical region, and (3) individuals from the same collection will demonstrate similar magnitude and pattern of sexual trait expression compared to the other skeletal collection. For each skeletal element, after landmarks were placed, data were subjected to a principal component analysis where the first five principal components (representing approximately 60% of the variance) were subjected to a matrix correlation test, strength of correlation test following definitions provided in Chan, and Pearson correlation tests to determine significance on correlations with greater than fair correlation strength (following Akoglu).[17,18]

Results indicate significant inter-skeletal element correlations in both skeletal samples, with the UTK Collection having a larger number of inter-skeletal element correlations than the Terry Collection. Different element correlations, with no clear pattern in pairings, were present in both collections, although cranium-mandible correlations were the most common and most statistically significant correlation pair observed in both. Other patterns for each collection are discussed. Interestingly, in both collections, females had more and more statistically significant correlations than males, with greater differences in correlations between sexes occurring in the UTK Collection than in Terry. Aside from integration, these results may suggest differential biocultural influences are impacting the magnitude and patterns of sexual trait expression across skeletal elements. Therefore, practitioners of forensic anthropology should consider dynamic nature of morphological traits and consider biocultural influences in studies of sex estimation.

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Sex Estimation; Interindividual Sexual Trait Expression; Human Variation

A15 A Test of the (hu)MANid Analytical Program

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Learning Objectives: After viewing this presentation, attendees will gain knowledge regarding the ability of the (hu)MANid analytical program to accurately estimate ancestry and sex from mandibular metric and morphoscopic data. Specifically, they will learn about the strengths and weakness of the method as observed in a large test of the program.

Impact Statement: This presentation will impact the forensic science community by providing a study that critically evaluates the ability of the (hu)MANid analytical program to accurately estimate ancestry and sex from data derived from mandibles. The results of this study may influence how forensic anthropologists chose to employ (hu)MANid in the future and how they interpret the results.

Abstract Text: Sex and ancestry are key components of the biological profile estimated during routine forensic anthropological casework. Recently, Berg and Kenyhercz Introduced a new freely available web-based computer program called (hu)MANid that utilizes mandibular metric and morphoscopic data to estimate both sex and ancestry.[1] Such an analytical program should be very useful, as mandibles are relatively resistant to taphonomic destruction, especially as compared to the fragile bones of the facial skeleton. However, only a small number of studies have tested the performance of (hu)MANid, and the results of these tests have been mixed. For instance, while one study of dry mandibles found the program to accurately (82.7%) identify mandibles of American Indian ancestry, another found ancestry classification for Asians, Blacks, Whites, and Hispanics to be relatively poor (33.57%) using mandibular Computed Tomography (CT) scans.[2,3]

In an effort to continue to add to the literature testing (hu)MANid, we have conducted a study using a sample of dry mandibles from White (n=96) and Black (n=115) individuals of known sex and ancestry from the Hamann-Todd Human Osteological Collection and from American Indian (n=52) individuals derived from prehistoric archaeological sites in Wisconsin housed at the University of Wisconsin-Oshkosh. For each mandible, data were collected following the instructions provided on the (hu)MANid website and processed using the linear discriminant function option and composite group reference samples. Overall, 43.4% of individuals were classified correctly in terms of both sex and ancestry, while 75.8% of individuals were classified correctly when only sex was considered, and 69.6% of individuals were classified correctly when only ancestry was considered. A more detailed consideration of the results indicates that (hu)MANid's performance varied by ancestral group. While 86.1% of Black individuals had sex estimated correctly, only 60.4% of White individuals were accurately classified for sex. In terms of ancestry, although 82.7% of Native American individuals were correctly classified, only 57.3% of White individuals and 52.2% of Black individuals were estimated to be of correct ancestry.

Secondarily, we ran a pilot study of the utility of 3D printed models of mandibles derived from CT scans (n=10) for sex and age estimation using the application. Mandibular CT scans were measured as a part of a prior study using the *(hu)MANid* variables.[3] Models derived from the CT scans were 3D printed in plastic and measured. Overall, we found high (>0.80) intraclass Correlations (ICC) between the CT scans and 3D printed models for most linear and angular variables, but low ICCs for the visually scored traits. This could be due to various factors such as sample size, printing quality, or differences in raters. More work is needed to refine a protocol for working with 3D printed mandibular models for use with the (hu)MANid program.

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Anthropology; Sex Assessment; Ancestry Estimation

A16 A Test of the (hu)MANid Program on Edentulous Mandibles

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Learning Objectives: The Human Mandible Identification [(hu)MANid] program is a free, web-based program for estimating sex and ancestry from metric and morphoscopic mandibular data from dry bone. The aim of this presentation is to investigate the applicability of the (hu)MANid program to edentulous mandibles and inter-observer error in mandibular data collection from dentulous and edentulous mandibles.

Impact Statement: This presentation will impact all anthropologists who utilize the (hu)MANid program by expanding the application of the program to mandibles that may have initially been excluded from analysis. Applied forensic casework often involves remains of edentulous individuals, and when the cranium and pelvis are not present for sex and ancestry estimation, the mandible becomes a critical element for analysis of the biological profile.

Abstract Text: The (hu)MANid enables anthropologists to estimate the sex and ancestry of unidentified skeletal remains from metric and morphoscopic variables from the mandible.[1] The program is suitable for a variety of anthropological contexts, including forensic casework and bioarchaeological or paleoanthropological analyses. As a free, web-based program, (hu)MANid is particularity beneficial for student development and training in mandibular data collection. The purpose of this research is to test the accuracy and applicability of the (hu)MANid program for edentulous mandibles, with inter-observer error estimates included. While the creators of (hu)MANid warn against data collection for edentulous mandibles, there are many circumstances in applied forensic casework where the unidentified person is edentulous. Edentulousness is often associated with elderly individuals, but people can experience tooth loss at any age for a variety of reasons, including genetic predispositions to periodontal disease, lack of access to regular dental care, and chronic drug use. Edentulous individuals experience alveolar resorption, which is the loss of bone structure following the extraction or traumatic loss of teeth. Therefore, it is expected that the alveolar resorption that is experienced by edentulous individuals will affect the outcome and accuracy of (hu)MANid because metric and morphological landmarks are remodeled through the processes of bone loss.

The test sample consists of 40 dentulous mandibles and 40 edentulous mandibles, with equal quantities of males and females, from the University of South Florida (USF) Donated Skeletal Collection. Data were collected following the protocols established by Berg and Kenyhercz for analysis in (hu)MANid.[1] Three variables, chin height (GNI), height of mandibular body at the mental foramen (HML), and dental arcade width at the third molar (XDA), were excluded from analysis of the edentulous sample because the landmarks of tooth crypts required for these measurements are not present in edentulous mandibles. Inter-observer error for the entire sample (n=80) was also investigated in this research by comparing data collected by three observers of varying experience levels. The data were analyzed using multiple quantitative methods to quantify the estimation results from (hu)MANid and the mandibular data sets.

The results show that sex combined with ancestry estimates are correctly classified in 77% of dentulous mandibles and 56% of edentulous mandibles, when seven population groups are selected for comparison in the program. The dentulous results of this research are higher than the 70% correct classification reported by Berg and Kenyhercz for six compared population groups.[1] When using the sex-only approach in (hu)MANid, estimations were correct for 90% of dentulous mandibles and 85% of edentulous mandibles. The results of inter-observer error indicate a high level of agreement between observers with almost all metric variables within 3mm of variation. The only statistically significant differences that occurred between observers were for four morphoscopic variables, and these differences may be tied to experience level. This research demonstrates (hu)MANid is a reliable program for sex and ancestry estimation and the program may be reliably used in edentulous cases.

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(hu)MANid; Biological Profile Estimation; Edentulous

A17 Evaluating a Greek-Cypriot Sample in FORDISC 3.1

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Learning Objectives: From this presentation, attendees will be able to: (1) understand the importance of the evaluation of FORDISC 3.1 (FD3) for realizing its fullest potential; (2) understand the necessity of incorporating contemporary and diverse population data sets into FORDISC to improve forensic analysis; and (3) examine the results of the study evaluating FD3's effectiveness in analyzing the contemporary Greek-Cypriot population.

Impact Statement: Our presentation will enhance the forensic anthropology community by highlighting the importance of using population-specific data for accurate sex and ancestry estimation when using specific software such as FD3. By demonstrating the limitations of current tools like FD3 and presenting evidence from the Greek-Cypriot population, we will drive improvements in forensic performance, ensuring more precise identification of human remains. Ultimately, these advancements will lead to aiding in the resolution of unidentified cases and supporting humanitarian effort.

Abstract Text: When technology is used without careful evaluation, its full potential cannot be realized. The most accurate method for sex and ancestry estimation utilizes discriminant function-based software leveraging morphometric data. Accurate classification of individuals requires population data from diverse global locations, outside of the United States. Due to this diversity, sex estimation can be biased if the data is insufficient.

FD3, a widely used software in forensic anthropology, calculates made-to-order discriminant functions using cranial and postcranial data. FD3 aids in constructing an individual's biological profile from unknown skeletal elements. Although FD3 is used worldwide, it currently lacks data outside of the United States, including samples representing a Greek Cypriot population. Representation for this population is crucial in humanitarian cases, such as the Turkish Invasion of Cyprus in 1974. This conflict left numerous individuals that are still unidentified. To remedy this, we evaluate a novel sample representing a contemporary Greek-Cypriot population using our sample and the data currently housed in FD3. Our study includes a contemporary (20th century) skeletal sample consisting of 202 Greek-Cypriot individuals (female = 101; male = 101) from the Cyprus Research Reference Collection (CRRC). An additional sample of 249 African American, 753 European American, and 222 Hispanic American individuals currently housed in FD3 were also included. Thirty cranial measurements were collected and implemented in the analysis. Utilizing the Custom Database function in FD3, we achieved an overall correct classification of 82.4%. The Greek-Cypriot sample correctly classified 90.7% of the cases. Incorrect classifications of this sample include 4 individuals as African American, 9 as European American, and 1 Hispanic American. Basion-Bregma height (BBH) and Nasal Breadth (NLB) were the most important variables in the discriminant function.

Our results will underscore the necessity of incorporating more contemporary, population-specific data sets to enhance the accuracy of analyses involving unknown skeletal remains. By addressing this gap, we aim to improve the identification process of human remains, thereby contributing to forensic anthropology and aiding in resolving historical and contemporary cases of unidentified individuals.

Anthropology; Software; Population

A18 Introducing FoRDent: A New Tool for Estimating Population Affinity From Dental Morphology

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Learning Objectives: After attending this presentation, attendees will be aware of the availability, strengths, and capabilities of a new tool for estimating population affinity in forensic anthropological casework. They can apply this tool to improve the biological profiles produced toward resolutions for cases of unidentified human remains.

Impact Statement: The impact of this presentation will include improving the diversity of data sources available for estimating population affinity by providing practitioners with a new, freely available, statistically sophisticated, user-friendly method that is built on samples appropriate for forensic cases and other analyses. This method leverages underutilized data from the most taphonomically resilient parts of the body, the teeth, to address one of the most complex components of the biological profile, population affinity.

Abstract Text: FoRDent is a new tool for estimating population affinity from the permanent dentition. It is web-based and free access. Its interface is user-friendly, simple to use, and rich with images to facilitate use by practitioners who may not be experts in dental morphology. This simple-to-use interface is coupled with a large sample (n=4,600) appropriate for forensic casework in North America, cutting edge statistics, and an approach to population affinity informed by contemporary anthropological thinking.

Dental morphology is an excellent data source for the estimation of population affinity in forensic casework. Teeth are hard, so they are often retained in cases even when taphonomic damage is significant to the skeleton. Dental morphological traits are heritable and generally neutral, so patterns of characteristics reflect biogeographic ancestry. These threshold characteristics are often scored categorically; however, traditional statistical methods for their analysis have required dichotomization of trait scores.

FoRDent draws on a near-global sample representing 111 modern nations, focused on the United States and Mexico. United States samples include recent and contemporary African, Asian, European, Hispanic, and Native Americans from 11 different states in different regions of the country. Additional contemporary samples represent Australia, Japan, and Mexico City, Tlaxcala, and Yucatán in Mexico. For contemporary samples, population affinities are as described by members of each local community from the samples were drawn.

Up to 65 dental variants can be scored and included in the analysis. All data are analyzed using the original (non-dichotomized) scores, preserving subtleties of variation and allowing for more fine-grained analyses. FoRDent employs a Gradient Boosting Machine (GBM) approach. GBM is an ensemble learning technique that builds a number of decision trees sequentially, with each tree correcting the errors made by previous models and boosting the final prediction as the sum of the predictions across all trees. GBM is highly effective in classifying this sample, robustly handling this complex, multidimensional data. The FoRDent approach showcases the power of machine learning techniques in dental research and underscores their potential for dental morphology in population affinity estimates going beyond more naïve approaches that do not weigh variable importance. Users can refine analyses through variable selection approaches emphasizing these measures. Further, users are provided with multi-level results, with probabilities associated with continental, national, and community level affinities. Classification accuracies vary depending on the variables and samples included in an individual analyses. Including all groups and all traits, which provides the lowest estimate of accuracy, still correctly classified nearly 50% of the sample. A more typical analysis comparing an unknown individual to four regional samples results in classification accuracies between 83.9 and 91.1 percent.

Anthropology; Population; Methods

A19 Digital Archives: An Investigation of Skeletal (In)completeness Using IRIS

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Learning Objectives: After this presentation, attendees will understand how digital databases and casework capture incomplete skeletal remains to inform forensic investigations.

Impact Statement: This research impacts the forensic science community by highlighting digital tagging in a cloud-based, Criminal Justice Information Services (CJIS) -certified, online case platform to capture the relationship between incomplete skeletal remains and identification of trauma in forensic casework. By tagging skeletal incompleteness within the database, the Forensic Anthropologist (FA)may help law enforcement and medical examiners identify the need for secondary or tertiary searches of a scene whether involving unidentified or unsolved cases, dismemberments, surface scatter scenes, and subaqueous sites.

Abstract Text: Consulting on a case-by-case basis, law enforcement, medical examiners, and coroners call upon forensic anthropologists to assist with the skeletal analysis of human remains irrespective of the decomposition state, skeletal completeness, or degree of fragmentation. Medicolegal cases require meticulous and detailed documentation that may be exchanged with the appropriate criminal justice agencies involved when permitted by law.1-3 Digital databases used as case and crime record repositories and as reporting entities (e.g., Uniform Crime Reporting [UCR] Program, National Missing and Unidentified Persons System [NamUS], Florida Unidentified Decedents Database [FluidDB], National Crime Information Center [NCIC], etc.) have long utilized forensic anthropological reports to supplement existing case data. Previous studies indicated that digital repositories often help solve cold (unsolved) cases.4 However, the utility of data tags in forensic anthropological casework (from initial consultation request through adjudication) has not yet been documented in the literature.

Researchers identified correlations between the percentage of elements recovered and subsequent identification and trauma analysis findings.5,6 Expanding upon previous literature that discusses the impact of skeletal incompleteness on forensic anthropological investigations, this study explores closed, adjudicated forensic anthropology trauma analysis reports (n = 486) in Florida from 2005–2022. In contrast to previous studies that relied upon physical case files, this study also incorporated a cloud-based, CJIS-certified, digital platform to assign digital tags of case attributes, including medical examiner district, quantity of elements evaluated, decomposition state, estimated biological skeletal sex, and trauma type. These data were culled using the respective tags for subsequent data analysis. When categorized by digital tag and evaluating only trauma cases, most cases consisted of incomplete (< 90%) skeletal remains (n = 375) compared to those with complete (>90%) skeletons (n = 111). Overall, an average of 86.18 elements (41.8%) were evaluated by the forensic anthropologist.

On average, the decomposition state tags allowed the FA to quickly sort cases that were skeletonized (31.9%), followed by fresh decomposition (27.4%), and advanced decomposition (21.6%). Age and sex were slightly positively correlated with the identification of trauma ($r = 0.011$, $p > 0.05$). The frequency of trauma type was the same for both sexes with no trauma present being the most common finding followed by blunt, sharp, and gunshot trauma ($r = -0.113$, $p > 0.05$). Of those without trauma (n = 74 females, n = 215 males), an average of 96.91 bones (or 47% of the skeleton) were evaluated. In contrast, when trauma was present, the FA was asked to evaluate only 33.1% (or approximately 69 skeletal elements) of the decedent (e.g., the medical examiner requested a focused analysis or limited the analysis to a particular part of the skeleton). Notably, 27.5% of females and 19.3% of males involved a focused analysis of the neck organ block (i.e., hyoid, thyroid cartilage, cricoid cartilage, tracheal rings). These findings help emphasize the need for FAs to have a secure digital repository with searching parameter capabilities to effectively aid in death investigations.

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Skeletal Incompleteness; IRIS; Case Archival

A20 Assessing the Applicability of Subadult Stature Estimation Methods for Japanese Subadults

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Learning Objectives: From this presentation, attendees will understand that this research aims to expand population-specific data and evaluate the applicability of four subadult stature estimation methods on Japanese subadults. The goal is to assess subadult stature estimation methods that may be population-specific to adapt and improve the biological profile component among Japanese subadults.

Impact Statement: This presentation will impact the forensic science community by expanding the anthropological knowledge of subadult stature estimation methods and their accuracy when applied to Japanese subadults. The evaluation will investigate the current stature estimation methods utilized in biological profile assessments to assist with identifying unknown human skeletal remains of Japanese subadults.

Abstract Text: Stature is one component of the biological profile with limited applicability for subadults due to the lack of data sources available.[1] Some methods require prior data collection of age, sex, and even assignment of population group affiliation that may not be available further complicating subadult stature estimation.[1] Furthermore, current subadult stature estimation methods are population-specific and may not accurately represent the reference samples of other populations.

This research aims to investigate and compare the reliability of various stature estimation methods for Japanese subadults. It is expected that the chosen subadult stature estimation methods applied will overestimate the stature for Japanese subadults due to a potential difference in limb proportions between popualtions.[2]

Postmortem Computed Tomography (PMCT) scans were obtained from 274 male (n = 160) and female (n = 114) individuals from ages starting at birth to 25 years. Eighteen long bone lengths and breadths were measured virtually using the 3D models generated by 3D Slicer.[3] The stature for every individual was calculated using the subadult stature estimation methods based on Murray, Chu and Stull, Smith, and Robbins Schug.[1,4-6] The known stature was then compared to the estimated stature for accuracy and precision. Linear and non-linear model performance assessed testing accuracy, Standard Error of Estimate (SEE), and the Mean Absolute Deviation (MAD).

The testing accuracy based on Chu and Stull non-linear fibula length model resulted in 97% correct within the 95% prediction interval range, a SEE of 3.76cm, and a MAD of 3.26cm.[4] Based on Murray linear models, the resulting testing accuracy ranged from 68% for the femur to 80% for the ulna of known stature falling within the 95% prediction interval range, the SEE ranged from 12.77cm to 24.37cm and the MAD ranged from 5.39cm to 6.16cm.[1] For Smith, the SEE ranged from 11.32cm to 23.18cm and the MAD ranged from 4.25cm to 7.24cm.[5] Lastly, for Robbins Schug, the SEE resulted in 12.17cm and the MAD is 5.37cm.[6]

Based on the results, the Chu and Stull subadult estimation method performed more accurately compared to the other published subadult stature estimation methods and with the lowest amount of error.[4] Though this is the most promising method currently available, the method put forth by Chu and Stull can be further adjusted to perform more accurately in estimating stature among the Japanese subadult population.[4] While it is not recommended to apply population-specific stature estimation equations to other populations, this research reveals that Chu and Stull is a reliable source to apply to the Japanese subadult population for forensic anthropological casework, until population-specific methods can be created.[4]

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Subadult Stature Estimation; Long Bones; Postmortem Computed Tomography

A21 CT Scan-Based Cranial Anthropometry for Stature Estimation: A Comparative Analysis Between Base of Skull and Front of Skull Parameters in a South Indian Population

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Learning Objectives: This presentation will provide the attendees with a better understanding of the role of the base of the skull and front of the skull parameter measurements taken from Computer Tomography (CT) scan images for estimating the stature in a defined native population. A comparative analysis will help in understanding whether the base or the front of the skull parameters give a better result when estimating the stature.

Impact Statement: This presentation will impact the forensic science community by exploring the importance of CT scan measurements of the base and front of the skull parameters for estimating the stature of a defined native population.

Background: One of the objectives of a medicolegal autopsy is establishing the identification of the individual, which may not be completely possible in the case of decomposed or mutilated bodies. Stature along with age, sex and race are the partial identification features of an individual. Researchers over the years have used different body measurements and bones for estimating the stature of individuals. The regression formula obtained from these studies is from different populations and may not apply to others because of the inherent genetic and geographic variations. This CT scan study was done to find out the importance of different skull measurements of the base and front of the skull for estimating the stature in a South Indian population.

Methods: This prospective observational study was started after obtaining the approval of the Institute Ethics Committee. All patients aged between 21 to 60 years posted for elective head and neck CT scans attending the Radiology department at our institute were included in the study. Patients who were unable to stand erect, sustained cranial fractures, or had visible cranial defects were excluded from the study. The CT scan was performed using the Somatom sensation 64-slice CT scanner (Siemens). The images were viewed using the Multiplanar Reconstruction and Volume Rendering technique (MPR-VRT). Fourteen parameters from the base of the skull and 11 parameters from the front of the skull were studied. The base of the skull parameters were Palatal Length (PL), Palatal Breadth (PB), Biauricular Breadth (BAB), Bimastoidal Diameter (BMD), maximum Length of Right Condyle (LCR), maximum Length of Left Condyle (LCL), maximum Width of Right Condyle (WCR), maximum Width of Left Condyle (WCL), maximum Bicondylar Breadth (BCB), Minimum Distance between condyles (MnD), Maximum Interdistance between condyles (MxID), Distance between two External Hypoglossal Canal opening (DEHC), maximum Length of Foramen Magnum (FML), maximum width of Foramen Magnum (FMB). Front of the skull parameters were Minimal Frontal Breadth (MnFB), Right Orbital Breadth (ROB), Left Orbital Breadth (LOB), Right Orbital Height (ROH), Left Orbital Height (LOH), Biorbital Breadth (BOB), Interorbital Breadth (IOB), Upper Facial Height (UFH), Bizygomatic Breadth (BZB), Nasal Height (NH), and Nasal Breadth (NB).

Results: Multiple linear regression analysis using backward elimination containing all the independent predictors revealed a statistically significant model, with an r2 value of 0.17 for both base of skull and front of skull parameters for the overall population. There was no violation in model assumptions such as linearity, constant variance and residual distribution. Multicollinearity was ruled out using the variance inflation factor. Among the various base of skull parameters studied, only MnD (< 0.001), DEHC (0.02), FML (0.03) in the males and BCB (0.002), BMD (0.01), FMB (0.03), MnD (0.03) in the females showed a statistically significant positive correlation with stature. The multivariate linear regression predictive model for base of skull parameters gave a statistically significant model with an r2 value of 0.10 for males and 0.15 for females. For the front of skull parameters, the observed r2 value was 0.104 with predictors BZB, NH and LOH for males and 0.06 with predictors NH and LOB for the females.

Conclusion: This study showed that there is no difference in the predictive value for male populations between base and front of skull parameters, whereas the predictive accuracy was more in the case of females for base of skull parameters in comparison to front of skull parameters.

Stature Estimation; Base of Skull Parameters; Front of Skull Parameters

A22 How Many Vertebrae Should Be Present to Estimate the Height of the Total Vertebral Column With Accuracy?

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Learning Objectives: After attending this presentation, attendees will learn that vertebral column height can be estimated with minimal errors if a certain number of vertebrae are present. Attendees will also understand the pros and cons of different methods used to reconstruct the vertebral column height.

Impact Statement: This presentation will impact the forensic science community by demonstrating that anatomical methods remain effective for stature estimation even with a partially incomplete vertebral column. This effort aims to broaden the applicability of anatomical methods, thereby enhancing the accuracy of stature estimation from skeletal remains recovered in a forensic context.

Abstract Text: Anatomical methods are known for their higher accuracy compared to mathematical methods in stature estimation. However, these methods require all 29 bones comprising height to be fully intact, so fragmented or missing elements, particularly the vertebrae, often limit their use. Two approaches commonly address this challenge: (1) estimating the heights of the individual missing vertebrae using adjacent vertebrae, which are then used to approximate the entire vertebral column height.[1,2] However, this approach may encounter limitations when multiple consecutive vertebrae are missing or fragmented; (2) regressing the height of vertebral region(s) with missing vertebrae or the entire vertebral column height against intact region(s) if any region(s) of the vertebral column (i.e., cervical, thoracic, or lumbar) remains intact. One drawback of this approach is that all vertebrae in the incomplete region(s) must be excluded from estimating the entire vertebral column height.

This study aims to test the efficiency of an alternative method where the entire vertebral column height is regressed against any remaining vertebrae. We tested whether it is more efficient to utilize all remaining vertebrae to estimate the entire vertebral column height directly rather than estimating individual vertebral heights first and then aggregating those estimates. Additionally, it aims to determine the minimum number of vertebrae out of 23 vertebrae (from C2 to L5) needed for accurate estimates with minimal errors.

This study utilized complete vertebral columns of 153 Koreans individuals (75 males, 65 females, 13 indeterminate) to explore 22 scenarios with varying number of remaining vertebrae (i.e., missing 1 to 22 vertebrae). Given the uncertainty of which vertebrae would remain in specific contexts, 10,000 random samplings were performed for each scenario. In each randomly selected sample, the height of the entire vertebral column was estimated using regression analysis based on the heights of the remaining vertebrae. The square root of the estimated variance of the random error (SEE) and adjusted R^2 were calculated from 10,000 iterations for each scenario, then averaged for further analyses.

The findings indicate that a greater number of remaining vertebrae corresponded to smaller SEEs and higher R^2 values. Specifically, with at least nine vertebrae remaining, SEEs less than 5mm and R^2 greater than 0.97 were achieved. To achieve SEEs less than 3mm or 2mm, a minimum of 14 or 18 vertebrae, respectively, were required.

Using the same random sampling approach, this study also evaluated various scenarios involving incomplete vertebral regions. For example, when missing vertebrae were only in the cervical region or only in the lumbar region, the SEE did not exceed 3mm, regardless of the number of remaining cervical or lumbar vertebrae. However, when only the thoracic region was incomplete, at least four thoracic vertebrae were needed to achieve SEEs less than 3mm. If only one of the three regions was intact, between five to ten vertebrae from the incomplete regions were required to achieve SEEs less than 3mm.

In contrast to Auerbach's suggestion that estimating the entire vertebral column height using intact region(s) may suffice when individual missing vertebral heights cannot be estimated, this study shows that using at least 14 remaining vertebrae to estimate the entire column height always yields smaller SEEs compared to estimation using only intact regions.[1]

This study demonstrates that incomplete vertebral columns do not need to be a significant barrier hindering the application of anatomical methods in stature estimation, thereby benefiting future research and practice with practical guidelines for using anatomical methods in stature estimation.

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Stature Estimation; Anatomical Method; Vertebra

A23 The Correlation of the Dimensions of Hands and Feet With Height in an Indigenous Population of the Tribal District of Himachal Pradesh, India

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Learning Objectives: Attendees will gain a clear understanding of advanced techniques used for measuring hand and foot dimensions, focusing on precision and reliability in forensic contexts. They will learn about statistical models and the relationships between these measurements and height, emphasizing how reliable these models are for estimating stature accurately in forensic cases. The presentation will cover gender-specific and regional differences within the indigenous tribal population of Himachal Pradesh (India) and their importance in forensic analysis. Practical applications will be illustrated through case studies, showing how the research findings can be used in real-world forensic investigations, especially for identifying stature from fragmented remains. Additionally, the discussion will highlight how these findings improve the accuracy and reliability of stature estimation, contributing to more precise identification processes in forensic anthropology. This will enhance the attendees' expertise in applying these techniques in their advanced forensic work.

Impact Statement: This presentation, by highlighting reliable statistical models and the relationships between these measurements and height, will enhance the community's ability to estimate stature precisely, even from fragmented remains. The insights into gender-specific and regional differences within the indigenous population of Himachal Pradesh, India, will offer valuable data that can improve the accuracy of forensic analysis in diverse populations. Furthermore, the practical applications and case studies presented will demonstrate how these findings can be applied in real-world forensic investigations, leading to more precise identification processes. Overall, this presentation will contribute to the advancement of forensic anthropology, promoting more accurate and reliable methods for stature estimation and identification in forensic science.

Introduction: Stature is considered as one of the parameters for personal identification and one of the "big fours" of forensic anthropology. The stature of an individual is an inherent characteristic, the assessment of which is considered pivotal in the identification of unknown human remains. The current study focuses on understanding the relationship between hand and foot measurements with height in the indigenous population of Kinnaur and Lahaul and Spiti districts of Himachal Pradesh, India. Stature estimation is a crucial aspect in forensic and anthropological fields, especially for identifying individuals from dismembered bodies. This study aims to fill the gap in the existing literature by focusing on a specific indigenous population, thereby enhancing the accuracy of stature estimation methods in forensic science and physical anthropology.

The primary objective of this research is to analyze the anthropometric relationships between the dimensions of feet and hands with the height of the individuals. The study also aims to estimate gender variations in these dimensions and analyze the correlation between hand and foot measurements. The study involved 383 individuals aged between 18 and 50 years from the tribal areas of Himachal Pradesh, ensuring that participants were residents for at least three generations.

Methodology: The study was carried out after obtaining the Ethical Approval from the Institute Ethics Committee. The participants were recruited through door-to-door surveys and random sampling methods. After obtaining their consent, height was measured using a stadiometer (in cm), while hand and foot dimensions of both the extremities were measured using a digital vernier caliper (in mm).

Results: The overall mean stature of the population was found to be 163.78 ± 9.68cm, with a mean right foot length of 241.09 ± 15.68mm and a mean left foot length of 241.66 ± 15.67mm. For males, the mean stature was 168.43 ± 8.84cm, with the mean right and left foot lengths being 248.93 ± 12.38mm and 249.82 ± 12.51mm, respectively. For females, the mean stature was 156.71 ± 5.91cm, with mean right and left foot lengths of 229.17 ± 12.30mm and 229.24 ± 11.22mm, respectively. Foot length emerged as the most reliable metric for estimating stature across both genders. However, the study found regional variations, with right foot length being more accurate for predicting stature in Kinnaur and Lahaul districts, while left foot length was more reliable in Spiti district. The mean right hand length was 183.15 ± 12.17mm and the left hand length was 183.11 ± 12.12mm. Of both the hands, the right hand was a more reliable predictor of the height of the individual.

Conclusions: This study offers significant insights into the estimation of an individual's stature using anthropometric measurements of the hand and foot. It demonstrates that foot length is the most reliable metric for stature prediction. The findings can be applied in forensic science and anthropological investigations, aiding in the accurate estimation of stature from hand and foot measurements, particularly in the unique indigenous population of tribal populations living in the Kinnaur and Lahaul and Spiti districts of Himachal Pradesh, India.

Stature; Anthropology; Identification

A24 The Effects of Tibial Nutrient Foramen Location on Biological Profile Construction

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Learning Objectives: This presentation aims to inform attendees how variability in the location of the tibial nutrient foramen can affect a metric approach to biological profile construction. It will address whether the position of the tibial nutrient foramen covaries with population affinity, biological sex, age at death, and limb length.

Impact Statement: Some measurements utilized for biological profile construction are taken from landmarks that are easy to define and locate, but for others, the landmark's absolute position may vary between individuals. Attendees will gain a better understanding of how the location of the nutrient foramen on an individual's tibia varies and the potential implications for the use of tibial metrics in biological profile construction.

Abstract Text: In the human skeleton, the pelvis and skull are considered the most useful indicators for estimating skeletal sex and the skull for estimating population affinity.[1] When these bones are unavailable, damaged, or incomplete, other skeletal elements must be considered. In such cases, forensic anthropologists will use long bones like the tibia and conduct metric analyses to estimate the living characteristics of the individual. Some commonly used tibial measurements are associated with the nutrient foramen (i.e., anteroposterior diameter, transverse diameter, circumference). In tibiae, there is typically a single nutrient foramen located on the posterior aspect of the diaphysis. However, the foramen's vertical position along the shaft can vary between individuals. Since the diameter of the diaphysis decreases as one moves away from the tibial plateau, an inferiorly placed foramen will yield absolutely smaller diameter measurements, potentially skewing analyses. Much of the previous literature addressing measurements taken from the nutrient foramen indicates that there is nearly always considerable overlap in the values obtained for males and females.[2,3] To better understand the effectiveness of measurements involving the nutrient foramen, this research explored how variables such as biological sex, population affinity, age at death, and limb length can influence the position of the foramen along the shaft of the tibia.

A contemporary sample of 180 adult individuals (102 males, 78 females) of European and African descent was analyzed from the osteological collection at the University of Tennessee – Knoxville. Tibiae were measured according to *Data Collection Procedures*, with the addition of a measurement from the posterior rim of the lateral condyle to the most inferior point of the nutrient foramen.[4] A ratio expressing the nutrient foramen's location along the shaft of each tibia was calculated by dividing the distance of the nutrient foramen from the proximal end by total tibial length. This ratio provides a standardized measurement of nutrient foramen position on the shaft. The following variance equation was used to express the relationships between the variables: measurement = biological sex + population affinity + biological sex*population affinity + tibial length + age + error.

Analysis of Covariance (ANCOVA) indicates the effects of biological sex, age at death, and the interaction between biological sex and population affinity do not significantly influence the position of the nutrient foramen. However, population affinity by itself does affect foramen position, with individuals of African descent tending to have a foramen positioned higher on the shaft. In addition, tibial length has a significant effect on foramen position, with longer left tibiae having a foramen positioned higher on the shaft; right tibiae showed no such correlation. The higher relative position of foramina in African and long-limbed individuals may skew metric analyses so that African and taller individuals of either ancestry are more likely to be called males regardless of their actual sex. However, these effects are absolutely small and account for only a small percentage of the total variance in foramen position, so corrections to existing metric methods may not be warranted.

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Biological Profile; Sex Estimation; Nutrient Foramen

A25 A Glimpse Through the Veil of Lost Identities: Identification Practices and Outcomes in Greece

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Learning Objectives: After attending this presentation, attendees will understand the importance of interdisciplinary investigation and the impact of advancing technologies to the identification of human remains in Greece, a country at the crossroads of migration into Europe experiencing migration flows across both land and sea borders.

Impact Statement: This presentation will impact the forensic science community by serving as an example of how incorporating advancing technologies such as a national DNA identification database, national missing persons investigative bureau, and digital record keeping into medicolegal casework can impact the medicolegal system's ability to successfully process and identify unidentified human remains particularly in regard to areas highly affected by the deaths of foreign nationals, including both migrants and tourists.

Abstract Text: The presentation uses retrospective case files of originally unidentified individuals over the span of 34 years (1988–2022) from across Greece to illuminate trends and patterns in identification methods and rates, demographic data, and technological advancements. A total of N=586 cases from the University and State Forensic Services of the Ministry of Justice were analyzed with the aim of recording information on the demography and country of origin of the deceased, the method of identification, the individual's status in the country (tourist, migrant, Greek national), the GPS location of recovery, as well as the cause and manner of death when available. Case files were collected from the Forensic Medicine Unit of the University of Crete (n=78), the Forensic Anthropology Unit, Department of Forensic Medicine and Toxicology at the National and Kapodistrian University of Athens (n=321), and the Forensic Pathology Division of Crete, Hellenic Republic Ministry of Justice Transparency and Human Rights (n=187). The caseload includes both anthropology and pathology cases from the island of Crete and mainland Greece.

For the entire dataset, 57.2% of individuals were identified. Identifications occurred most often through circumstantial evidence and DNA analysis (70%). The identified non-Greek nationals pertained to citizens of 21 different countries across four continents. Despite high levels of migration through Greece, the majority of non-Greek nationals in this dataset were tourists (55%), while migrants made up only (45%) of the identified non-Greek cohort. Results demonstrate regional differences within Greece regarding caseload types (primarily due to large tourist influxes on the island of Crete), as well as temporal and demographic patterns regarding identification methodology and approach. Specifically, case data highlights that after the introduction of the national DNA database of the Hellenic Police in 2003, the rate of identifications in these departments decreased (by 18%) likely due to a combination of over-reliance on DNA data on behalf of the investigative authorities and a reduction in the acceptance of circumstantial identifications. The introduction of the national Missing Persons Bureau within the National Hellenic Police in 2008 also appears to have had limited effects on the overall identification rates in this dataset, likely due a lack of centralized access to the system for departments outside of the Attica region and other intra- and inter-agency communication pitfalls.

This study also discusses the impact of digital casefile database systems on a department's ability to retrospectively analyze data as well as to extract and share information with investigative authorities and other relevant stakeholders. Together, this presentation shows that despite the introduction of technological advancements, without the implementation of protocols that encourage appropriate use of technology and interdisciplinary communications, identification outcomes are not necessarily improved by the introduction of technology alone. Continued research on the impact of technology, including the implementation of postmortem imaging in the protocol for migrant identification, will also be addressed.

Identification; Migrants; Unidentified Persons

A26 Applying Postmortem Fingerprinting Into Disaster Victim Identification in Maverick County, Texas

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the significance and utility of fingerprinting techniques following various postmortem changes in the positive identifications of unidentified migrants. This study was made by a collaboration with Operation Identification (OpID) and the Disaster Victim Identification (DVI) Unit with the Federal Bureau of Investigation (FBI).

Impact Statement: This presentation will impact the forensic science community by increasing the awareness of fingerprinting techniques following various postmortem changes for improving identification outcomes of unidentified remains from Maverick County, TX.

Abstract Text: In 2012, Texas surpassed Arizona as having the highest number of migrant deaths. According to the United States Customs and Border Protection statistics, a total of 1,976 migrant deaths occurred in Texas from 2012 to 2020, though more than 4,400 migrant individuals have died in the state since 1981.[1] Arizona has centralized medical examiner's offices like Maricopa County Office of the Medical Examiner (MCOME) and the Pima County Office of the Medical Examiner (PCOME) to investigate unidentified migrant deaths; whereas investigations in Texas are county dependent and rely heavily on elected justices of the peace. Of the 254 counties in Texas, only 14 have medical examiner's offices. Forensic experts are often not involved in the investigation of unidentified migrant deaths, despite the Texas Criminal Code of Procedure (TCCP) mandating that all unidentified remains be investigated, fingerprinted (if applicable), sampled for DNA, and tracked for a minimum of ten years. The degree to which South Texas counties are compliant is inconsistent, resulting in the burial of hundreds of unidentified persons without identification efforts. Shifts in migration routes have resulted in record-breaking deaths in Maverick County, making the county the new epicenter of migrant deaths. Medicolegal authorities and law enforcement there typically do not take fingerprints or DNA prior to burial due to the overwhelming number of deaths and exceeding local capacity, creating a mass disaster.

Decedent fingerprints are typically taken with ink and paper or powder and an adhesive for identification purposes. However, unidentified migrants recovered in advanced stages of decomposition make it challenging to process postmortem prints, and alternative methods must be employed. OpID therefore sought to investigate the question: can fingerprints help facilitate identifications of decedents recovered in Texas border counties, especially for decomposed individuals?

To address this question, in March 2024, OpID personnel participated in a fingerprint training workshop taught by the FBI's DVI Unit. The OpID team learned about the different methods by which to obtain fingerprints of decedents in various stages of advanced decomposition. Following the fingerprint training, the OpID team traveled to Maverick County to help collect and process fingerprints with the head of the FBI DVI Unit. The fingerprints of 26 deceased migrants were collected using various methods, such as an automated fingerprint identification system, wearing the degloved epidermis, injecting liquids beneath the dermal tissue, or submerging the hand into near-boiling water and printing the dermal ridges using fingerprint powder and adhesive lifters.[2,3]. Fingerprints were searched in various databases by the FBI DVI Unit, leading to the identification of 14 individuals.

A few months later, an additional 25 cases from Maverick County were transferred from the Webb County Medical Examiner's Office to OpID. Of the 25 individuals, 7 were identified prior to the transfer through fingerprint comparison, fingerprint database, or a DNA cold hit. OpID was able to use the boiling method to collect fingerprints from 14 of the 18 unidentified individuals, of which 2 were identified.

The objective of this presentation is to demonstrate that these methods should become standard practice for unidentified deceased persons in Texas and for any decomposed remains found in any jurisdiction. Further, the importance of international collaborations during the identification process will be discussed.

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Migrants; Human Rights; Disaster Victim Investigation

A27 Unveiling the Role of Tattoos as Ink Trails in Forensic Anthropological Identifications: Exemplified From a Survey of a Northwest Indian Population

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Learning Objectives: After attending this presentation, the attendees will be informed about the pivotal role of tattoo marks in mapping out the identity affiliations of missing persons or unknown corpses found in medicolegal or cultural settings.

Impact Statement: This presentation will impact the forensic community by informing attendees that tattoos can be used as adjunct soft biometric traits in forensic death investigations as well as identifying missing persons.

Abstract Text: Tattoos serve as powerful tool of cultural marker and identifier in the realm of cultural and forensic anthropology in situations when routine channels of inquiry like facial features or fingerprints are not feasible to identify an individual.[1] Tattoos are a common type of body modification to express the aesthetic, socio-cultural, and religious affiliations of the bearer. They can play a crucial role in identity affiliations of a person as to certain specific regional or ethnic groups. The recognition and analysis of individualistic tattoo patterns have been commonly used to discern gang affiliations, religious beliefs, criminal records, and periods of incarceration.[2,3] Historically, tattoos were employed for criminal identification and slave branding, with evidence of their existence discovered on ancient mummified corpses.

In the present review-cum-interview study, the relevant literature was searched from different scientific databases and scrutinized for the use of tattoo marks for different forensic purposes. Also, 155 Northwest Indian subjects were interviewed for an anthropological understanding of their psychology, motivations, and symbolism of having engraved tattoos on different body parts. Questions were drafted as to the intentional or unintentional, permanent or temporary, professional or amateur, traumatic or cosmetic, decorative, cultural, medical or religious, etc. thrust for having these marks on their bodies. The systematic review showed that tattoo marks have helped in postmortem victim identification, identifying missing persons and unidentified corpses, mapping-out affiliation of individuals to certain specific ethnic backgrounds, beliefs and cultures, and even to certain gangs or hate groups. Scars, Marks, and Tattoos (SMT) have been used as soft biometric traits in crime scene analysis by providing multifaceted advantages in suspect identification, mapping subcultures, and facilitating decedent identification to the law enforcement agencies.

The study results revealed that the majority of persons having inked tattoos belonged to the age group of 20–29 years, represented almost equally by both sexes (suggestive of comprehensive understanding of tattoo marks). Significant occupational and workplace-specific differences were noticed among the participants. Females preferred tattoos on their lower arms and "letter-number" combinations outnumbered the figures, portraits, or symbols in their marks. Northwest Indians tattooed different body parts to display their affiliation to certain specific organizations, ideologies, or religious and social groups, to hide a scar, and to commemorate important life events and individuality. These results can supplement other methods of forensic identification of northwest Indians found missing or dead in some mass-disaster events. A population- and region-specific catalog of common tattoo marks and scar patterns can be generated for envisioning an expedited and accurate human identification, thus providing culturally sensitive solutions for victim identification in post-disaster scenarios.

The detailed results will be presented in textual, tabular and graphical/diagrammatic form.

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Forensic Anthropology; Tattoo Marks; Identification

A28 Using Images of the Ear for Postmortem Human Identification

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Learning Objectives: After attending this presentation, attendees will understand the potential for using comparisons of human ear images to aid in the identification of unidentified human remains.

Impact Statement: This presentation will impact the forensic science community by demonstrating that the human ear continues to show comparable features well into decomposition and that comparisons of ear images can contribute to the suite of available approaches employed to aid in the identification of unidentified decedents.

Abstract Text: Numerous studies, based on everything from low-tech one-to-one image comparison to machine learning algorithms, have established the human ear as a viable comparable biometric that can be used as a means for assisting in the identification of unknown individuals. The ear is also among the facial components taken into consideration when practitioners are faced with comparing facial images for the purpose of identification verification, such as at a border crossing or during a police traffic stop, providing investigators with an investigative lead, in situations where images of persons of interest are captured by surveillance cameras, and when forensic examiners are asked to give a professional opinion regarding whether or not two images depict the same individual.

The facial image comparison community, however, does not always handle cases involving living persons. In situations where there may have been a mass-fatality event, whether it is human-caused, such as the detonation of an explosive device, or natural, such as a catastrophic hurricane or earthquake, victim identification is essential. It is helpful, therefore, to have multiple means for establishing disaster victim identification, depending on what is available to investigators and what is feasible.

This study was conceptualized as a pilot study to first establish whether or not the human ear is helpful as a biometric that could be used to facilitate human decedent identification in situations where the remains may be in an advanced state of decomposition. This pilot study focused on evaluating whether or not untrained examiners, mainly students at Northern Michigan University who have self-identified as having an interest in forensic science, are able to correctly determine whether images of human ears in varying states of decomposition depict the same or different ears and, by extension, the same or different individuals. All ear images involved in the study were taken of human remains that were donated to the Northern Michigan University (NMU) Center for Forensic Anthropology and placed in the Forensic Research Outdoor Station (FROST) as part of routine data collection procedures between 2018 and 2023. Students were invited to voluntarily participate in a Qualtrics survey through which the data for this study were collected. Participants were asked to provide some demographic information and to answer several questions aimed at ascertaining their self-evaluated ability to notice details and their familiarity with both image comparison and external human ear anatomy. They were also asked to compare ten sets of one-to-one image comparisons and ten sets of one-to-many image comparisons. For each one-to-one comparison, participants were also asked to rank each named anatomical feature of the ear for its usefulness in helping them reach their decisions.

Results of this pilot study indicate that external anatomical features of the ear persist well into decomposition, although there is a point at which many become unidentifiable, especially when the ear has been affected by insect activity. The untrained participants in this research demonstrated high success rates in both the one-to-one and one-to-many scenarios, meaning that the ear seems to be a viable biometric for aiding in the identification of unidentified decomposed human remains.

Victim Identification; Decomposition; Facial Identification

A29 An Intersectional Approach to Developing a Prediction Model for Adult Missing Persons Off a Major United States Corridor in Idaho Using Novel Computational Techniques and Multidisciplinary Data

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Learning Objectives: This presentation will report on the outcome of our pilot project entitled Predictive Locator for Missing Person's I15 Corridor. Specifically, this presentation will go over the creation of the inclusive and multidisciplinary database, which includes a combination of categorical, numerical, and spatial data and the computational techniques used to create the prediction model. Last, we will discuss the results of testing the database, the resulting data analysis, and its future impacts.

Impact Statement: This program has the potential to impact communities at local, state, and national levels by handling large amounts of data, enabling widespread use across various jurisdictions in the United States. It can be applied to various cases, including child abductions and crimes against vulnerable groups, reducing the financial burden on law enforcement and public services by narrowing search areas and saving time and resources. The goal is to make this program available to agencies, non-profits, and humanitarian groups to aid in search and recovery efforts for missing persons.

Abstract Text: Forensic anthropologists are uniquely positioned to work on missing person cases due to our expertise in the search and recovery of human remains, skeletal analysis of unidentified remains, and our ability to liaise between law enforcement and community groups or families with cultural awareness and competency. There are more than 500,000 people reported missing in the United States.[1] In Idaho, the Idaho Missing Persons Clearinghouse has reported over 35 adult missing persons since 2020, while NaMus has over 125 open cases in the state.[2,3] These cases often remain unsolved due to a lack of information, funding, resources, and various biases, conscious or unconscious.

Previous research has predominantly focused on children and White individuals, deemed as vulnerable and "important" subsets within American demographics, thus attracting more attention and resources.[4] Unfortunately, most missing persons do not fall into these categories. Existing repositories for missing persons and unidentified remains primarily focus on open cases, neglecting the potential of using data from closed cases to predict the location of individuals. Johnson highlights that despite the valuable information these repositories contain, they often do not connect, and there is no centralized database[5]. One goal of this research was to create a more centralized, culturally inclusive, and easily accessible database.

Law enforcement faces numerous challenges in resolving missing person cases, including limited funding, lack of community engagement, and insufficient manpower and resources. In Idaho, where many law enforcement agencies are rural and understaffed, these challenges are even more pronounced. Unidentified remains add another layer of difficulty, as they are hard to connect with open cases and often cannot be identified due to the high cost of bone tissue DNA analysis. Another goal of this project was to narrow down missing person cases to those most likely to be found in a specific location in order to make investigations more financially viable and increase the likelihood of solving these cases.

This research focuses on adult missing persons in Idaho who went missing or were found near US Highway I-15, a corridor known for human trafficking that runs from Canada to Mexico. This presentation introduces the creation of an inclusive, multidisciplinary reference database encompassing approximately 200 closed cases of missing adults from counties along the corridor from 2018 to the present. Ongoing projects like the National Missing and Unidentified Persons System (NaMus), the National Crime Information Center (NCIC), the New Mexico Decedent Image Database, and Operation Identification were analyzed to determine current data collection practices. The authors introduced new data points to create a comprehensive reference database compatible with a machine learning algorithm. The final goal of this research was to identify the specific characteristics that aid in locating the individual. Trials were conducted to identify the more significant data points. By using an intersectional lens, this project addresses inequities and biases in missing person cases, specifically regarding data collection on individuals. It demonstrates how incorporating new, inclusive data points can enhance law enforcement's ability to locate missing persons.

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Anthropology; Missing Persons; Modeling

A30 Enhancing Forensic Human Identification in Emergencies and Disasters: Leveraging Technology for Improved Efficiency, Cost Reduction, and Ethical Practices

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Learning Objectives: After attending this presentation, attendees will understand the benefits and ethical considerations of using advanced data collection techniques, Artificial Intelligence (AI), and data analytics in the recovery and identification of human remains in emergencies and disasters. This presentation will impact the forensic science community by demonstrating how these technologies can reduce operational costs, enhance efficiencies, alleviate stress on first responders, and provide quicker answers to grieving families, particularly in Disaster Victim Identification (DVI) settings.

Impact Statement: This presentation will impact the forensic science community by improving competence in using advanced technologies for the recovery and identification of human remains in conflicts and disasters. It will enhance performance by providing practical solutions to reduce the overall identification costs and the stress on first responders while expediting identification processes and ultimately improving outcomes for grieving families and communities.

Abstract Text: The deployment of AI in forensic human identification leverages vast amounts of available data to support forensic experts. This technology not only enhances accuracy but also ensures that resources are allocated more effectively, supporting human expertise in the most complex cases.

Key improvements include enhancing the chain of custody and evidence continuity, ensuring that remains are managed with greater precision and less risk of contamination, commingling, or misidentification. In addition, the expedited identification process facilitated by AI-driven data analysis and predictive algorithms allows victims' families to receive answers more quickly, providing crucial emotional and psychological relief.

Reducing the pressure on first responders is a critical aspect of these technological advancements. By automating routine tasks and providing real-time data analysis, first responders can focus on immediate rescue and recovery efforts rather than being overwhelmed by the intricacies of data collection and management. This not only improves the overall efficiency of disaster response but also contributes to the mental and physical well-being of first responders, who often operate under extreme stress and time constraints. Integrating portable data collection devices and real-time communication tools enables rapid information sharing and coordination, further enhancing the effectiveness of response efforts.

This presentation addresses the ethical implications of these advancements, emphasizing the importance of implementing technologies in a manner that is both just and respectful of the deceased and their families. The balance between probative value and fundamental fairness is examined, highlighting the need for robust protocols and oversight to prevent potential abuses. The ethical considerations include ensuring data privacy, obtaining informed consent where possible, and maintaining transparency in the identification process.

Finally, the presentation discusses strategies for effectively communicating and educating about the responsible use of these technologies. By fostering a better understanding of the capabilities and limitations of AI and related tools, we can ensure their integration into forensic science is both beneficial and ethically sound. Training programs for forensic professionals, workshops for legal and governmental stakeholders, and public awareness campaigns are crucial to achieving this goal.

This research underscores the transformative potential of technology in forensic science. It advocates for the use of technological advancements to provide timely, accurate, and respectful handling of human remains and upholds justice and dignity for all involved. The continued development and refinement of these technologies promise to revolutionize the field, making forensic identification more efficient, cost-effective, and dignified.

Artificial Intelligence; Disaster; Technology Transition

A31 Understanding the Role of CODIS and Familial DNA in Positive Human Identification for the Louisiana Repository for Unidentified and Missing Persons

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Learning Objectives: After attending this presentation, attendees will understand the complexity of DNA identification facilitated by forensic anthropologists though a retrospective case analysis. This study highlights the differences and variability in the time from receiving a case to having positive DNA identification in cases handled by the Louisiana Repository for Unidentified and Missing Persons

Impact Statement: This presentation will impact the forensic science community by demonstrating a method for better understanding how individual identification methods like DNA fit into a broader workflow for forensic anthropologists. By understanding the extrinsic and intrinsic factors that affect the average length of time for DNA identification, it is possible to implement plans for other assistive methods like facial approximation in remains that are truly unknown.

Abstract Text: Forensic anthropologists have a diverse toolkit that can assist in identification when unidentified human remains are recovered; however, it can be difficult to determine which methods should be employed to expedite positive identification. DNA is often used as a primary identification method and can be a logical approach for skeletal remains. This research explores the role of DNA in identifications handled by the Louisiana Repository for Unidentified and Missing Persons (Louisiana Repository) to build an efficient identification workflow for forensic anthropologists at the Louisiana State University Forensic Anthropology and Computer Enhancement Services (FACES) Laboratory where time-consuming methods like facial approximation are generated when other methods are unsuccessful.

Information from 156 cases (1980 to 2024) was collected from the Louisiana Repository. Seventy-seven of the positive identifications were generated from Combined DNA Index System (CODIS) "hits" and 79 cases were identified through comparisons to familial DNA samples. The following data was collected for each case: (1) date the case was received in the FACES Lab, (2) date the DNA sample was submitted to a DNA laboratory for processing, (3) date completed DNA report was received from the DNA laboratory, and (4) date of positive identification.

Cases that were identified through assistance from CODIS were true unidentified persons with no leads on a possible or putative identification. The earliest CODIS hit case was received as remains in 2001, a DNA sample was submitted in 2006, a DNA report was generated in 2008, and positive identification was established in 2017. This timeline is an outlier because most cases are resolved within months. With this outlier removed, CODIS cases averaged 92.14 days from the date the case was received to the date that an identification was made. The average time between submission to a DNA laboratory to identification was 65.49 days. On average, it took about 26.66 days from the time that a case was received by the FACES Lab to the time that a DNA sample was submitted to a DNA laboratory.

Cases where possible or putative identifications were established during the early stages of the investigation are compared to family reference samples collected for the assumed missing persons. Sometimes putative identifications are suggested long after a remains case has been analyzed by an anthropologist. The Louisiana Repository assisted with a case that was received in 1980, DNA samples were submitted for analysis over the years but were unsuccessful. In 2010, a final DNA sample was submitted, and a DNA report was generated the same year. The DNA profile was compared to familial DNA, and a positive identification was made in 2019. This cold case is an outlier with 14,120 days between the date the case was received to identification; however, it demonstrates the wide variation in time that identification through familial DNA can take. With three of the coldest cases removed from the data, the average days from that date a case was received in the FACES Lab identification was 283.21 days.

Our analysis revealed that CODIS hits facilitated faster identifications, with an average turnaround of 92.14 days from case receival to final identification, and reference sample cases tend to be more time-consuming, averaging 283.21 days. The advent of DNA technology has undeniably revolutionized forensic science by significantly enhancing the speed, cost-effectiveness, and precision of identifying individuals. Despite the advanced capabilities of DNA profiling, forensic anthropologists remain indispensable, particularly when dealing with complex cases involving unidentified and missing individuals. This study underscores that forensic anthropologists who rely on DNA identifications need to understand the realistic timelines for these identifications so that other methods can be used when it is apparent that a CODIS hit is unlikely.

Personal Identification; DNA; Unidentified Persons

A32 Forensic Age Progression as a Support for the Search for Missing Persons: A Test of the Accuracy of the Aging Method

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WITHDRAWN

A33 Vertebral Bone Density Variations in Scoliotic vs. Non-Scoliotic Juveniles and Their Implications for Schmorl's Node Development: A CT-Based Analysis Using the New Mexico Decedent Image Database

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Learning Objectives: Attendees of this presentation will learn specific insights into vertebral bone density variations in scoliotic versus non-scoliotic juveniles and the implications for Schmorl's node development. They will gain a comprehensive understanding of how scoliosis affects bone density, particularly as to how it correlates with the presence of Schmorl's nodes as observed through Computed Tomography (CT) scans from the New Mexico Decedent Image Database. The presentation will cover the methodology used for the CT-based analysis, the statistical significance of the findings, and the implications of these findings for clinical practices and future research. Attendees will also learn to evaluate and interpret statistical data related to bone density and scoliosis, enhancing their ability to apply these insights in their own professional practice. The measurable objectives include understanding the relationship between scoliosis and bone density, the prevalence of Schmorl's nodes, and the impact of these findings on potential treatment and prevention strategies for spinal pathologies. Attendees will also explore how advancements in CT imaging technology and data analysis can transform our understanding and management of scoliosis and related spinal pathologies, demonstrating the potential of technology as a powerful tool for medical transformation.

Impact Statement: Presenting this research at the AAFS conference will significantly impact the forensic science community by highlighting the transformative role of CT analysis in forensic contexts. By avoiding destructive methods, CT imaging allows for non-invasive examination of skeletal remains, preserving valuable evidence. This research underscores the importance of pathology analysis in postmortem investigations, offering insights into why individuals may exhibit unusual traits, such as lower vertebral density, which can be attributed to genetic or medical conditions rather than forensic factors. Additionally, comparing postmortem findings with CT scans can enhance identification accuracy, particularly when dealing with rare pathologies like Schmorl's nodes in children. This approach not only advances forensic analysis but also aids in distinguishing between medical conditions and forensic evidence, thereby improving overall case assessments.

Abstract Text: Scoliosis, characterized by an abnormal lateral curvature of the spine, is predominantly idiopathic, underscoring the need to delve into its underlying causes for effective treatment and preventive strategies. This study investigates a potential correlation between scoliosis and Schmorl's nodes (intervertebral disc herniations) influenced by Wolff's law, which posits that bones adapt to external pressures. We analyzed CT scans from 108 juvenile decedents, including 56 with scoliosis and 52 without. After running multiple statistical tests, there was no significance between the mean bone density when compared to having scoliosis. An independent t-test provided a t-value of 0.041, which, when compared to the original significance level of 0.05, is statistically significant, although weak. When compared to the Bonferroni correction level of 0.008, it throws out the significance to give a result of not being statistically significant. It was the same in the cases of L3 ($t = 0.103$), L2 ($t = 0.084$), and L1 ($t = 0.053$). If compared to the regular significance level of 0.05, T12 ($t = 0.012$) and T11 ($t = 0.042$) had weak significance, but that was then excluded when the Bonferroni correction was applied. When looking at any significance of densities in different vertebral regions, the results from a one-way Analysis of Variance (ANOVA) (p-value = 0.213) suggest that it is likely that the results are due to random variability or chance, and that there is no statistical significance. With a value of 0.273 from a Chi- squared ($\chi 2$)/Fisher's exact test, it suggests that there is no statistically significant correlation or difference between the variables of scoliosis and Schmorl's nodes. The general pattern seems to follow that as the spine ascends, the density increases, and this is true in both scoliotic and non-scoliotic individuals. As a whole, it is evident that those with scoliosis have a lower vertebral density than those without, in all of the vertebral regions. There is, however, a weak negative linear relationship between bone density and age in both scoliotic and non-scoliotic individuals. A p-value of −0.229 obtained from a Pearson correlation coefficient analysis in non-scoliotic individuals, as well as a p-value of −0.069 in scoliotic individuals, was obtained. Overall, the findings of this study are comparable to some existing studies on similar topics, but there are few results that hold statistical significance, so this would be interesting to research further.

3D Technology; Spine; Juvenile

A34 Vertebral Variation in Forensic Anthropology Casework

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Learning Objectives: After attending this presentation, attendees will have an increased awareness of the frequency with which anatomical variation within the vertebral column is encountered in forensic anthropology casework.

Impact Statement: This presentation will impact the forensic science community by providing a comparison between the rates at which spinal anomalies are observed in casework to the frequencies of such variations as reported in the clinical literature. Familiarity with this topic can help alleviate concerns related to recovery and commingling as well as contribute to the generation of a biological profile and identification.

Abstract Text: A series of recent forensic anthropology cases analyzed at the King County Medical Examiner's Office (KCMEO) in Seattle, WA, exhibited anomalous numbers of vertebral elements, prompting a review of cases examined in the past two years for which at least two regions of the vertebral column were observable. Of the 52 cases meeting these criteria, 15 exhibited some form of developmental anomaly of the vertebral column, with the majority (n=13) involving a shift of one or more segmental borders.

To ascertain whether the observed frequency of developmental anomalies is unusual, a review of the clinical literature was conducted. Of the developmental anomalies observed in casework, Lumbosacral Transitional Vertebrae (LSTV) are the most commonly studied. Owing to differences in imaging modality and definition, the frequency with which this variation is observed is recorded as being between 5.4% and 35.6%.[1-3] When definitions are controlled and individual studies are combined into regional samples, population frequencies for this anatomical variant are more stable, falling between 9.06% and 14.15%. Five cases (9.6%) with LSTV were observed in the KCMEO sample, consistent with reported population frequencies.

By contrast, the presence of 13 thoracic elements is rarely addressed in the clinical literature. Most studies that mention this segmentation anomaly report population frequencies of less than 1%.[4-6] Four cases (7.7%) with 13 thoracic elements were observed in the KCMEO sample. Interestingly, the only study reviewed that analyzed skeletal samples7 reported a population frequency of 5.3% for this anomaly, suggesting that the prevalence of this anatomical variant is underestimated in the clinical literature.[7]

Together, these results suggest that forensic anthropologists can expect to routinely encounter vertebral variation within their casework. When such variation results in the gain or loss of vertebral and/or costal elements, it may raise concern over recovery methods and/or commingling. Awareness of the expected frequency of such variations, together with careful articulation of skeletal elements and observation of taphonomic characteristics, can help resolve such anxieties. Additionally, the identification of anatomical variants of the vertebral column may aid in the formation of the biological profile and the establishment of a positive identification.

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Anatomical Variants; Vertebrae; Population Frequency

A35 The Ontogenetic Development of the Glabella and Its Association With Maturation of the Humeral Proximal Epiphysis in Accurate Subadult Sex Estimation

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Learning Objectives: After attending this presentation, attendees will have increased knowledge regarding the ontogenetic development of the glabella and its association with patterns of fusion of the Humeral Proximal Epiphysis (HPE).

Impact Statement: This presentation will impact the forensic science community by providing information regarding the utility of the HPE as a maturity indicator associated with puberty and in generating accurate sex estimates from the subadult skull.

Abstract Text: Forensic anthropologists have historically used a minimum age standard as the threshold on which to base whether a sex estimate should be attempted in immature individuals. However, if the age estimate is incorrect, this approach could also result in an inaccurate sex estimate. Further, several studies have shown that skeletal maturity is more closely linked to pubertal stage than chronological age.[1,2] Since the onset of dimorphism in most sex traits is associated with puberty, using maturity as the parameter on which to base a sex estimate is more appropriate than an age-at-death estimate.

The authors have previously shown that ossification of the iliac crest, which is associated with menarche in females, improves classification accuracy from the pelvis.[3] The goal of the current study is to evaluate skull changes associated with puberty to further illustrate the utility of skeletal maturity indicators in estimating subadult sex, but with a focus on male-based developmental processes (i.e., changes occurring in the skull rather than pelvis). The glabella was selected as the sex trait because it is the most accurate trait for estimating sex from the skull.[4] The HPE was selected as the maturity indicator due to its association with peak height velocity, or mid-stage puberty.

Postmortem computed tomography scans of known individuals (n = 563, m = 348, f = 215) aged 8 to 20 years from the Subadult Virtual Anthropology Database were used to evaluate the development of the glabella with refence to maturation stages of the HPE. Trait expression of the glabella was assessed following the five-stage scoring procedure outlined in The OnSEt User Manual, where a score of 1 represents the most gracile expression of the trait and a score of 5 represents the most robust expression of the trait.[5] Maturation of the HPE was assessed following the four-stage scoring procedure outlined in Stull and Corron, where Score 0 indicates absence of the epiphysis, Score 1 indicates presence without fusion, Score 2 indicates active fusion, and Score 3 indicates complete fusion.[6]

Differences in glabella score frequencies between males and females for each maturation stage of the HPE were first evaluated using Chi-square tests. The mean score of the glabella for each HPE maturation stage was also visualized with line plots for each sex to further illustrate the stage of maturity at which the glabella becomes dimorphic. Next, Random Forest (RF) modeling was used to evaluate classification accuracies for individuals with each HPE score to determine if maturation improves sex classification accuracy. Training and testing samples were generated using 75% and 25% of the original data, respectively.

Sex differences in trait score frequencies of the glabella were significant with active fusion of the HPE (p = 0.002), which is reflected in the high classification accuracies obtained by the RF models. When HPE is scored 1, the classification rate and sex bias were unacceptable for sex estimation (C = 67%, M = 100%, F = 0%). When HPE is scored 2, the classification rate was 91% and sex bias was greatly improved (M = 100%, F = 73%). Results were maintained when HPE was scored 3 (C = 89%, M = 96%, F = 75%).

The findings lend further support that maturity indicators associated with puberty can be used to guide the practitioner in deciding if a subadult is sufficiently mature for sex to be accurately estimated without needing to rely on age. Importantly, these results were obtained using only one sex trait and one maturity indicator. The incorporation of multiple sex traits and maturity indicators should improve confidence around the maturity estimate and accuracy of the sex classification.

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Juvenile; Puberty; Sex Assessment

A36 Intra-Observer and Inter-Device Error in Osteometric Data

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Learning Objectives: After attending this presentation, attendees will understand the impact that inter-device and intra-observer error can have on osteological measurements and forensic anthropological estimates.

Impact Statement: This presentation will impact the forensic science community as it will guide the refinement of future forensic science standards and methods. This will contribute to the continued standardization of the discipline.

Abstract Text: With the implementation of the *Daubert* standards, the forensic science community is required to identify potential error rates of different methodologies to demonstrate they are reliable and have been tested.[1] Within forensic anthropology, osteometric data is commonly utilized to estimate the stature, age at death, and biological sex of unidentified individuals. Despite the frequent use of osteometric data, little research has been conducted to establish the intra-observer and inter-device error rates of the commonly utilized instruments for gathering these data. Inter-device error is the difference between multiple measurement devices performing the same measurement, and intra-observer error refers to the difference in repeated measurements taken by one individual.[2,3] The establishment of these error rates is critical as osteometric measurement devices are utilized by the forensic anthropological community without knowledge of their ability to produce consistent results. Additionally, intra-observer variability can influence inter-device reliability, and factors such as replicability, can affect the consistency of results.

This study aims to determine the error rates of intra-observer measurements and the inter-device reliability between two different sets of calipers, designated Caliper A and Caliper B, through standardized craniometric measurements as established in the Data Collection Procedures for Forensic Skeletal Material 2.0.[4] The craniometric data of two high-quality skeletal casts were collected from six different observers. Twenty-four cranial measurements were taken with reference to the Central Identification Laboratory (CIL) standard measurements.[5,6] Each observer took the measurements twice with both devices, equaling 96 measurements per cranium. The observers performed the measurements independently and with no outside assistance during an academic semester, and each observer had prior educational experience in osteometrics. Data were analyzed in RStudio by conducting one-way Analysis of Variance (ANOVA) to examine the inter-device and intra-observer error rates. This was done through a comparison of the spread of the measurements taken by both sets of calipers for each observer. Presently, an acceptable error range is for osteometric measurements to be within a 2-millimeter range of the known and/or repeatable measurements.

Results of this research demonstrate that Observer One was within the standard error range for 22.72% of the osteometric measurements for cranium one and 59.09% of the osteometric measurements for cranium two. Observer Two had 81.82% of their osteometric measurements for cranium one within the standard error range and 86.36% within the range for cranium two. Observer Three had 79.16% of their osteometric measurements for cranium one within the acceptable error range and 83.33% for cranium two. For Observer Four, 69.56% of measurements for cranium one and 75% for cranium two were within the standard acceptable error range. Observer Five had 75% of their measurements for cranium one and 70.83% for cranium two within the acceptable error range. Finally, Observer Six had the lowest error rates, with 95.45% and 87.50% of measurements falling in the acceptable error range for crania one and two, respectively.

Additionally, it was observed that for cranium one, 6.48% of the osteometric measurements taken with Caliper A were outside the acceptable error range, whereas 15.83% of measurements taken with Caliper B were outside the standard error range. The results were similar for cranium two, with 6.34% of the measurements taken with Caliper A and 14.69% of measurements taken by Caliper B falling outside the acceptable error range. This research demonstrates that intra-observer and inter-device error rates should be considered when gathering osteometric data as they may affect the accuracy of measurements and the outcomes of forensic anthropological estimates such as biological sex, age at death, and stature. Additional research is required to determine the error rates for postcranial measurements as this will further supplement current osteometric data research.

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Osteometry; Intra-Observer Error; Inter-Device Error

A37 Morphological Patterns of Corresponding Atlanto-Occipital Joint Surfaces: Potential Applications for Sorting Commingled Human Remains

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Learning Objectives: Attendees of this presentation will come away with a better understanding of shape patterns between articulating surfaces of the Atlanto-Occipital Joint (AOJ) and how these patterns may inform segregation of commingled human remains.

Impact Statement: This presentation will impact the forensic science community by identifying morphological patterns between corresponding joint surfaces that may be useful when assessing the articulating pair match potential at the AOJ. Recognition of these patterns during analysis may increase analyst competency and confidence in evaluating this joint in commingled scenarios.

Abstract Text: Assessment of joint articulation is a subjective process with little published guidance regarding normal variability for joint congruency. However, visual assessment of joint articulation is utilized as a traditional anthropological approach to sort commingled human remains. While visual evaluation of a joint fit is often perceived as reliable, the strength of the association between articulating elements is highly dependent upon the elements in consideration.[1,2] In particular, the AOJ has been documented as an articulation with a "moderate" level of analyst confidence during the visual assessment of fit and a "moderate" level of reliability, measured within a rate of 60%–90% correct classification when observation of fit is cross-checked with DNA results.[1,2]

This study presents an examination of patterns of matching outline shapes between articulating AOJ surfaces: the Occipital Condyles (OCs) and C1 Superior Articular Facets (SAFs). The objective of this study is to provide a foundation where analyst confidence and method reliability may be improved during visual evaluation of potential occipital-to-C1 matches by elucidating the rates and trends of shape matches between known articulating surfaces.

In this study, 78 individuals (35 female, 43 male) with at least one intact, articulating pair of AOJ surfaces were analyzed from the William M. Bass Donated Skeletal Collection. Of these, 73 individuals (31 female, 42 male) had bilateral AOJ pairs. Each articular surface was visually assessed and categorized as a shape type. The observed variation of joint surface outlines was not well-represented by extant typological methods, resulting in uncertainty of type assignment.[3,4] To accommodate this, a modified system defining six shape categories (five monopartite: ovoid, sigmoid, reniform, clepsydral, irregular; and one representing all bipartite presentations) was developed in this study and used to score each articular surface. Articulating shape patterns were examined by categorical shape type, sex, side, and AOJ type using R v.3.6.1.[5]

Clepsydral was the most prevalent shape (33.6%) for pooled sexes, sides, and OC-SAF types. Few deviations from this pattern were observed when analyzed by sex, side, or AOJ type, such as bipartite surfaces are the most frequently observed shape for pooled-sex left SAFs (33.6%). A Fisher's Exact test of paired AOJ shape patterns between sexes yielded no significant results ($p > 0.05$). Twenty-one combinations of articulating shapes were observed, with the same articular shape types matching in only 32.5% of the total joint pairs. The most frequent pair-matched types are clepsydral OC-to-clepsydral SAF (35.9%), followed by clepsydral OC-to-bipartite SAF (19.2%). Of particular interest are the broad patterns of matching between bipartite and monopartite surfaces. Bipartite OCs paired with bipartite SAFs but were not observed to be paired with monopartite SAFs. However, the converse of this latter scenario does occur; monopartite OCs commonly articulate with bipartite SAFs. When bipartite SAFs were present, they were observed to be paired with either bipartite (16.7%) or monopartite OCs (83.3%), most frequently with the monopartite clepsydral OCs (62.5%).

This study identifies two general patterns that may be particularly useful to the analyst when evaluating articulating match potential at the AOJ. More often than not, the outline shapes of articulating AOJ surfaces are not mirrored by one another, suggesting joint surfaces cannot be excluded as potential matches if their outline morphologies grossly differ. Additionally, the absence of observed bipartite OC-to-monopartite SAF articulating matches suggests observation of the partite nature of the AOJ surfaces has strong potential as a morphological exclusionary criterion, particularly in generating short lists of potential matches in large-scaled commingling scenarios.

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Joint Outline; Articulation; Bipartite Surface

A38 Two Instances of Unusual Non-Human Remains Encountered in Routine Medicolegal Significance Casework

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Learning Objectives: The goal of this presentation is to demonstrate the importance of expertise in zooarchaeology/non-human osteology within forensic anthropology as well as the importance of detailed inspections of morphologies and peer review to avoid cognitive bias.

Impact Statement: This presentation will impact the forensic community by providing two unrelated cases of rare non-human remains encountered by law enforcement that were resolved by forensic anthropologists via assessment of medicolegal significance.

Abstract Text: While forensic anthropology is popularly considered to focus on the analysis of human remains in order to establish identity and contribute to the determination of the cause and manner of death, a routine aspect of forensic anthropology is the examination of questioned items in order to determine if they have forensic and/or medicolegal significance. The number of questioned item cases a forensic anthropologist encounters varies; however, forensic anthropologists traditionally perform many medicolegal-significance cases every year. Typically, these types of cases are relatively predicable and include the skeletal remains of local animals, often deer, bear, dogs, pigs, racoons, etc.

Case #1 represents a single skeletal element that was carried by a dog onto a private residence in Transylvania County, NC. Because of the large size of the element, the owner of the dog was concerned the remains may have been human and contacted the local Sheriff's Office, who subsequently reached out to Dr. Passalacqua for further assistance. The Sheriff's Office digitally sent a number of images of varying quality of the element for analysis. Based on these images, the skeletal element was clearly a large, long bone (400+mm long, estimated). While the proximal end of the element was obscured by adhering desiccated tissue and debris, the rest of the element was relatively clean and in good condition with minor taphonomic alterations. The shaft of the bone did not exhibit any notable morphological features aside from being very straight; however, the distal end presented with two large articular condyles with deep lateral grooves. The size and morphology of the skeletal element was consistent with an avian tibiotarsus; however, the size indicated that it was from a very large avian species, likely an ostrich or emu. The Sheriff's Office stated that they were unaware of any farms in the area, and the origin of the remains is unclear. Final species identification via genetic testing was not performed because the Sheriff's Office did not retain the element.

Case #2 represents a single skeletal element found in the yard of an individual in Fulton County, GA. Due to its size, the individual who found the element thought it may have been human. The medical examiner was unable to determine medicolegal significance and asked Dr. Tersigni-Tarrant for assistance. The bone had distinct epiphyseal morphology that was inconsistent with any of the bones of the human lower limb. Similar to Case 1, the morphology of the bone was suggestive of a large avian species. Emus and ostriches are known to have been farmed in this area of Georgia, although not on the property in question.

These cases represent two instances of similar, uncommon non-human remains being encountered in forensic anthropology casework. Most questioned items cases are typically relatively straightforward and come from the same few local non-human species (e.g., the femur of a deer). Being confronted with a large, long bone with unusual morphologies can lead to cognitive bias (i.e., "only a human long bone would be this large," etc.), which could result in erroneous conclusions. In both instances, the practitioners were surprised by the unusual size and morphologies of the remains that in some ways mimicked human morphologies (i.e., long tubular shaft with distal articular condyles). However, in both cases potential cognitive bias was mitigated via a critical assessment of the bone morphologies and unofficial peer review. Additionally, because the remains were relatively complete and in good condition, the analysts were able to assess the full gestalt of the remains, which assisted in reaching conclusions of non-human; however, if the remains had been damaged or incomplete, the assessment would have been much more difficult.

Medicolegal Significance; Non-Human Remains; Skeletal Morphology

A39 An Analysis of Non-Human Skeletal Remains in the Digital Age: An Examination of Temporal Trends (2014–2024) and Taxonomic Diversity in Northern California

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Learning Objectives: This presentation will examine ten years of non-human casework submitted to the Human Identification Laboratory at California State University, Chico (2014–2024), including the mode of human vs. non-human identification, taxon diversity, and skeletal element representation.

Impact Statement: After viewing this presentation, attendees will gain a better understanding of the non-human taxa commonly submitted to forensic anthropologists for identification in northern California and how the use of digital technology can provide rapid expertise to the law enforcement community.

Abstract Text: A large portion of forensic anthropology casework involves differentiating between human and non-human skeletal remains, and the ability to do so quickly and confidently is critical to law enforcement and in mass fatality scenarios. By determining which non-human taxa are most commonly encountered in the western United States, practitioners can prepare for analysis by keeping reference exemplars on hand for comparison with case material. This is particularly useful as non-human requests have become increasingly digital.

The Human Identification Laboratory (HIL) at California State University, Chico, has a large case load, providing services to over 87% of the counties in California and occasionally other states as well. Of the 1,075 cases submitted to the HIL from 2014–2024, 52.9% of them were requests for human vs. non-human identification. Most cases are found by the public or during search and rescue operations for missing persons or wildfire victims.

The records of 569 non-human cases were examined for characteristics such as mode of consultation, taxonomic identification, and skeletal elements represented. Possible modes of consultation were in-person, via email, via text, and a combination of digital and in-person assessments. Taxonomic identification was split first into large and small animals, with more specific identifications made when possible. Juvenile remains were also noted by the presence of open or unfused epiphyses. Elements were defined as axial, appendicular, or cranial (skull and dentition), or a combination of these.

Of the non-human cases included in this analysis, 74.9% consisted of digital consultations. In 2021, the number of text-based consultations surpassed the number of email consultations, and that trend has continued. An additional 3.0% included primary digital consultations but required in-person analysis for confirmation. Only 2.1% of cases consisted of solely in-person assessments. While technology has aided in casework efficiency, photo quality is often a limitation. Of the cases assessed, 33.0% were photos without the inclusion of a scale, 46.9% of cases included a proper scale, while the remaining 20.1% included improvised scales using objects such as pens, currency, water bottles, and shoes. While the majority of the photos were of high quality, a number of them were too blurry to examine detail on the remains or to observe the scale for reference.

Large mammalian remains were present in much higher frequencies than small animals, with 66.8% of cases including elements from large animals (most commonly deer, cow, or bear) and only 13.0% of cases including elements from small animals (typically bird, cat, or dog). Large animals are likely represented in higher frequencies because their size is more similar to humans compared to small animals such as birds. Juvenile animals were represented in 6.5% of cases. Appendicular elements were the most represented elements in casework at 52.6%. In contrast, only 11.1% of cases contained axial elements alone, while 2.3% contained only cranial elements. The remaining 24.1% of cases represented some combination of these.

The widespread use of technology – smart phones in particular—throughout the United States has greatly increased law enforcement access to forensic anthropologists for human vs. non-human identification. The HIL, located in northern California, regularly consults with law enforcement agencies hours and even states away through digital consultations, which in the past could have only been conducted through mailing cases or direct delivery. Instead, officers now send photos from the scene and receive a response within minutes, most often confirming remains as non-human, a practice that has also been used in wildfire contexts. It is important that the forensic community continues to optimize the use of technology to expand non-human casework capabilities and improve efficiency so as to allot resources to other cases of forensic significance.

Forensic Anthropology; Non-Human Remains; Casework

A40 A Multi-Isotope Approach for Reconstructing Residence Patterns of Massacre Victims of the Indian Uprising in Ajnala, Amritsar, India

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Learning Objectives: The goal of this presentation is to highlight the application of stable isotopes analysis for predicting possible region-of-origin for unidentified remains cases from India.

Impact Statement: This presentation will impact the forensic science community by presenting novel data to aid in predicting region-of-origin of Indian sepoy victims whose remains were recovered from a disused well in Ajnala, India, in 2014. This research will further aid in efforts to reconstruct the biohistories of these individuals who were killed by British colonizers.

Abstract Text: In 2014, the commingled remains of hundreds of individuals were unexpectedly discovered in an abandoned well in the town of Ajnala in Amritsar, India. The remains were excavated non-scientifically by amateurs, which resulted in the loss of much of the archaeological context. Ongoing research by Punjab University and collaborators has resulted in a long-term study of the remains, including studies of oral health, skeletal trauma, commingling, Minimum Number of Individuals (MNI), and dietary patterns and region of origin using isotopic analyses. Recently, the remains have been linked to the massacre of 282 Indian sepoys who were killed by British colonizers during the Indian Uprising of 1857.

Stable isotope analysis has been shown to be a useful identification tool in forensic anthropology that can provide investigative leads toward personal identification. Carbon and nitrogen isotope values ($\delta^{13}C$ and $\delta^{15}N$) of dentinal collagen provide important geocultural information reflecting dietary practices during infancy and childhood whereas carbon and oxygen isotope values ($\delta^{13}C$ and $\delta^{18}O$) of the carbonate of tooth enamel bioapatite provide information on whole diet and the source of drinking water during childhood, respectively.

This study presents isotope results for dentinal collagen $\delta^{13}C$ and $\delta^{15}N$ and tooth enamel bioapatite $\delta^{13}C$ and $\delta^{18}O$ of 101 individuals recovered from the Ajnala well. In total, 101 tooth samples were selected from separate dentitions, ensuring that no individuals were double sampled. Tooth enamel was separated from dentin, and each was ground to a fine powder for isotopic analysis. A stereoscopic microscope was used to remove soil and other potential contaminants. Dentinal collagen was demineralized in hydrochloric acid, contaminants were removed with sodium hydroxide, then solubilized, frozen, freeze dried, and weighed for Isotope Ratio Mass Spectrometry (IRMS). Enamel powder was soaked in hydrogen peroxide to remove organics, treated with a dilute solution of acetic acid to remove diagenetic contaminants, and dried and weighed for IRMS. Fifteen dentin samples failed to yield collagen and were excluded from the data set, leaving 86 well-preserved samples.

The mean collagen $\delta^{13}C$ value is –17.8‰ (±1.3‰, 1 SD; *range*=6.4‰) and the mean $\delta^{15}N$ value is +8.9‰ (±1.2‰, 1 SD; *range*=5.8‰) enamel bioapatite $\delta^{18}O$ value is –4.5‰ (±0.7‰, 1 SD; *range*=4.2‰). The collagen and bioapatite $\delta^{13}C$ results reflect a childhood diet consisting of a mix of both C_3/C_4 resources, and the collagen $\delta^{15}N$ values suggest consumption of terrestrial meat protein. The enamel bioapatite $\delta^{18}O$ results show minimal variation, suggesting that these individuals may all be from a similar geographic area. $\delta^{18}O$ values are elevated relative to previous research conducted on a smaller sample of individuals, suggesting some heterogeneity in region of origin within the broader sample.[1] Currently, there is limited baseline data and isoscape prediction maps for India. However, previous $^{87}Sr/^{86}Sr$ work on Ajnala remains suggests that some individuals may have been from the Varanasi region (Uttar Pradesh), the Gangetic plain, and the Bihar, Orissa, West Bengal.[2] The relationship between $^{87}Sr/^{86}Sr$ ratios and $\delta^{18}O$ values will be explored in further detail to better reconstruct probable region of origin for the Ajnala massacre victims.

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Stable Isotope Analysis; Massacres; Human Remains

A41 A Stable Foundation for the Use of Tooth Enamel Oxygen Isotopes in Human Identification

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Learning Objectives: Attendees of this presentation will learn the foundational principles, practical limitations, and best practices for the reconstruction of geographic life history using oxygen stable isotopes in human tooth enamel. They will learn the status of Project FIND-EM, a major collaborative program to develop an open database and the data science tools that support it, including how to access these resources.

Impact Statement: The impact of the presentation will be the development of a community that is aware of and able to confidently and robustly apply this technology in forensic casework. These capabilities will advance causes, including cold case resolution, humanitarian investigations, and identification and repatriation of the remains of American personnel lost in foreign conflicts.

Abstract Text: Stable isotope ratios of elements such as carbon, strontium, and oxygen in human body tissues have been shown to vary geographically and are potentially useful in reconstructing life history and identifying unknown decedents.[1] The oxygen isotope composition ($\delta^{18}O$ value) of tooth enamel carbonate is a particularly promising target for this work, but we lack a large, well-documented, standardized database of human tooth enamel $\delta^{18}O$ values as a foundation for forensic applications.

We report on Project FIND-EM, a collaboration between the academic research community and the United States Defense POW/MIA Accounting Agency to build such a database. We hypothesize that tooth enamel $\delta^{18}O$ values exhibit strong geographic variation that closely match previously documented patterns of tap water $\delta^{18}O$ values (a primary source of oxygen to growing teeth) across the contiguous United States. Third molars were collected from several hundred human donors through acquisition of existing collections, new sampling from skeletal collections, and contributions from living participants. Life history information (residence location and several health and demographic variables) spanning the period of third molar formation was also collected from donors, and all samples were prepared and analyzed using standardized, experimentally supported laboratory methods.

Comparisons show that most variation in enamel $\delta^{18}O$ values was explained by drinking water $\delta^{18}O$ values at the location(s) where individuals lived during tooth growth (adjusted $R^2 > 0.85$, residual error < 0.6‰ for individuals who reported a single location of residence during tooth formation). Given this result, analysis using the software package *assignR* suggests that on average, third molar oxygen isotopic composition could be used to exclude approximately 70% of the contiguous United States as a potential residence location for an individual with 90% confidence. Collectively, these results suggest great potential for high-quality tooth enamel oxygen isotopic data, interpreted in the context of the FIND-EM database, as a source of investigative information supporting human identification.

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Identification; Anthropology; Stable Isotopes

A42 Intra-Individual Isotopic Variation Within Teeth: Refining Forensic Applications Using Isotopes

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Learning Objectives: After attending this presentation, attendees will have an improved understanding of variation of oxygen and carbon isotope data values within individuals. Understanding these variations improves the use of stable isotopic data in geolocation and provenancing of unidentified persons.

Impact Statement: This presentation uses data compiled through Project FIND-EM, which is focused on developing robust methodology and a nationwide oxygen isotope dataset to aid in human identification efforts using oxygen isotopes in tooth enamel. Part of Project FIND-EM's mission is to inform and establish standardization in the use of isotopic measurements for forensic human identification.

Abstract Text: Individual teeth of the same type (e.g., central incisors, third molars, etc.) are generally understood to have similar development times within each dental arcade (maxillary and mandibular). However, there is some variation between the arcades, particularly in third molars.[1] Several studies have looked at isotopic variation between tooth types or intra-tooth isotopic variation within individuals.[2-6] To our knowledge, however, none have looked at isotopic variation with the same tooth type within or between arcades.

To examine intra-individual variability, we used oxygen and carbon isotope data from teeth collected for Project FIND-EM. We hypothesized that the variability between the teeth from different dental arcades would be greater than teeth from the same dental arcade due to somewhat larger differences in the time of tooth formation. We measured and calculated differences between teeth within an arcade (either maxillary-maxillary or mandible-mandible, n=14) and between different dental arcades (maxillary-mandibular, n=36). The range of differences for oxygen isotope δ-values for teeth from different dental arcades was -1.83 to 0.51‰, and for those within the same arcade was -0.54 to 0.56‰. The equivalent ranges for carbon isotope δ-values were -0.41 to 0.8‰ for teeth from different arcades and -0.37 to 0.3‰ for teeth from the same arcade. An F-test found the variance for teeth from different arcades was not significantly different from that for teeth from the same arcade (oxygen: $F(17,6)=3.09$, $p=0.17$; carbon: $F(17,6)=1.94$, $p=0.42$). Although we are unable to test with the current sample size, we hypothesize that higher variance between dental arcades would be more likely in individuals who lived in two or more locations during third molar crown development than in individuals who had static residential history. Real Interpretable Differences (RID; see) calculated by Stantis et al. for our lab protocol treatment (1.4‰ for $\delta^{18}O$ and 0.3‰ for $\delta^{13}C$) suggest that only 8% (n=2) of the between-tooth oxygen differences and 28% (n=7) of the carbon differences would lead to meaningful differences in the interpretation of the data.[7]

This initial assessment indicates that oxygen and carbon isotope values of enamel carbonate exhibit limited within-individual variation within the same tooth type, regardless of arcade sampled. That said, the existence of interpretable differences for some individuals, particularly for carbon, implies that within-tooth-type variation and its impact on forensic applications warrants further investigation.

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Anthropology; Stable Isotope Analysis; Identification

A43 The Impact of Chemical Maceration on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ Values in Bone Collagen and Bioapatite

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Learning Objectives: After attending this presentation, attendees will be aware of how chemical maceration, the removal of soft tissue from bones, may affect $\delta^{13}C_{coll}$ and $\delta^{15}N_{coll}$ values in collagen and $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ values in bioapatite of bone samples.

Impact Statement: This presentation will impact the forensic science community by demonstrating how commonly analyzed stable isotope systems in bone are altered by chemical maceration. Because maceration is a routine protocol prior to sampling for isotopic analysis, this presentation will assist in determining if stable isotope analysis of bone collagen and bioapatite is suitable for a particular sample.

Abstract Text: Stable isotope analysis of bone collagen and bioapatite provides dietary and geographic information to assist with identification of human remains. Because altered isotope data can provide inaccurate life history inferences, understanding if a process such as chemical maceration alters the isotopic composition of a bone is important to determine if isotopic analysis is appropriate for a particular sample.

This study evaluated carbon and nitrogen isotopes of collagen ($\delta^{13}C_{coll}$ and $\delta^{15}N_{coll}$) and carbon and oxygen isotopes of bioapatite ($\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$) in bone for five chemical maceration treatments adapted from forensic anthropologists and medical examiners: (1) Alconox at 100°C for 24 hours, (2) Biz laundry detergent with sodium carbonate at 90°C for four hours, (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] Five racks of pig ribs from a single farm were obtained. From each rack, two samples were set aside as controls while seven samples were macerated using one of the methods above ($n = 7$ for each method). Experimental rib values were baseline normalized ($\Delta^{X}Y = \delta^{X}Y_{exp} - \delta^{X}Y_{avg,control}$) to the average value of the two controls from the same rack. The largest difference between two intra-individual control samples ("largest control difference") is used as an example of natural intra-individual variation

For both stable isotope systems of collagen, the Greased Lightning method displayed the largest average ($\pm 1\sigma$) shifts ($\Delta^{13}C_{coll} = 0.41 \pm 0.23$ ‰, $\Delta^{15}N_{coll} = 0.12 \pm 0.21$ ‰) and was also the only maceration method to bias $\delta^{13}C_{coll}$ values of macerated samples (all positive shifts). The largest average shift for both stable isotope systems fell below the respective largest control difference ($\Delta^{13}C_{coll} = 0.58$‰, $\Delta^{15}N_{coll} = 0.19$‰).

For $\delta^{13}C_{ap}$ values in bioapatite, average shifts were: $\Delta^{13}C_{ap} = 0.88 \pm 0.17$‰ (Alconox), $\Delta^{13}C_{ap} = 0.18 \pm 0.18$‰ (Biz laundry detergent with sodium carbonate), $\Delta^{13}C_{ap} = 0.45 \pm 0.12$‰ (Greased Lightning), $\Delta^{13}C_{ap} = 0.18 \pm 0.18$‰ (Adolph's with Palmolive), and $\Delta^{13}C_{ap} = 0.45 \pm 0.15$‰ (Tergazyme). All average shifts fall above the largest control difference ($\Delta^{13}C_{ap} = 0.14$‰). For oxygen, the Alconox ($\Delta^{18}O_{ap} = -1.24 \pm 0.46$‰) and Tergazyme ($\Delta^{18}O_{ap} = -1.33 \pm 0.40$‰) methods displayed average shifts in $\delta^{18}O$ values beyond the largest control difference ($\Delta^{18}O_{ap} = 0.74$‰), while the Greased Lightning method displayed the largest variability ($\Delta^{18}O_{ap} = -0.63 \pm 0.80$‰). In general, shifts induced by all maceration methods biased toward more positive $\delta^{13}C_{ap}$ values and more negative $\delta^{18}O_{ap}$ values.

Shifts in $\delta^{13}C_{coll}$ and $\delta^{15}N_{coll}$ values fell within the largest control difference, indicating any effects of chemical maceration were within intra-individual variation and did not meaningfully alter isotope ratio values. Bioapatite was less robust as average shifts in $\delta^{13}C_{ap}$ values for each method were larger in bioapatite than in collagen. Additionally, the Alconox and Tergazyme methods produced relatively large average shifts in $\delta^{18}O_{ap}$ values while all maceration methods biased data toward more negative $\delta^{18}O_{ap}$ values indicating a similar effect across methods. A better understanding of natural variation of $\delta^{18}O_{ap}$ values in pigs is needed to assess if these shifts induced by maceration are meaningful. Overall, carbon and nitrogen isotope analysis of collagen in bone chemically macerated under the parameters of this study are unlikely to meaningfully alter inferences while carbon and oxygen isotope analysis in bioapatite requires caution, particularly for the methods that produced the largest shifts.

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Stable Isotope Analysis; Chemical Maceration; Bone

A44 The Role of Cadaver Decomposition Islands on Soil Chemistry During Carrion Decomposition: Eco-Forensic Implications in a Southern Nigerian Ecosystem

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Learning Objectives: At the end of this presentation, attendees will understand the role of cadaver decomposition islands and soil chemical properties in Postmortem Interval (PMI) estimation in southern Nigeria with respect to climatic differences.

Impact Statement: This presentation will impact the forensic science community by providing data for research and also for solving homicidal forensic cases.

Abstract Text: PMI is the time of death of an organism based on the body decay changes that have occurred.[1] Different factors affect the decay process of carcasses, especially climatic conditions, biotic factors, soil, carcass size, and age.[2-5] Studying the soil chemical properties could provide a taphonomic approach of carrion decomposition.[6] This study aimed to examine the relationship between soil chemical properties and decomposition timeline of remains in both dry and wet seasons of a southern Nigerian (Port Harcourt) ecosystem.

Using an observational and analytical design, the study observed the PMI of carcasses in dry season (December 2022 to February 2023) and wet season (April 3–18, 2023). A total of six healthy *Sus scrofa domestica* (domestic pigs) were used (three for each season) for this study and weighed between 40–60 kg. The pigs were euthanized using sodium pentobarbiturate injection that was administered intramuscularly as recommended by the Research Ethics Committee of the University of Port Harcourt and placed aboveground upon death confirmation. Decomposition was observed and scored quantitatively using Keough et al. Total Body Scoring (TBS) method based on the morphological appearance of three body regions; the head and neck, trunk, and limbs.[7]

Soil samples related to Cadaver Decomposition Island (CDI) were collected at weekly intervals, air-dried, sieved, and analyzed for chemical properties. Soil pH was determined using the standard water extraction method.[8] Soil exchangeable potassium (K) was extracted with neutral normal ammonium acetate.[9] Exchangeable calcium (Ca), magnesium (Mg), and sodium (Na) were determined using EDTA complexometric titration.[10] Exchangeable Acidity (EA) was determined from 0.1NaCl extracts and titrated with 1.0N hydrochloric acid. Effective Cation Exchange Capacity (ECEC) was determined by summing up total exchangeable bases and total exchangeable acidity. The control soil sample of the study location not affected by CDI (at day 0) was collected. Differences between control and CDI-affected samples at intervals were statistically examined using one-way Analysis Of Variance (ANOVA) and Post hoc test. Level of statistical significance of less than 0.05 was accepted.

Comparing with day 0 for dry seasons, the pH of CDI soil samples increased at day 7 (mean TBS = 16.3), day 14 (mean TBS = 21.3), and day 21 (mean TBS = 26.7). Both Ca and Mg concentrations increased at days 7 and 21 while K levels increased at days 14 and 21. EA levels increased at days 14 and 21 while ECEC values elevated significantly at both days 7 and 21. For wet seasons, pH values of CDI soil samples increased significantly at day 14. Concentrations of K were significantly higher at day 7 (mean TBS = 19.3) and day 14 (mean TBS = 31.3) while Na levels reduced at day 7. The study showed that CDIs significantly influenced soil chemistry as pig decomposition progressed for both seasons, thus serving as a reliable tool for PMI estimation. Furthermore, pH, Ca, Mg, and K levels were better PMI indicators during dry seasons while those of wet seasons were pH, K, and Na.

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CDI; Chemistry; PMI

A45 Reassessing Forensic Significance With Radiocarbon Dating at the Oklahoma Office of the Chief Medical Examiner: A Cautionary Tale

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Learning Objectives: After attending this presentation, individuals will better understand the limitations of assessing forensic significance from observational characteristics of skeletal remains and the risk of misclassifying modern unidentified cases as historic or archaeological. Individuals will also possess information on how radiocarbon dating can provide a more accurate assessment and mitigate misclassification risk.

Impact Statement: This presentation emphasizes the need for scientific evaluation of all remains of questionable forensic significance. These data can be used to advocate for the resources necessary to perform radiocarbon analysis and to facilitate discussions regarding the necessity of destructive testing to ensure that missing persons are not overlooked.

Abstract Text: Per state statute, the Office of the Chief Medical Examiner (OCME) must be informed when suspected human remains are discovered. Historically, the postmortem interval for skeletal cases was evaluated using observational data (e.g., color, texture, and density), combined with informant information and proximity of the discovery location to known cemeteries and archaeological sites. Given the extreme diversity of Oklahoma biomes and the poor documentation for many older cases, the Forensic Anthropology Division (FAD) became concerned that modern unidentified individuals might have been incorrectly classified as not being of forensic interest.

In July 2023, the FAD began reevaluating forensic significance for all cases that had not yet been reburied or put into the repatriation process. Cases exhibiting at least one of the following were classified as historic/archaeological: (1) direct evidence of a non-clandestine interment and/or the presence of non-modern artifacts; (2) artificial cranial deformation not practiced by modern communities; and/or (3) extensive dental wear typical of a pre-contact Native American diet. One hundred nine cases of questionable forensic significance were identified, falling into three overlapping categories: (1) isolated bones, commonly found in or around rivers/bodies of water; (2) remains with no provenience and limited information regarding acquisition, and (3) cases where the discovery circumstances, visual characteristics, and/or exam findings are unclear or conflicting. Between October 2023 and June 2024, 42 cases were reevaluated. Bone characteristics traditionally used to estimate postmortem interval were documented and cortical bone samples (~2g) were submitted to the University of Georgia Center for Applied Isotope Studies radiocarbon accelerator mass spectrometry facility.

Based on the calibrated radiocarbon data, 20 individuals (47.6%) were identified as having died prior to 1650 AD. Based on the population history of Oklahoma, these individuals are assumed to be Native American and will be turned over to the appropriate tribal representative or State Archaeologist for repatriation. Fifteen individuals (35.7%) were identified as dying between 1650 AD and the mid-twentieth century. These individuals include imported anatomical specimens, "trophies/souvenirs" from overseas conflicts, historic burials, and remains of uncertain provenance. These cases will be evaluated on a case-by-case basis and may require additional investigation or testing before final disposition. Seven medicolegal cases (16.7%) were identified: two suspected forensic cases confirmed to be modern and five cases (11.9%) previously misclassified as "not significant" in the 1990s that are likely modern unidentified persons.

Investigations have been reopened for these newly identified forensic cases. However, efforts are hindered by the loss of original personnel and investigative documentation. Given the likelihood of additional unidentified individuals in our possession, the OCME expects to continue testing remains of uncertain significance and has updated the evaluation processes for new cases to include radiocarbon analysis. Based on the findings from the first year of this project, the OCME strongly urges other medicolegal agencies and forensic anthropologists to use caution when visually assessing skeletal remains and to consider radiocarbon testing as part of the scientific evaluation of forensic significance.

Forensic Significance; Radiocarbon Dating; Anthropology

A46 The Biocultural Dynamics of Postcranial Skeletal Variation: A Focus on Ontogeny, Socioeconomic Status, and Population History

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Learning Objectives: After attending this presentation, attendees will understand the potential biocultural contributory forces of postcranial Macromorphoscopic (MMS) traits. Age at time of appearance and sociocultural factors are investigated at various stages of growth and development throughout childhood and well into adulthood, exploring how they may account for skeletal differences.

Impact Statement: This presentation will impact the forensic science community by illustrating how technological advances, such as Computer Tomography (CT) databases, can allow researchers to understand the human skeleton from a more biocultural perspective. Utilizing the New Mexico Decedent Image Database (NMDID), associated metadata are paired with individuals to assess whole body CT scans to explore the embodiment of sociocultural factors on skeletal biology. Understanding the impact of biocultural factors will allow attendees to better understand the various pillars of the biological profile in forensic anthropology through the lens of postcranial MMS traits.

Abstract Text: The human skeleton offers insights into an individual's lived experience, which can be quantified as morphological changes brought about by intrinsic and extrinsic forces acting on aspects of human variability. This interplay can leave imprints on the human skeleton in a patterned (and thus predictable) way, reflecting environmental adaptation and the embodiment of an individual's social history over time, especially at their youngest age.

Considering growth rates and timing, particularly relative to developmental processes and puberty, this project utilizes a model designed to capture, measure, and quantify the age-of-appearance/age-of-stability of postcranial MMS traits. Age is a significant contributing factor in the ontogeny of postcranial traits, particularly when focusing on puberty and age-of-attainment for adult morphology. In this sample of juvenile individuals, sex and social race, in tandem, do not seem to contribute to differences in trait manifestation. These results suggest postcranial MMS traits should not be collected from individuals who have not reached puberty for estimation of an individual's population affinity or sex. The stabilization of traits occurs at distinct points in time; puberty is the best indicator of stability for most of these traits.

The embodiment of the health-wealth gradient and social racism are explored from an osteological perspective. Impacts of sociocultural factors—social race, Socioeconomic Status (SES), and education—are modeled to examine whether these influence postcranial skeletal morphology. Studying social categories in relationship to biology and forensic anthropological approaches to population affinity is important, especially when studies confound race and SES-related health disparities. Patterns of embodiment of structural racism can be correlated in various postcranial MMS traits as variation within and between social races and SES and/or education. Three traits show significant differences between social race alone. This does not mean that social race is biological in relation to these three traits, rather there should be further exploration of all postcranial traits in an evolutionary model related to geographic ancestry, climate, genetic flow, and genetic drift.

Overall, this presentation's aim is to better understand what influences the variability of skeletal morphology. Whether that is evolutionary processes of climate impacts and migration correlated with geographic ancestry or structural violence influencing the health of groups of individuals and, thus, the production (or lack thereof) of bone in certain patterns, this research shows that there is a call for a more comprehensive approach of mixed-methods to incorporate more multivariate, refined, multiregional approaches to understand the full picture of human variation across the human skeleton.

Anthropology; Biocultural Theory; Postcranial Skeleton

A47 Antemortem Tooth Loss as a Biomarker of Structural Vulnerability in a New Mexico Forensic Sample

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Learning Objectives: After attending this presentation, attendees will understand how structural vulnerability approaches can enable the recognition of population-level patterns in forensic casework.

Impact Statement: This presentation will impact the forensic science community by showing one such pattern—the correlation between poverty and tooth loss—and arguing for its redress.

Abstract Text: The theory of structural vulnerability builds on the idea of structural violence—that inequitable social systems can themselves enact violence against human bodies—to describe how some individuals may be particularly vulnerable to (i.e., at greater risk for) negative health outcomes. In any society, the differing circumstances under which people are born, grow, live, and work can produce health inequities; those inequities are structured by that society's institutions, policies, and belief systems, often along social and economic fault lines like class, gender, race, and sexuality.

Structural vulnerability has been deployed in clinical medicine to direct attention toward the upstream, societal-level factors that produce poor health outcomes, rather than the biological and behavioral factors that are often perceived to be individual choices.[1] Recently, forensic anthropologists have proposed that viewing skeletal and dental variation through a similar lens would enable the aggregation of population-level data revealing socially produced patterns of suffering that could be reported to the state.[2-4]

Periodontal disease is one area where such socially structured patterns emerge, with previous anthropological researching linking it with experiences of marginalization among Mexican and Central American migrants to the United States and White Americans living in poverty in Eastern Tennessee.[5,6] This study proposes that correlations between poverty and Antemortem Tooth Loss (AMTL) will also be evidenced in a forensic sample from New Mexico.

A sample of 350 identified, anonymized forensic case decedents was selected from the New Mexico Decedent Image Database (https://nmdid.unm.edu), an open-access repository of CT imagery. All decedents in the sample were contextualized with antemortem demographic data by their next of kin, including age, gender, race, and Socioeconomic Status (SES). The sampling strategy was to select 25 age-matched low-SES and high-SES females and males who identified as Black, Hispanic, Native American, and White; however, due to low numbers of Black and Native females whose next of kin provided contextual data, these categories were not fully populated. Age-matched, roughly equal samples were amassed for the remaining groups (n=25 for Hispanic and White individuals and Native males; n=24 for high-SES Black males; n=18 for low-SES Black males).

All software used in this study was open source. Each individual's CT images were cleaned using DicomCleaner and analyzed using 3D Slicer. Two-dimensional slices and the 3D volume renderings created from them were scored for AMTL. The *R* statistical language was used to run non-parametric Wilcoxon signed-rank tests to assess differences in distributions of scores between low- and high-SES groups within each gender and race category.

For Black males, Hispanic females and males, and White females and males, median AMTL was higher in low-SES individuals (9.5, 8, 4, 6.5, and 6, respectively) than in their age-matched high-SES counterparts (1, 2, 2, 0, and 0, respectively). These differences were statistically significant for Black males ($p=0.0006$), White females ($p< 0.0001$), and White males ($p=0.04$). High-SES Native males exhibited more AMTL than their low-SES counterparts (median of 4 versus 1.5), but the difference was not statistically significant. When edentulism was considered, this pattern was reproduced, with most low-SES groups exhibiting higher frequencies of complete AMTL (low-SES BM 17%, HF 16%, HM 8%, WF 13%, WM 25%; high-SES BM 4%, HF 0%, HM 0%, WF 0%, WM 8%).

The power of a structural vulnerability approach is that, because the problems it highlights are socially created, they are also ultimately solvable. Poor dental health is linked with poor physical and mental health, but the poor dental health repeatedly evidenced among people living in poverty is solvable with changes to social policy and medical infrastructure. This research contributes to a growing body of scientific data that can inform social change.

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Forensic Anthropology; Oral Health; Social and Structural Determinants of Health

A48 Mapping Oahu's Cold Cases: Evaluating the Role of Structural Barriers in Missing and Unidentified Cases Through Geospatial Analysis

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WITHDRAWN

A49 The Impact of Substance Misuse Behaviors on Skeletal Physiology and Premature Skeletal Senescence in an Autopsy Population

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Learning Objectives: This presentation demonstrates variable effects of substance misuse behaviors on skeletal physiology and aging. The study tests the hypothesis that effects on the noradrenergic system are consequential to skeletal physiological function and are patterned by substance of choice. Attendees of this presentation will have a better appreciation of the importance of underlying physiological processes affected by human behavior, and the resulting skeletal morphological variation.

Impact Statement: Forensic anthropologists have traditionally focused on skeletal morphology to estimate the chronological age of an individual with little attention given to the interaction of environment and biology influencing skeletal phenotype. The results of this study suggest that skeletal physiological function, particularly related to the noradrenergic system, is patterned by drug(s) of choice and affects bone quality and rate of skeletal aging.

Abstract Text: In the past ten years, the United States has experienced an unprecedented rise in drug-related morbidity and mortality. Simultaneously, important advances in research on the endocrine functions of the skeletal system have occurred. While superficially unrelated, both are critically important to forensic anthropology. Skeletal tissues like bone marrow adipose tissue (bmat) have recently been discovered to have osteoclastogenic and insulin-resistance properties.[1] Furthermore, accumulation of bmat near nerve fibers innervating bone correlated with Sympathetic Nervous System (SNS) activity has also been observed in murine models.[2] Increased SNS tone also promotes release of norepinephrine, which stimulates osteoclastogenesis in addition to its effects on blood sugar levels.[3,4] Since select drugs induce their subjective effects (the "high") by variable influences on release and/or reuptake inhibition of norepinephrine, serotonin, and dopamine bmat volumes (and bone quality by proxy) should also vary by substance use behaviors.

Volumes of bmat and bone marrow in the sternal body were calculated from postmortem Computed Tomography (CT) imaging data for 60 adult males (mean = 39.48 years; range 18-75 years) who received a postmortem exam at the Western Michigan University Homer Stryker M.D. School of Medicine (WMed) in Kalamazoo, MI. Tissues were isolated and volumes calculated following Suchacki et al.[1] Ratios of bmat to bone marrow volumes were used to control for size variation. Volume ratios were evaluated by drug of choice as indicated in the death investigation materials and include alcohol, cocaine, methamphetamine, opioids, opioids and methamphetamine, and a control group. The opioids, cocaine, and methamphetamine groups had the lowest values. Interestingly, the group that concomitantly used opioids and methamphetamine had the highest relative bmat volumes. A Kruskal Wallis test of ratio values by group was significant ($p = 0.00004$). And a Dunn test of pairwise differences with a Bonferroni correction resulted in multiple significant comparisons. The opioids and methamphetamine group had significantly higher ratios (relatively more bmat) than the cocaine ($p = 0.0037$), opioids ($p = 0.0041$), and control (0.05420) groups. Both opioids and methamphetamine individually affect noradrenergic system function, but the apparent cumulative effects of chronic exposure to both on bmat accumulation and skeletal physiological function is unclear.

Skeletal senescence was tested using the fourth sternal rib end as an age indicator. An autopsy population at WMed and donors from the WMed Skeletal Series were sampled and scored following Hartnett.[5] The mean age at transition from one phase to the next was evaluated for the collapsed substance misuse group (all substances; n=126) and control group (n=76) using transition analysis. As expected, differences between groups were more pronounced in older adults. The probability that an individual at a specific age would be in a particular phase was calculated from the cumulative probit model following Konigsberg.[6] Results indicate premature skeletal aging in the substance misuse group. For example, the probability of a 40-year-old in the control group being in phase 4 (43.3%) is higher than the probability of being in a later phase (35.7%). For the substance misuse group, the probability of being in phase 4 (31.6%) is lower than the probability of being in a later phase (50.0%). Therefore, a 40-year-old person with a substance misuse history has a higher probability of exhibiting advanced skeletal aging than someone with no misuse history.

The results of this study highlight the importance of understanding how skeletal physiological function is affected by human behavior with respect to drug and alcohol use. Premature skeletal senescence correlated broadly to substance misuse is also demonstrated. These data are compelling and should motivate forensic anthropologists to question the mechanisms influencing the morphological variation that age estimation methods are based upon.

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Substance Abuse; Bone; Age Estimation

A50 Establishing a Skeletal Atlas of Elder Abuse: A Bayesian Examination of the Complex Relationships Between Age, Sex, and the Probability of Abuse or Accident as Related to Skeletal Trauma

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Learning Objectives: After attending this presentation, attendees will be aware of the associations between decedent age and sex among elderly individuals (65+) and the probability their skeletal fractures noted at autopsy are due to abuse/neglect or a witnessed accidental fall.

Impact Statement: This presentation will impact the forensic science community by further describing the skeletal manifestations and patterning of elder abuse, contributing data to the ongoing effort to unmask elder abuse at a state and national level.

Abstract Text: Increasing rates of elder abuse in the United States produce an urgent need for improved diagnostic criteria.[1] Physical abuse represents the most severe manifestation of this trend, yet is difficult to prove.[2] Skeletal expressions of abuse offer key indications of inflicted injuries, but may be 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] Previously, we identified a significant difference in skeletal patterns of injury between those individuals involved in accidental falls and those for whom abuse/neglect is suspected, as well as differences in fracture patterning in non-ambulating individuals in both categories.[4,5]. We examined differences in osteoporosis between these categories and found a disproportionately high frequency of osteoporosis among individuals sustaining fractures in accidental falls.[6] Here, we further explore the skeletal patterns of abuse vs. accidental falls in cases from the Maricopa County Office of the Medical Examiner by examining age and sex as potential predictor variables.

Investigative summaries of 388 individuals over the age of 65 who sustained skeletal trauma were included in the study (ca. 2014–2021). From these, 64 individuals were included as representative of cases of abuse/neglect based on the following criteria. Decedent must: (1) have been admitted for exam, (2) be associated with an Adult Protective Services and/or law enforcement investigation, and (3) have radiographs/samples available for review. Given these strict criteria, this sample is known to be an underestimation of cases of abuse/neglect. Three hundred eighty-four cases of witnessed ground-level falls from the same pool were reviewed.

In sum, results reveal an age effect in the skeletal manifestation of injury for individuals involved in ground-level accidental falls, but not for those where abuse is suspected. There is no sex effect on either category.

We examined age associations by performing logistic regression in which the decedent's age was treated as a predictor variable for the binary dependent variables, having experienced skeletal fracture associated with documented or suspected abuse (model 1) or witnessed accidental fall (model 2). The results of model 1 suggest no age affect among victims of abuse (SlopeAbuse = -0.01346, SEM = 0.012, p=0.285). The results of model 2 suggest a significant positive relationship between age and the probability of a skeletal fracture due to an accidental fall (SlopeAccident = 0.03026, SEM = 0.014, p=0.029). To further examine the relationship between abuse and biological sex, we performed Bayesian multilevel logistic regression models. Models 3 and 4 examine the random (sex) and fixed (age) effects abuse (model 3) and accidental (model) breaks. The results are consistent with the logistic regression.

Bayesian Models 5 and 6 examine the fixed effect of *sex* (model 3) and accidental (model) breaks while treating *age* as a random effect and examine incidents of abuse and accidental falls respectively. The results of model 5 suggest no fixed sex effect (SlopeSex = -0.35, 95%CI: 0.29, 0.93). Similarly, the results of model 6 suggest there is no age effect with the probability of Accidental injuries (SlopeSex = 0.25, 95%CI: -0.34, 0.86).

Fractures remain the most common musculoskeletal condition requiring hospitalization among individuals aged 65+ in the United States, and rigorous diagnostic criteria must be developed to differentiate accidental injury from abuse.[7] Data that are easily obtained as part of a death investigation—sex and age—are here found to be significant variables in diagnostic criteria and could provide further probabilistic weight to a diagnosis when considered in light of the results described. 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; Skeletal Trauma; Age and Sex

A51 The "Missing Migrant": Schrodinger's Cat of Legal Status and Applicable Frameworks

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Learning Objectives: After attending this presentation, attendees will: (1) gain a more comprehensive understanding of some of the main issues caused by the lack of a clear legal definition for the "missing"; (2) learn how and why "missing migrants" in particular fall through the cracks of existing legal frameworks both at national and international jurisdictional levels; and (3) discover how a global redefinition of the status of "missing" could help countless individuals and families worldwide.

Impact Statement: This presentation will impact the forensic science community by raising awareness about how the lack of a defined status for the "missing" (a widespread, yet seldom-discussed issue) impacts numerous aspects of the search process and practitioners' ability to resolve cases, as well as directly impacting the family of those missing. Through gaining a more comprehensive understanding of these issues, the forensic science community can begin to advocate for changes to the existing legal frameworks so that fewer missing individuals fall through the cracks in the future.

Abstract Text: "Missing migrants," as a demographic, epitomize a legal conundrum reminiscent of Schrödinger's cat: neither definitively alive nor dead until their fate and whereabouts are determined. Slipping between the cracks of existing legal frameworks, missing migrants are engulfed in a perpetual state of legal ambiguity because significant elements of their status are only determinable through the discovery of their body (alive or dead).[1] This paper makes three main arguments that seek to redefine how the "missing" are recognized across international and domestic legal frameworks.

First, this paper identifies that several legal regimes may apply to the missing. Identifying the applicable law requires determining status, and status cannot be determined without positive identification. As such, initiation of the search process might be inhibited or prevented due to unclear frameworks leading to systemic accountability gaps.[2]

Second, this paper argues that a universally recognized legal status for the "missing" is necessary to ensure families have access to their fundamental rights and that states uphold their obligations to search for the missing and identify the dead.[3-5] In law, being "missing" is not generally a distinct legal status. Factually, however, being "missing" has consequential effects. If a person's status is unknown, even if they are presumed dead, no death certificate can be legally issued. This causes families additional harm, including: legal problems related to the unclear status (alive/dead); impacting their ability to access social assistance and fundamental rights; and preventing emotional closure due to the prolonged trauma of ambiguous loss.

Third, this paper argues that there is a need for more comprehensive search processes and a broadening of the acceptable parameters for legally determining a missing individual's fate and whereabouts.[1,2]

This paper will discuss how the overlapping legal frameworks applying to migration, missing persons, and treatment of the dead can lead to different legal obligations dependent upon an individual's status. Domestically, laws governing border control are often inconsistent with international legal frameworks such as International Human Rights Law (IHRL), Refugee Law, and the Law of the Sea (LOTS).[6] As concerns the missing, under IHRL, states have a procedural obligation to investigate disappearances within their jurisdiction. When International Humanitarian Law (IHL) applies, this extends to heightened duties toward the missing and deceased.[5] In both cases, when at sea, obligations related to rescue and assistance apply. These complexities are more sharply pronounced again in disaster situations, especially when natural disasters occur alongside armed conflicts.

Overall, this paper suggests redefining how the "missing" are currently defined and recognized in law. A proposed legal definition for the "missing" as a unique category of person would aid in clarifying which state obligations toward the missing and the dead should be applicable in all situations. This necessary paradigm shift would allow for more equal protections and access to fundamental rights for the missing, the deceased, and their families, in all contexts.

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Missing Persons; Migrants; Search and Recovery

A52 A Brief Introduction to Artificial Intelligence in Forensic Anthropology: Core Concepts and Ideas

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Learning Objectives: After attending this presentation, attendees will have a better understanding of why and how Artificial Intelligence (AI) has come to be integrated in contemporary forensic research. Attendees will also be introduced to key terminology, methodology, and best practices related to AI utilization, including a brief case example that highlights using AI in forensic contexts.

Impact Statement: This presentation will impact those developing novel research and identification methods within the forensic sciences. With this, we intend to highlight the utility of multidisciplinary research that is, currently, not widely incorporated into forensic anthropology. Methods incorporating AI have a demonstrated potential to increase the accuracy and reliability of many existing methods. This presentation will provide a succinct introduction to this likely unfamiliar mode of research and data analysis to help better inform the forensic community.

Abstract Text: Machine learning and AI have seen rapid growth in their development since the turn of the century. A rise in personal computing power has allowed complex processing to be completed in minutes and become integrated in daily life, where these tasks used to be limited to supercomputers. In tandem with this, the sciences have entered an era of "big data," where these same advancements in personal computing are used to curate large amounts of data. Curation of big data allows scientists to analyze large quantities of data with robust algorithmization to help answer novel research questions or create predictive models with high accuracy, reliability, and resolution. While methods incorporating AI and machine learning have shown promise, they have primarily been integrated into electrical engineering and computer science. Forensic anthropologists have not routinely tested these methods, putting the field at risk of falling behind in the current technological revolution. A handful of forensic anthropologists have begun to explore AI methodologies in recent years; however, there is still much debate about the utility, ethics, and operationalization of these techniques.

This presentation will provide a brief introduction to AI history and terminology, highlighting the nuanced differences between AI, machine learning, and deep learning. We will also explore how each can be uniquely incorporated into forensic research and training. Open-access resources that can allow any forensic researcher to incorporate AI into their research design regardless of background or funding will also be introduced. Best practices for utilizing AI within forensic anthropology will also be suggested with full transparency in methodology and algorithmization being encouraged for all forensic scientists, particularly when utilizing "black-box" methodology where the exact reasoning for algorithm-made decisions is unknown.

The presentation will conclude with a discussion surrounding the ethical concerns that have been raised surrounding AI-based research, particularly surrounding classroom usage, data curation, and "black-box" methodology. While acknowledging that the best ethical practices for utilizing AI methodology will take time to develop and implement, it is essential to highlight that there is utility in these methods to better serve individuals and communities, necessitating forensic anthropologists' involvement. The open-access nature of many machine learning and AI toolkits is also an advantage to underprivileged individuals who may lack access to much of the specialized software (e.g., MatLab, IBM SPSS) currently used to develop methods and predictive models. Ultimately, AI will continue to be developed and incorporated into nearly every field, and a base understanding of methods related to AI will soon become a necessity.

Artificial Intelligence; Machine Learning; Method Development

A53 3D Biological Profile: The Application of 3D (STL) Models of Skeletal Remains to Assist With Human Identification

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WITHDRAWN

A54 Using Artificial Intelligence to Automate Human Stage of Decay Identification

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Learning Objectives: After attending this presentation, attendees will have a better understanding of how Artificial Intelligence (AI) can be applied to the field of forensic anthropology. Specifically, attendees will learn about the feasibility of using AI techniques to automate human stage of decay identification methods from digital photos.

Impact Statement: This presentation will impact the forensic science community by demonstrating an approach that utilizes AI, specifically deep learning methods, to automate human stage of decay identification methods. In doing so, it leverages a large-scale archival collection of human decomposition photos.

Abstract Text: Determining the stage of decay is an important and common task in human remains cases. Knowing the degree of decomposition is vital for estimating the postmortem interval and identifying human remains. Currently, labor-intensive manual scoring methods are primarily used to establish the stage of decay of a decedent. These methods, which rely on subjective interpretation made by humans, are more susceptible to bias and error, consequently affecting the accuracy of downstream tasks, such as estimating the postmortem interval. Additionally, due to their labor intensity, these methods do not scale well for the emerging large-scale archival collections of human decomposition photos. Therefore, this study explores the feasibility of automating two common human decomposition scoring methods proposed by Megyesi et al. and Gelderman et al. using AI.[1,2]

Evaluated were two popular deep learning model architectures, such as Inception V3 and Xception, by training them on a large dataset of human decomposition images to classify the stage of decay for different anatomical regions, including the head (and neck), torso, and limbs (including the hands and feet).[3,4] Additionally, an interrater study using the Fleiss' Kappa statistic as the evaluation metric was conducted to assess the reliability of the developed AI models compared to human forensic examiners for stage of decay identification. The Xception model achieved the best classification performance, with macro-averaged F1 scores of .878 for the head, .881 for the torso, and .702 for the limbs when predicting Megyesi et al.'s stages of decay, and .872 for the head, .875 for the torso, and .76 for the limbs when predicting Gelderman et al.'s stages of decay.[1,2,4] The interrater study results supported AI's ability to determine the stage of decay at a reliability level comparable to a human expert. This work demonstrates the potential of AI models trained on a large dataset of human decomposition images to automate stage of decay identification.

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Anthropology; Artificial Intelligence; Machine Learning

A55 Forensic Comparative Radiography Using Transformer-Based Neural Networks and Large Language Models

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Learning Objectives: After attending this presentation, attendees will understand how advanced neural-network transformers and large language models can be applied to scientific identifications via comparative chest radiography.

Impact Statement: This presentation will benefit the forensic community by presenting a unique approach to scientific identifications utilizing a Vision Transformer and a Large Language Model that can assist in comparing antemortem and postmortem chest radiographs for mass fatalities, missing persons databases, or routine forensic casework, while reducing practitioner bias

Abstract Text: Scientific identification is an integral component of forensic investigations, ensuring accurate death certification and that the correct remains are returned to loved ones. Forensic anthropologists routinely aid in scientific identifications utilizing comparative medical radiography. In typical forensic casework, antemortem medical imaging of the presumed identity is provided for comparison with postmortem medical imaging of the decedent to assess whether the images originated from the same individual. However, practitioners may be implicitly biased to believe that the antemortem medical imaging should match the postmortems. This research aims to develop an AI-assisted approach to comparative radiography to: (1) reduce practitioner bias; (2) allow for applications to mass fatalities, unidentified persons databases, and typical forensic casework; and (3) provide an explanation of the decision process.

Previous research demonstrated the utility of deep Convolutional Neural Networks (CNN) models to automatically generate a ranked list of chest radiographs that most closely match the input chest radiograph image.[1] A rank-1 accuracy of 92.38% was obtained when utilizing the entire chest X-ray, meaning that in 92.38% of the cases, the true match was ranked first on the list of potential matching identities. When an ensemble method combining the feature vectors from different models pertaining to different regions of interest was used, an accuracy of 94.08% was achieved. Despite the success of the CNNs, there were two notable limitations: (1) lack of explainability—it was not possible to ascertain the specific features the models were using to compare radiographic images to generate the ranked lists; and (2) absence of attention modules—the relationship between patches in the images was not explicitly exploited in the CNN framework.

To address these limitations, a new approach was explored utilizing Vision Transformers (ViTs), a neural network architecture that uses the so-called "attention module" to automatically determine correlated regions in an image.[2] Further, we combined this with a Large Language Model (LLM) to provide a descriptive output of the features utilized to compare radiographs.[3] The goal is to improve the accuracy of the comparison process while imparting explainability to the decision process.

A large sample of 5,165 anonymized chest radiographs of 760 individuals from casework from the Michigan State University Forensic Anthropology Laboratory and the National Institutes of Health (NIH) Chest Xray Dataset were used. The sample was split into training (n = 3,289 radiographs) and testing (n = 1,876 radiographs) subsamples. Two ViT-based models with a patch size of 16 and 32 were trained on complete chest radiograph images. Preliminary results demonstrate that the patch 16 model has an 84.21% rank-50 accuracy, the patch 32 attains 80.43% accuracy, and the ensemble has the highest accuracy with 84.75%. These promising results show the power of ViTs, whose performance is expected to improve with increased sample sizes and training. In addition, a preliminary experiment involving an LLM demonstrated its ability to analyze and describe the constituent images using medical terminology, thereby providing additional evidence about the similarities and dissimilarities in the shape, structure, and texture between the postmortem and antemortem images.

Our ongoing work involves integrating the ViT with the LLM in order to develop an automated system to assist practitioners in comparative radiographic identifications and provide rationale for the provided matches. The use of such a system could facilitate faster decedent identification in cases of mass fatality or could be used to identify decedents in large unidentified databases by decreasing the potential identity pool.

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Anthropology; Positive Identification; Artificial Intelligence

A56 Applying Machine Learning Methods to Age Estimation in Forensic Anthropology—From Glass Box to Black Box

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Learning Objectives: After attending this presentation, the attendees will learn the difference between explainability and interpretability in Machine Learning (ML) for forensic anthropology and how glass and black box ML methods are currently applied to age estimation in forensic anthropology. The attendees will gain an understanding of using 3D medical image files in ML for forensic anthropology.

Impact Statement: The presentation will provide the forensic science community with an understanding of the differences between explainability and interpretability in ML for forensic anthropology. The presentation will present applied examples of how medical images can be used in ML for biological profile estimations in forensic anthropology.

Abstract Text: The application of ML and Artificial Intelligence (AI) within the forensic science discipline has increased in recent years. The rise of ML-focused research has led to concerns and discourse over algorithmic opacity and transparency issues in governmental reports and literature. The application of new technologies in forensic anthropology is still relatively new, with ML in adult age estimation relatively unexplored.

The use of ML in forensic anthropology and biological profile estimation is, however, not new and has been increasing in recent years. ML algorithms vary in mathematical complexity, which impacts upon the understandability of their outputs.

This presentation will provide an overview of what interpretability and explainability are, and how these concepts relate to algorithmic opacity, understandability of output, and calls for transparency in forensic science. This will be showcased using results from three ML studies investigating the feasibility of using 3D ML for adult age estimation in the living in forensic anthropology. In this research, 3,093 CT studies containing the pelvis from University College London Hospital were converted to NIfTI file format to create a training, validation, and testing dataset to develop ML models varying in algorithmic opacity (glass box to black box). The ML algorithms used include Decision Tree (DT), Random Forest (RF), and Convolutional Neural Network (CNN), and a "voxel-feature" approach was used on the health care-derived dataset. The "voxel-feature" approach solely utilizes the individual voxels of the 3D pelvic NIfTI image to train 3D ML models, avoiding the use of nonmetric scoring.

The results of these studies confirms the feasibility of this approach to the pelvis and across three types of ML models from glass-box (DT) to black box (CNN), which was previously shown feasible by Joshi and Tallman in NIfTI files of the postmortem skulls using CNN.[1] In addition, the results indicate that classification accuracy increases as the ML algorithm become more complex and opaque.

Using the results, this presentation will discuss future responsible applications of glass-box and black box ML algorithms; raise questions on how algorithmic opacity can and should be related to the needs of transparency in forensic science; highlight potential ethical issues of using health care-derived data in ML model development for forensic science and by extension forensic anthropology

The insights gained from this research contribute to the ongoing discourse on the responsible development and application of AI in forensic contexts, highlighting the importance of open dialog that considers ethics, application contexts, and interpretability of ML and AI tools in forensic science to build trust and effectiveness of science evidence in the justice system.

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Machine Learning; Age Estimation; Transparency

A57 The Potential Applications and Uses of Neural Radiance Fields (NeRF) and Gaussian Splats: Artificial Intelligence in Forensic Anthropology

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Learning Objectives: After viewing this presentation, attendees will gain an understanding of the inner workings of Neural Radiance Fields (NeRF) and Gaussian Splats, their relationship to Artificial Intelligence (AI), and how they can be applied to projects and technologies relevant to the forensic anthropology community.

Impact Statement: This presentation will impact the forensic science community by highlighting how utilizing NeRF and Gaussian Splats technology can benefit forensic anthropologists.

Abstract Text: NeRFs and Gaussian Splats are emerging types of imaging that utilize AI to rapidly convert Computed Tomography (CT) scans into Augmented- and Virtual Reality-compatible (AR/VR) 3D representations. The rapid automated conversion process made possible by these AI technologies allows forensic researchers to create 3D AR/VR images from CT, significantly cutting down lengthy processing times, while reducing the manual manipulation that introduces bias and inconsistency into the data.

NeRF AI technology allows for the creation of AR and VR 3D meshes that can be used to reconstruct fractured and fragmented remains with minimal manual processing. Gaussian Splats, a more recent development, offer a complementary approach by representing 3D scenes as collections of oriented 3D Gaussian functions, providing faster rendering and potentially higher quality results in some scenarios. From a forensic anthropological standpoint, these technologies allow for the manipulation of fragile, burned, and fragmented remains while minimizing the possibility of damaging bony evidence due to overhandling. Bony elements can then be reconstructed within the virtual space, making it easier to determine traumatic injuries, such as gunshot wounds or blunt force trauma, that are not instantly detectable due to the fragmentary nature of the remains. Additionally, assuming the join is undamaged, and the bone is not warped, reconstruction using these AI technologies may also make measurements of skeletal elements possible.

NeRF AI and Gaussian Splats can be built into computer applications that convert CT scans of fragmentary skeletal elements into 3D AR/VR representations designed to aid in reconstruction. Here, the authors examine how these AI technologies can benefit forensic anthropologists via Cranial Tool AR, a computer application they designed that utilizes LUMA AI NeRF and Gaussian Splats as a means of reconstructing fragmented craniofacial images so they can be reconstructed in AR to assess the cranial and facial skeleton for signs of trauma as well as aspects of the biological profile, such as biological sex and population affinity.

In this case, craniofacial CT scans were imported into 3D Slicer to represent the scans as 3D surface volumes, which were then captured via videoframe recording and uploaded into Luma AI NeRF and a Gaussian Splats processing pipeline. The AI programs processed the videoframe captures as NeRFs and Gaussian representations, respectively, and exported them as 3D representations of the fragmented craniofacial remains that could be used in craniofacial reconstruction, trauma assessment, and analysis of the biological profile. These representations were imported into Cranial Tool AR, which utilizes the 3D gaming engine Unreal Engine to render them into manipulable holograms.

While there are other ways of employing CT scans, such as more traditional manual selection non-AI workflows, the authors found that utilizing NeRF and Gaussian Splats AI decreased processing time from a matter of hours to one of minutes, reduced workflow complexity related to creations of these images, and made it simpler for non-specialists in 3D Slicer to use, all while rendering highly precise images of the craniofacial fragments. Both NeRFs and Gaussian Splats produce 3D representations with higher levels of fidelity when it comes to the intricacies of the details captured by a CT scan, meaning the fracture margins produced by these AI technologies provide a more accurate picture of each fragmented element.

The integration of Gaussian Splats alongside NeRFs offers additional benefits, such as faster rendering times for real-time interaction, potentially improved preservation of fine details, and more efficient storage of large datasets. This combination of technologies provides forensic anthropologists with a versatile toolkit for analyzing and reconstructing fragmentary remains, enhancing the accuracy and efficiency of their work in both research and practical forensic applications.

Anthropology; 3D Technology; Artificial Intelligence

A58 Machine Learning and Artificial Intelligence in Forensic Anthropology: Ethical Considerations, Challenges, and Future Applications

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Learning Objectives: After attending this presentation, the attendees will learn about the development of technology in forensic anthropology, specifically the use of Machine Learning (ML) algorithms and Artificial Intelligence (AI) using human remains. The attendees will learn how the use of any technology involving human skeletal remains poses challenges related to ethics, privacy, consent, and the potential for dehumanization in addition to the risk of bias within algorithms. The attendees will gain an understanding of the importance of glass-box algorithms and explainable AI models in the development of new and existing methods within forensic anthropology. Attendees will also learn how to responsibly apply technological solutions to address some of the existing challenges within forensic anthropological assessments and interpretations.

Impact Statement: The presentation will provide the forensic science community with an understanding of ethical and responsible use of ML algorithms and the differences between glass-box and black-box approaches. The presentation will provide the community with applied examples of how ML algorithms have been utilized across other domains, including medicine. The presentation will also explore how statistical modeling, ML, and AI algorithms could be part of one entity with human decision making, not replacing, but by extending the utility of experts.

Abstract Text: With technological developments, there has been increased incorporation and application of AI and ML algorithms within forensic science.[1] Research within this field has focused on the expansion of new automated approaches to some of the methods used, showing promising results in efficiency and accuracy across different forensic identification fields. In forensic anthropology, the utilization of ML algorithms to the estimation of a biological profile has begun to focus on the application of AI and Deep Learning (DL) in classification decisions through imaging.[2] However, the integration of AI/ML also raises critical ethical and legal concerns. While current literature has shown that it is possible to utilize the approaches, there is also a notion that the accuracy and performance of the algorithms currently used are relatively complex with varying degrees of explanation accuracy.[3] The need for transparent and explainable models is akin to the call for transparency in forensic science in terms of understanding the role of the expert and their decision making and the inferences and conclusions drawn from the evidence. Even though the forensic science community has seen the positive attributes of ML and AI across various disciplines, universally there is a need for caution when building and applying these systems within a forensic context.[4,5]

This presentation will provide an overview of the recent developments of ML and AI in forensic science broadly, and forensic anthropology more specifically. The presentation will highlight some of the key research studies in forensic anthropology where ML and AI algorithms have been used successfully to estimate sex, age at death, and population affinity from modern populations. The presentation will also emphasize some of the key challenges facing the forensic anthropology community for when developing automated models such as biases within the data (systemic biases), the algorithm (statistical/computational biases), or by the user themselves (human biases).

A step-by-step guideline will be presented on what to consider for when utilizing digital osteology for the use of incorporating decedents into ML and AI models. The presentation will discuss the path forward for ethical utility of ML and AI in forensic anthropology and how these systems could contribute to the decision-making process of the expert

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Machine Learning; Anthropology; Artificial Intelligence

A59 Fracture Characteristics of Human Ribs Vary With Intracortical Porosity

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Learning Objectives: After attending this presentation, attendees will understand the importance of considering bone tissue microstructure when investigating sources of variation in skeletal trauma.

Impact Statement: This presentation will impact the forensic science community by showing that cortical structure, and particularly intracortical porosity, is associated with variation in fracture characteristics in the same loading scenario.

Abstract Text: Skeletal trauma analysis often focuses on the description of fracture patterns, while little is known about the mechanisms that influence their variability. Rib fractures are particularly common and important in forensic trauma analysis, so their characterization should extend beyond simple presence or absence. Fracture characteristics of ribs have been shown to vary even within controlled loading conditions. The best predictor of rib structural properties in these bending scenarios is cross-sectional geometry, especially when calculated from a cortex with porosity removed. The objective of this study is to investigate whether variation in rib fracture characteristics is also associated with porosity.

Human midthoracic ribs (n = 189, [males = 118, females = 71], 22–108 years old, mean = 57.6 years) were dynamically loaded to failure in a simplified bending scenario representing a frontal thoracic impact. Rib behavior was classified as brittle (n = 23) or ductile (n = 166), fracture number as one (n = 144) or two (n = 40) fractures, fracture type as simple (n = 133) or wedge (n = 47), and fracture group as simple oblique (n = 41), simple transverse (n = 92), wedge partial (n = 27) or wedge intact (n = 20).[1] Fracture characteristic groups with < 10 ribs (three fractures, incomplete, or multifragmentary fractures) were excluded. Histological cross-sections adjacent to the fracture location were imaged at 100x magnification. A custom ImageJ macro, Pore Extractor 2D, quantified the morphometry of total, cortical, and trabecularized pores, and calculated cross-sectional geometry from each solid cortex and porous cortex.[2] Logistic regressions tested pore morphometry and cross-sectional geometry as predictors of rib behavior and fracture type, group, and number.

Fracture type, fracture group, and rib behavior significantly ($p < 0.05$) varied with pore morphometry, largely due to trabecularized pores, while number of fractures showed no significant association with porosity. Simple fractures showed significantly elevated trabecularized percent porosity ($p = 0.007$, pseudo-R^2 = 9.3%, Simple mean = 4.6 ± 5.2%, Wedge mean = 2.3 ± 2.3%) and trabecularized pore density ($p < 0.001$, pseudo-R^2 = 12.9%, Simple mean = 1.3 ± 0.8 pores/mm^2, Wedge mean = 0.8 ± 0.5 pores/mm^2), compared to wedge fractures. Analysis of fracture group suggested this was driven by simple transverse fractures, as simple oblique fractures also showed increased trabecularization but did not significantly exceed partial or oblique wedge fractures. Rib behavior also significantly varied with pore morphometry, with brittle ribs showing significantly higher trabecularized percent porosity ($p = 0.001$, pseudo-R^2 = 10.0%, Brittle mean = 7.3 ± 8.6%, Ductile mean = 3.5 ± 3.6%), trabecularized pore density ($p < 0.001$, pseudo-R^2 = 14.6%, Brittle mean = 1.8 ± 1.0 pores/mm^2, Ductile mean = 1.0 ± 0.7 pores/mm^2), and mean pore area ($p = 0.009$, pseudo-R^2 = 7.6%, Brittle mean = 0.01 ± 0.01mm^2, Ductile mean = 0.005 ± 0.004 mm^2) compared to ductile ribs. Simple fractures and brittle ribs were concurrently associated with significantly ($p < 0.05$) smaller cortical area and cortical bone distribution. More variation (Nagelkerke pseudo-R^2) was explained in fracture type (pseudo-R^2 = 4.5–10.8%) and behavior (pseudo-R^2 = 11.0–24.3%) when predictors were calculated from a porous cortex, compared to a solid cortex. Total area and external dimensions were weaker or insignificant predictors, suggesting that fracture characteristics are more influenced by cortical area and distribution than cross-sectional size.

Trabecularization of the cortex with large, convergent voids may facilitate the direct path of a simple transverse fracture, rather than deviating obliquely or along multiple paths into a wedge fracture. Weakening the cortex with trabecularization may also minimize the plastic energy absorbed before failure, resulting in brittle rather than ductile behavior.

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Cortical Porosity; Fracture Type; Fracture Behavior

A60 The Application of Forensic Fractography to Thermally Altered Bone

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Learning Objectives: The goal of this presentation is to determine if and how forensic fractography can be applied to thermally altered bone. After attending this presentation, attendees will have a greater understanding about how fractographic features present in a sample of thermally altered human femora and how these features may be used to reconstruct crack initiation and propagation.

Impact Statement: This presentation impacts the forensic science community by addressing a current gap in knowledge about the utility of fractography for analyzing thermally altered bone.

Abstract Text: Forensic fractography of bone was recently introduced as a materials science-based approach for examining and interpreting skeletal fractures.[1] This approach is premised on the concept that fracture surface features reveal information about bone failure such as the origin, direction, speed, and stability of the propagating crack.[2] Fractography has been applied to identify crack origins and evaluate patterns of crack propagation in biomechanically fresh human bone.[1-3] Fractography is a potentially useful tool for analyzing thermally altered bone, which presents various analytical challenges, including complex fracture patterns and fragmentation.

The goals of this exploratory research were to: (1) evaluate the presence of fractographic features observed in the fracture surfaces of thermally altered bone, and (2) assess potential applications of fractography for analyzing fractures in thermally altered bone.

To achieve these goals, 39 fracture surfaces from 37 femora were evaluated for fractographic features. The femora are part of a commingled human remains assemblage from the **Defense POW/MIA Accounting Agency**'s (DPAA's) Tokyo Prison Fire project. Tokyo Military Prison was utilized during World War II to hold prisoners awaiting trial for war crimes. In May 1945, the prison was hit during a nighttime incendiary raid and burned down. It is estimated that 62 United States service members were imprisoned at the time, all of whom perished in the fire.

Fracture surfaces were photographed to facilitate scoring by observers without direct access to the samples and were scored for the following: presence of thermal (dry bone and/or heat induced) fracture; presence of perimortem (wet bone) fracture; presence of bone mirror, bone hackle, and arrest ridges; and direction of crack propagation. Scoring was completed independently by CNH, RTH, and MII and inter-rater agreement was calculated using Fleiss' kappa.

The three observers scored thermal damage present in 94.9% to 97% of fracture surfaces, with fair agreement among observers ($\kappa = 0.223$, $p = 0.0156$). They scored perimortem trauma present in 12.8% to 20.5% of fracture surfaces, with fair agreement among observers ($\kappa = 0.216$, $p = 0.0195$).

Observers identified bone mirror in 38.5% to 74.4% of surfaces, bone hackle in 25.6% to 71.8% of surfaces, and arrest ridges in 38.5% to 53.8% of surfaces. Inter-observer agreement on the presence of bone mirror was fair ($\kappa = 0.218$, $p = 0.0186$). While there was slight agreement among observers for bone hackle ($\kappa = 0.175$, $p = 0.0589$) and arrest ridges ($\kappa = 0.135$, $p = 0.145$), agreement was not statistically significant, indicating the observed agreement is not better than chance. The results of this study contrast with published data indicating higher frequencies of fractographic features and good interobserver agreement when observing fracture surfaces of biomechanically fresh, unaltered human femora.[1,3]

This study highlights challenges in applying fractography to the analysis of burned bone. One challenge was that fracture surfaces evaluated in this exploratory study of thermally altered bone appeared dissimilar to the fracture surfaces reported in fresh, unaltered bone.[1-3] A likely reason is that crack initiation and propagation are more complex in thermally altered bone, which may influence the presence or appearance of typical fractographic traits.

Before fractography can be appropriately applied to the analysis of burned bone, research is needed to understand how fractures form in thermally altered bone and how thermal exposure may alter perimortem fracture surfaces. While current guidelines center primarily on fractographic features in fresh bone, additional guidelines may be needed to help standardize identification of fractographic features in thermally altered bone.[2]

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Forensic Anthropology; Forensic Fractography; Thermal Alteration

A61 Differential DNA Preservation in Soft and Osseous Tissues After Fire Exposure

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Learning Objectives: Attendees will acquire a foundational understanding of the ways in which the material properties of muscle and bone differentially affect tissue burning and how these factors affect DNA preservation within the respective tissue types. This baseline can be operationalized to inform sample selection for use in identification efforts of fire-related fatalities, with specific consideration for the operating abilities of the ANDE Rapid DNA System.

Impact Statement: This presentation will impact the forensic science community by providing data-driven guidance for fire-related disaster victim identification efforts.

Abstract Text: Over the past two decades, the frequency and scale of wildfires has increased dramatically, nearly doubling globally.[1] Wildfire-related deaths have paralleled this global increase, necessitating the development of specialized recovery and identification strategies in response to fire's unique taphonomic process.[2] DNA is often the only means to identify victims of these fire-related fatalities, with rapid DNA technology increasingly replacing traditional DNA for use in wildfire responses. While extensive deployment of this rapid technology in recent fires has demonstrated its efficacy as a Disaster Victim Identification (DVI) tool, no formalized recommendations for sampling exist.[3,4] Anecdotal yield patterns reflect the role that tissue shielding plays in DNA preservation, but the mechanisms are not well understood by forensic anthropologists. Despite this gap in our own field's literature, a large body of literature from other scientific disciplines can be collated to establish a scientific basis for how fire affects tissues. When muscle and bone are understood as distinct materials, with distinct physiological responses to heat, and inherent differences in thermal capacity and conductivity, their material properties can be utilized to develop sampling protocols that are scientifically substantiated.[5-13]

In order to test this assumption, a series of experimental, simulated wildfires were conducted with temperatures recorded at both intramuscular and intraosseous sites for the duration of the burn. Both osseous and soft tissue samples were collected and subsequently tested using the ANDE Rapid DNA System. DNA was successfully amplified for 72.22% of soft tissue and 36.54% of osseous samples, reflecting muscle's material resilience to heat. Notably, where tissue color is frequently used as a proxy for tissue condition, in muscle especially, the color of the sample collected *in situ* bore almost no correlation ($r = -0.179$) with the color of the dissected portion of the sample ultimately used for amplification, providing evidence for tissue shielding. When considering the color of the ultimate dissected portion, muscle performs as expected. Bone color, however, is a less accurate predictor of DNA amplification success, with multiple samples initially assessed to be too calcined to yield having amplifiable DNA, and multiple charred samples failing to yield. These discrepancies between expected and actual yield in bone samples indicate a variable beyond tissue shielding is also likely contributing. Ultimately, results produced were in line with performance expectations as established by the larger body of non-anthropological literature, which contravened the literature from molecular anthropology regarding the performance of degraded DNA samples.

This research serves as part of a longitudinal study contributing to the development of data-driven recommendations for best sampling practices in response to fire-related fatalities and affirms that, when available, soft tissue samples have significantly superior DNA preservation than osseous samples. It positions interdisciplinary research as an invaluable tool to bolster the legitimacy of forensic anthropology practice and establishes a more robust theoretical basis for fire research to maximize efficacy of novel technological interventions for DVI work.

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DNA Preservation; Thermal Damage; Disaster Victim Identification

A62 Re-Evaluating Temperature Data in Human Tissues Exposed to Fire

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Learning Objectives: Attendees will acquire a foundational understanding of temperature changes of osseous and soft tissue exposed to fire. The results of this research demonstrate that tissues (soft and osseous) do not reach the ambient temperature of the fire as quickly as has previously been reported in literature.

Impact Statement: This presentation will benefit the forensic science community by providing intramuscular, intraosseous, and interosseous temperatures for soft and osseous tissues exposed to fire. Our results indicate that tissue color does not strongly correlate with temperature, as previously suggested.

Abstract Text: In fire research, temperature measurements are routinely used to quantify fire behavior in various burn scenarios. For example, temperature measurements give valuable information on the performance of building materials and firefighter equipment, fire spread and patterns in vehicles, and correlation of bone and tissue temperatures with the observed level of thermal damage in forensic fire-death scenarios.[1-5] Most types of temperature sensors would be rapidly destroyed by fire and cannot be used in these environments. Properly selected and utilized thermocouples are an exception, though many models are unsuitable for fire exposure. Recent research has demonstrated that use of inadequate thermocouples can cause errors in reported temperature data that vary constantly and can range from -270°C to over 1,093°C.[6,7] With such erroneous data, no meaningful correlations or conclusions about tissue temperatures, tissue shielding, or DNA preservation can be reached. Improper equipment selection and practices in the past have caused an accumulation of invalid temperature data and interpretations in the literature.

Thus far, three experimental burns on human donors using a new water-cooled thermocouple system have been conducted. We have successfully produced accurate temperature plots from muscle, mirroring expected trajectories based on theoretical thermodynamics laws. We have seen that the simulated burn process can take multiple hours, and that the measured temperature will not increase past the boiling point of water until the tissues (osseous and bone) are directly exposed to the fire or the water boils away from the tissue. These results are contrary to much of the published literature.

We have successfully and repeatedly recorded temperatures from probes placed in areas of more robust muscles (biceps, quadriceps, vastus lateralis, gastrocnemius, etc.). In our most recent burn, we also successfully acquired medullary temperatures from thermocouples implanted bilaterally in both humeri. The donor was placed on two side-by-side stacks of four wooden pallets that were packed with paper and dry straw before ignition. The left humerus temperature stayed stable for 6.7 minutes from fire start, then gradually rose to a plateau around 100°C that lasted between 26.4 to 40.7 minutes. The right humerus temperature stayed stable for 11.5 minutes from fire start, then gradually rose to a plateau around 100°C that lasted between 22.2 to 48.3 minutes. Post-plateau, the left humerus temperature rose much more steeply than the right and displayed rapid swings after 50.8 minutes while the right humerus temperature did not. Post-burn inspection revealed that the left humerus had fractured mid-shaft and directly exposed the probe to fire. The right humerus had remained intact and shielded the probe from fire for the duration of the burn. The fire temperature in this scenario was recorded in the pallet stack under the pelvis of the donor and reached a maximum of 1,055.4°C, 14.2 minutes after fire start.

Between the intramuscular, intraosseous, and interosseous placements, we have begun to accumulate accurate temperature data for the appendicular body. Our results also suggest that tissue color does not strongly correlate with temperature, as previously suggested. With this new data, we can begin to reassess the relationship between temperature, tissue preservation, and DNA yield.

The temperature data acquired from this research will allow us to improve our understanding of how fire alters human remains, and ultimately impacts identification efforts. Accurate temperature data, paired with DNA quantification, will allow a suite of protocols to be established to guide tissue sample selection for any fire-related event. These protocols will ensure the most efficient use of time and resources when working with thermally altered remains, thus bringing closure to surviving family members as quickly as possible.

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Temperature; Fire; Identification

A63 Locating Clandestine Burial Grounds: A Forensic Approach

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Learning Objectives: The learning objectives of this presentation include discussion of archival, ethnographic, bioarcheological, forensic, and remote sensing methods used in combination to identify possible unmarked burials and cemeteries. Strategies for georeferencing historic maps and images with current GIS data are demonstrated. Finally, the use of a demographic approach to reconstructing the population structures is shown as a highly effective way to locate communities and burial grounds that have been erased from the landscape or destroyed through predatory land practices.

Impact Statement: A significant number of lost, abandoned, and erased cemeteries exist in every state, most on government-owned properties affecting a disproportionate amount of Black Communities. A 1999 Florida State Task Force estimated there were as many as 100 unmarked burial grounds in every county. Participants will learn how a multi-method approach and demographic analysis can yield significant results for finding unmarked burials. This presentation is relevant to forensic anthropologists, bioarcheologists, and other investigators tasked with locating clandestine burials, unmarked burial grounds, or abandoned cemeteries throughout the country.

Abstract Text: In 2019, two African American cemeteries built over and lost to history in Tampa, FL, in the 1950s became known: the Zion Cemetery, located on property owned by the Tampa Housing Authority, and the Ridgewood Cemetery, located on the grounds of King High School, owned by the Hillsborough School Board Authority. The re-discovery of these cemeteries prompted the Hillsborough Board of County Commissioners (HBCC) to question whether there were additional unmarked cemeteries on county-owned property. An investigation into the location of possible unmarked graves was undertaken. A combination of archival, ethnographic, bioarcheological, forensic, and remote sensing methods were used to identify possible unmarked cemeteries and burials throughout the county. As a result of this approach, 44 additional unmarked burial grounds were located, just over half of which were African American or "Colored" cemeteries used and destroyed during the Jim Crow era, in large part by predatory land purchases that targeted Black and Afro-Cuban communities.

Unlike traditional archeological surveys typically used during construction surveys on government land, this project used a broader framework based on methods in forensic anthropology and incorporated several different lines of evidence and a demographic approach, resulting in the discovery of a high number of burial grounds. This approach starts with an understanding of the population structure and mortality trends for the community ca. 1500–today and combined a wide variety of archival documentation with GIS spatial analysis utilizing historic maps. In other words, it begins with asking the question of who was living in the area and *who should be buried* within the county. This approach enabled the identification of unmarked cemeteries based on historic land use such as homesteaded farms, pioneer towns, convict leasing and slavery, migration patterns, and ghost towns now abandoned or incorporated into larger communities. Reconstructing the population structure meant understanding the burial practices for diverse groups within the population, including those historically marginalized and consequently left out of the written record. It also considered the changing laws and practices over time used to marginalize certain groups of people based on the perception of race. Using a framework based on population structure builds on these considerations: the population size and ethnic diversity of the county; previously unmarked homestead or family burial plots; inclusion of the 1,000+ enslaved persons buried in the county, ca. 1830-1865; the settlement patterns of freed men and women following emancipation; changing settlement patterns over time associated with industry, technology, and population growth; and identification of convict lease contracts and possible burial areas within the county. A population approach also utilized data from undertakers, funeral home directors, and cemetery owners as these individuals significantly shaped mortuary practices throughout the past century. Similar patterns as identified here can be found in most cities throughout the Eastern United States. This presentation shares the results of a two-year, county-wide investigation and explores the unique approach and range of methods used and has relevance to a number of different applications in forensics and archaeology.

Anthropology; Clandestine; Archaeology

A64 Photogrammetric Documentation of the Changing Surface Morphology of Buried Human Remains

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Learning Objectives: After viewing this poster, observers will understand some characteristics of how the ground surface above a human burial change over time. A timeline of observed morphological differences will be presented.

Impact Statement: This presentation will impact the forensic science community by enhancing investigators' ability to evaluate a site for the location of a clandestine burial. Moreover, an improved ability to estimate the antiquity of a human burial will guide and improve investigative efficiency.

Abstract Text: Considerable effort has been applied to determining the postmortem interval of human remains in both surface and burial contexts; however, the Post-Burial Interval (PBI) of human remains continues to elude a comprehensive understanding.[1] This study focuses on the evaluation of the antiquity of a burial based on visual observations and the measured change in volume of surface soil mounds or depressions.

The use of emerging technology, such as Light Detection And Ranging (LiDAR), to locate lost or forgotten cemeteries is becoming increasingly popular; however, guidance for the use of LiDAR to document crime scenes has only been published in the past few years.[2,3]. Not all law enforcement agencies have access to the necessary equipment or training required to deploy this technology during surveys for a clandestine grave. Thus, identifying potential grave locations with a simple pedestrian survey can increase the efficiency of subsequent surface and sub-surface investigations.

Although many surface characteristics of burials have been described, little is understood about the timing of the appearance and disappearance of these features.[4] Eleven burials at the University of Tennessee's Anthropology Research Facility were included in this longitudinal study to provide a reference for burial aging. Utilizing photogrammetry software, the settling of the soil was quantified via volumetric measurements and correlated with PBI.

Rain events correspond with alterations to the appearance of the ground surface of a burial: darkening of the color of the grave soil, fewer visible cracks on the soil surface, and a more homogenous appearance to the soil. However, these alterations are temporary, and the characteristics of the soil surface revert to their previous appearance as the ground dries. Therefore, it is important to consider local weather conditions the week prior to a visual search for clandestine burials.

Within five months, most initial surface characteristics indicative of a burial have diminished to the point of near invisibility. Other characteristics associated with a longer PBI, like a depression in the soil or changes in vegetation, did not appear for at least one year post-burial.

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Photogrammetry; Burial; Characteristics

A65 Cellular Degradation and Migration in Bone as a Tool to Investigate the Postmortem Interval and Skeletal Break Timing

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Learning Objectives: After attending this presentation attendees will have a better understanding of how laser scanning confocal microscopy can be used to visualize and quantify the degradation and migration of osteoclasts in human bone as possible tools in assessing the postmortem interval and possible skeletal break timings.

Impact Statement: The presentation will impact the forensic science community by demonstrating a novel method for assessing the postmortem interval and the timing of skeletal damage using the degradation and migration of osteoclasts visualized using a laser scanning confocal microscope.

Abstract Text: Two of the more difficult assessments anthropologists are tasked with is assessing the Postmortem Interval (PMI) as well as any skeletal break timing. During the early skeletonized period, the assessed PMI can be wide as the soft tissue indicators have exuviated yet the skeletal elements have not undergone taphonomic changes. With break timing, while the assessment of damage that has taken place long postmortem can be easily distinguished from injuries that occur antemortem, damage occurring in the early postmortem period while the bone still maintains its "fresh" properties can be difficult to ascertain. The purpose of this study was to investigate the use of cellular degradation and migration as a means of assessing both the PMI as well as the skeletal break timing in the early postmortem period.

There are four stages of fracture healing beginning from the time of propagation to the terminal stage of remodeling. For the purposes of this project, it is the initial stage of induction that was of interest. This stage lasts a few weeks, beginning with fracture propagation and ending at the appearance of new bone. Due to the vascular nature of bone, once bone reaches the failure point, thousands of capillaries rupture, leading to a massive hemorrhage. Within minutes of fracture propagation, the vasculature will undergo blood clotting procedures and a hematoma will develop. Osteoprogenitor cells located in the periosteum will migrate, convert the hematoma to granulation tissue and ultimately osteoid. Once the hematoma develops, two processes initiate: inflammation of soft tissues and resorption of necrotic tissues.

Two major sample groups were used: a perimortem group of nine iliac biopsies from the Centre Hospitaller de l' Université de Montréal, and a postmortem group of nine iliac sections from donated remains collected from the University of Toronto Division of Anatomy. Each sample from the latter was further divided into three time groups: early-postmortem, 7-day, and 14-day postmortem. Samples were sectioned to 100µm, then labeled following the Coxon method.[1] They were imaged using a laser scanning confocal microscope using the 555nm emissions wavelength and analyzed using histology imaging software. The cortical bone section was isolated, allowing just an analysis of the cortical bone. Bone volume was standardized across all of the samples with cell counts occurring with both the raw numbers and standardized by volume. A Kruskal-Wallis *H*-Test and Spearman correlation were run to test if there was a significant variation and correlation in both degradation and migration between the four time groups. To verify if there was a significant variance of cells present in the migration portion of the study, a 700µm region of interest was placed over the break margin and the center of the cortical bone, with the results analyzed using a paired *t*-test.

The results of this study found that there was a sharp declination of cell presence (80%), and migration appeared to have ceased in most cases after the early postmortem period, with these results being both significant and strongly correlated ($p < 0.001$; $r = -0.815$, $p < 0.001$) when standardized by volume. Migration, though promising, however, was not statistically significant ($p = 0.051$), with migration seen in the perimortem and early-postmortem (< 72 hour) group, yet after this migration appears to halt. The results of the paired *t*-test found that there was a significant difference in those samples, which demonstrated migration ($p < 0.001$).

The results of this study have found that imaging osteoclasts could be useful in establishing PMI, particularly in fragments of an early (< 72 hour) period, and that there is a potential to use this cellular migration for establishing early postmortem injury timing, yet more research is necessary.

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Osteoclast; Postmortem Interval; Skeletal Damage

A66 3D-CT-Based Estimation of Facial Soft Tissue Thickness in the Punjabi Population for Craniofacial Reconstruction

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WITHDRAWN

A67 The Intra- and Inter-Observer Reliability in Morphological Extractions of the Maxillofacial Region

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WITHDRAWN

A68 Adapting Facial Approximations to a Modern Audience: The Use of Augmented Reality Filters and Profile Apps

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Learning Objectives: After attending this presentation, attendees will be able to evaluate the potential of using Augmented Reality filters for facial approximation obtained with analogical techniques.

Impact Statement: This presentation will impact the forensic science community by demonstrating the possibilities of using Augmented Reality filters in conjunction with standard analogical forensic art techniques to create a more suitable facial approximation for the modern population.

Abstract Text: Facial reconstruction, more appropriately facial approximation, aims to outline the physical features of an unidentified subject's face from their cranial remains. This branch of Forensic Art involves a combination of artistic and anatomical techniques developed to recreate the appearance of an unknown deceased individual to aid their recognition; however, it is essential to clarify that it is not an identification method, and it is a non-accepted practice to compare the recreated face with photographs of one or more missing individuals and directly identify the subject. Instead, if appropriately disseminated, the result obtained through Forensic Art serves as an investigative aid by helping acquaintances and family members of a missing individual to recognize the features in the reconstruction, directing the investigative effort toward a potential identification candidate. The confirmation of the identity of the remains must then be verified by appropriate scientific means, such as genetic, anthropological or dental comparisons.

Based on the memory of the missing person's relatives, one key factor that makes a facial approximation successful is the wide dissemination through media and social media and the possibility that it is seen and recognized by those who know the missing. Following this and considering how the modern population has become accustomed to seeing pictures edited with "beauty filters," facial approximations could be presented by adopting a similar graphic style to be better received and recognized by modern society.

The author proposes revising a series of facial approximations achieved with 3D sculpting methods and two-dimensional drawing by applying popular Augmented Reality filters. Each reconstruction has been digitalized by photographing the sculpted face or by optically acquiring the drawing and converting it to a digital format. A reference scale has been included in the picture to check the final result with the original image against any undesired alteration of the facial proportions and features obtained with the standard and scientifically recognized methods.

The digital pictures of the facial reconstructions were processed using popular applications available for mobile devices. These applications, widely used by the general public, allow the addition of realistic texture to various parts of the face. The use of Augmented Reality filters was driven by the need to enhance the lifelike appearance of the image without altering the somatic features reconstructed following Forensic Art principles. At every step, the new textured image was cross-referenced with the original source image and to a 1:1 scale photograph of the skull by photo editing software, ensuring the integrity of the reconstruction.

The research demonstrates the potential of using Augmented Reality filters to enhance the realistic appearance of facial approximations achieved through traditional Forensic Art analogic techniques. This approach not only maintains the scientific reliability of the original result but also provides more modern-looking images that can be better perceived and positively identified by modern society. The author recommends conducting statistical tests to evaluate the impact of Augmented Reality filters on the recognition process, specifically assessing the effectiveness of Augmented Reality filters in enhancing the recognition of forensic art facial approximations by the public.

Facial Reconstruction; Artificial Intelligence; Forensic Art

A69 Comparative Identification in 3D: What Virtual Anthropology Can Do for You

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Learning Objectives: This presentation will outline a workflow for skeletal comparative identification that harnesses the power of the Virtual Anthropology (VA) toolbox. We will describe an assortment of equipment and computer software that varies in cost and the steepness of the technical learning curve. The workflow can either be abbreviated at the point of visual qualitative comparison or, for the more statistically minded practitioner, continued to advanced Three-Dimensional Geometric Morphometric analysis (3D GM).

Impact Statement: The terms "Virtual Anthropology" and "Virtual Morphology" refer to a collection of computer-based tools for: (1) generating and manipulating digital imagery, and (2) quantitatively describing differences in size and shape.[1] While the tools associated with digital imagery are becoming increasingly valued in medicolegal settings, the second component of the VA toolbox (morphometric analysis) has yet to be used to its greatest potential.[2-6] We use two case studies to highlight how 3D GM tools could expand the range of anatomical regions and "features" used for individualization.

Abstract Text: The goal of comparative identification is to document features that are either unique or rare enough in a population to be useful in individualization.[7] For this reason, evidence of medical procedures, trauma, and pathology accumulated over the lifetime are ideal individualizing features. Extreme morphological anomalies can also be considered individualizing, along with aspects of non-pathological skeletal variation like sinus shape and trabecular pattern. This variety of approaches highlights the gray area between individualizing features and normal skeletal variation. After all, every individual is theoretically biologically unique. The difficulty in comparative identification comes in the methodological details of demonstrating that uniqueness, given the antemortem data at hand.

The comparative identification workflow involves evaluating similarities between antemortem medical images and postmortem images collected by the forensic anthropologist or medical professional.[7] Traditionally, one or both of the images are two-dimensional (2D) radiographs, which imposes the well-documented challenge of replicating bone orientation as precisely as possible in post- and ante-mortem images.[4,5,8,9]

The orientation challenge is eliminated when both post- and ante-mortem images are 3D datasets. The superimposition of antemortem and postmortem digital models can be incredibly precise, which facilitates qualitative comparison and illustration of individualizing skeletal features.

More importantly, these 3D imaging tools can be combined with 3D GM to quantify overall shape similarity between a digital bone model derived from antemortem image and its postmortem counterpart. State-of-the-art capabilities include the measurement and comparison of 3D curves and surfaces.[10,11] Because these methods permit measurement of morphology in such detail, we suggest that overall bone morphology could be used as an individualizing feature.

We evaluate the utility of cranial morphology in individualization, using the real-life scenarios from two recent cases. In both examples, the target dataset consists of (*x*,*y*,*z*) landmarks derived from an antemortem Computed Tomography (CT) scan of the suspected decedent. The comparative dataset consists of the same configuration of cranial landmarks, collected from 50 cranial surface scans curated at the Mercyhurst Forensic Anthropology Laboratory and one additional scan of the unidentified cranium under study (total *n*=51). The landmark configurations from target and comparative datasets were subjected to a Generalized Procrustes Analysis, then pairwise comparisons conducted. Procrustes Distances were used to quantify overall shape difference between the antemortem target and every individual in the comparative sample. The resulting distribution of pair-wise Procrustes Distances can be used to estimate error rates of the identification.

With future research, the approach described here could be expanded to a variety of anatomical regions, thus enabling the forensic anthropologist to tailor the comparative identification to any anatomical region for which 3D medical imagery exists.

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3D Technology; Computed Tomography; Identification

A70 Forensic Investigative Genetic Genealogy: A New Opportunity to Resolve Unidentified Cases

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Learning Objectives: After attending this presentation, attendees will be made aware of the potential, and success, of Forensic Investigative Genetic Genealogy to provide new investigative leads in the effort to identify both short- and long-term unidentified persons that are under the jurisdiction of medical examiner and coroner's offices.

Impact Statement: This presentation will impact the forensic science community by illustrating the importance of properly retaining postmortem tissue samples, or the prior DNA extracts from these biologic tissues, as well as advocating for the routine publication of these unidentified person cases in the National Missing and Unidentified Persons System (NamUs).

Abstract Text: Perhaps no new investigative tool within the past decade has revolutionized our potential ability to identify previously unidentified John and Jane Doe cases as Forensic Investigative Genetic Genealogy (FIGG) as has already been demonstrated.[1] The Pima County Office of the Medical Examiner (PCOME) curates investigative records, and in many cases the skeletal remains, of more than 1,600 currently unidentified cases all entered into NamUs. Many have had their fingerprint records compared in the Automated Fingerprint Identification System (AFIS), most have DNA profiles in the Combined DNA Index System (CODIS), and a fair amount have viewable facial images. When these methods fail to generate an investigative lead through fingerprints, Single Tandem Repeat (STR) DNA profiles, mitochondrial DNA (mtDNA) profiles, or facial photographs, in the past there was little chance for identification.

FIGG has changed that scenario through the use of publicly available databases that contain Single Nucleotide Polymorphism (SNP) DNA profiles from an increasing number of people who are purchasing Direct-To-Consumer (DTC) genealogy kits. There are scores of these providers operating in the United States and a growing number of FIGG organizations who are contacting medical examiner and coroner's offices throughout the country offering to employ this familial DNA technology after the individualizing STR assays produced no matches in CODIS. These FIGG organizations are offering to accept (as well as arrange funding) select unidentified cases where traditional methods have not produced an identification. The FIGG groups contract with various DNA laboratories to perform SNP profiling, many times via whole genome sequencing, to produce a record that then can be entered into one of several publicly available SNP databases in the search of blood-relatives. If successful, usually at a level of distant cousins, traditional genealogical methods aided by the internet, can produce a "candidate" for a particular unidentified individual. From there, a one-to-one comparison is typically conducted using a closely related family reference sample to confirm the hypothesized identification. While it is well-known that past "abuses" of selected SNP data have occurred, it appears that the DTC genetic ancestry companies have now implemented the appropriate safeguards.

The PCOME has, as of August 1, 2024, been contacted by ten different FIGG groups resulting in 60 of our unidentified cases being subjected to this exciting new tool. Of these 60 cases, ten have been identified after the PCOME confirmed the identification of the candidate proposed by the FIGG group. These ten identifications have included an elder-abuse homicide from 1988, a child homicide case from 2020, and a third homicide case from 2012. These three identifications are now providing law enforcement with a crucial investigative lead in pursuit of the perpetrators of these still unresolved homicides. Nearly all 60 of these cases were chosen by the FIGG group or by the PCOME from our 1,600-plus NamUs unidentified cases. Maintaining a publicly viewable list in NamUs has proved to be a tremendous time-saver for case selection. Furthermore, the collection and retention of biological samples throughout the decades has proven fruitful in providing the necessary tissue or extracts to perform these powerful new investigations.

Although a few of the 50 cases that have not yet resulted in a proposed candidate have reached a "dead-end" as far as personal identification, important ancillary information regarding nationality and/or ancestry has still been provided to the PCOME. We hold out great hope that many of our 1,600 currently unidentified cases may someday be resolved through FIGG analyses. We advocate that the necessary funding sources be made available to keep this now-indispensable tool available to those medical examiner and coroner offices that have yet to be able to identify Jane and John Doe cases by conventional methods.

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Genealogy; Genetics; Unidentified Persons

A71 The Sugar Land 95: A Forensic Genetic Genealogical Approach to Restoring Individual Identity

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Learning Objectives: Attendees at this poster session will learn about the process of using Forensic Investigative Genetic Genealogy (FIGG) to identify individuals in clandestine burials. Attendees will also learn about the usefulness of the Qiagen Forenseq Kintellegence kit in identification of degraded teeth/bone samples.

Impact Statement: This presentation will give insight and support regarding the capabilities of FIGG in identifying unknown skeletal remains. It will also allow professionals to have a framework when attempting to restore identity to large numbers of unknown decedents from cemeteries or mass graves.

Abstract Text: The Sugar Land 95 are individuals who were victims of the convict leasing era in the United States that began after the ratification of the 13th Amendment abolishing slavery in 1865. This amendment, which is still in place today, made forced labor illegal except, notably, in the case of incarcerated individuals. As a result, the Sugar Land 95 were leased to sugar plantations, replacing slave labor in Sugar Land, Texas. This was not exclusive to the state of Texas as many other states also participated in convict leasing. These individuals sustained numerous injuries, including gunshot wounds, bone infections, fractures, head injuries, and many other ante- and perimortem injuries.

The remains of the Sugar Land 95 were disinterred in 2018 and subsequently analyzed to create biological profiles, as well as to assess traumas, pathologies, and taphonomic conditions. These analyses initiated the identification process, at which point historic records were examined to try and find the names of the interred individuals. Today, after receiving permits from the Texas Historical Commission, the Principal Research Group and the Snow Molecular Anthropology Laboratory have begun the process to genetically identify the Sugar Land 95 using FIGG.

Overall, the Sugar Land 95 cemetery represents a unique opportunity not only to bring justice to the Sugar Land 95 but also to test the limits of forensic technology. In turn, this will contribute significantly to the forensic community, using the insights gained from this research to inform modern day identification of mass graves and victims of natural disasters. Research will highlight best practices for large sample numbers, difficult sample types, and exclusively kinship-informative Single Nucleotide Polymorphism (SNP) markers. All work is being done with the ultimate goal of reliable identification of both the deceased and their descendants to uncover and restore the history of the Sugar Land 95.

Genetics; Genealogy; Human Remains

A72 The Wounds Time Does Not Heal: A Case of Historical Pitfalls and the Present-Day Identification of a United States Marine From World War II

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Learning Objectives: The goal of this presentation is to provide a real-world example of the benefit that a multidisciplinary approach can bring to the segregation and resolution of a commingled remains case, particularly when the recovery context and prior analyses introduce additional complications to the identification process. After attending this presentation, attendees will better understand how different lines of evidence, including historical records, anthropological and dental analysis, and DNA, can interplay to bolster an identification from a commingled assemblage.

Impact Statement: This presentation will impact the forensic science community by showing the contributions that a multidisciplinary approach can make to the resolution of commingled skeletal assemblages. It will also impact the forensic science community by demonstrating and emphasizing the importance of training in the recovery and identification process.

Abstract Text: In the heart of World War II, the United States military began an island-hopping campaign across the Pacific with the intention of capturing strategic strongholds from which to launch attacks against the Japanese homeland. As part of this initiative, from November 20–23, 1943, an amphibious assault was launched against the Japanese-controlled Betio Island, located in the southern portion of the Tarawa Atoll. The battle (known as the Battle of Tarawa) lasted approximately 76 hours and resulted in the loss of over 1,000 United States service members. Following the battle, casualties were buried in expedient cemeteries across the island; these cemeteries were subsequently "beautified," resulting in incorrect and incomplete records. In 1946, the American Graves Registration Services attempted to recover and identify the Tarawa casualties but faced several barriers to this effort, including the inaccurate burial records, a lack of archaeological or anthropological training, and a heavy reliance on the presence of personal effects to support identification. Individuals that were unable to be identified in the 1940s were buried as Unknowns in the National Memorial Cemetery of the Pacific (NMCP). In 2016, the Defense POW/MIA Accounting Agency (DPAA) began to disinter the Tarawa Unknowns from the NMCP for analysis and identification efforts, an effort supported by present-day excavations on Betio Island by a DPAA strategic partner.

This presentation details the process of segregation and subsequent identification of a United States Marine killed during the Battle of Tarawa. Identification was made using a multidisciplinary approach, including an examination of historical records, anthropological and dental analysis, and DNA. This individual was listed as historically identified in the 1940s; however, the identification was largely based on the presence of an identification tag. This individual was disinterred by DPAA in 2016; additional skeletal remains were recovered from Betio Island and turned over to DPAA in 2015 and 2023 and could be associated with the disinterred remains through articulation, pair matching, refitting of fragments, dental analysis, and DNA. Additionally, tracing the historical records and understanding the historical context of the historical identification, as well as the historical analysis and movement of the unidentified remains, DPAA was able to correctly identify this service member and return him to his next-of-kin.

Anthropology; Commingling; Personal Identification

A73 The Use of Antemortem Trauma in Identifications at the Defense POW/MIA Accounting Agency

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Learning Objectives: The Defense POW/MIA Accounting Agency's (DPAA) mission is to "provide the fullest possible accounting for our missing personal to their families and the nation."[1] Antemortem trauma can collaborate with other evidence for an Identification (ID).[2]

Impact Statement: The presentation will impact the forensic science community by discussing how using antemortem trauma can be applied outside of the DPAA

Abstract Text: DPAA casework between 2016–2023 was reviewed and cases from four projects selected based on their workflow and specific documentation, including a project level preliminary report, a Forensic Anthropology Report (FAR), antemortem records, a report comparing the FAR and antemortem records (BCR), and a medical examiner report that compiles the information to make a final identification. The overall question this research seeks to answer is how the DPAA utilizes antemortem trauma in their identifications.

From the four projects, there were 308 cases that met the requirements: Cabanatuan (71), Tarawa (46), Korean War (164), and Pearl Harbor Ships (27). Skeletal trauma was noted in DPAA reports in 74 (24%) of cases with the majority written in FAR reports only. Seventeen cases (15%) with both skeletal trauma and antemortem records used the trauma in the final identification by the medical examiner. This was medical-examiner specific. Based on DPAA reports, the most common elements affected were nasals and clavicles (15% each). Thirty-nine cases (12.7%) had trauma noted in antemortem records only mostly due to those elements, such as the hands and feet, not being present for analysis (25/39 cases).

There was a significant difference in the amount of trauma ($p = 0.015$) between projects; Tarawa had the highest percentage (80.8%). The type of antemortem record that the trauma documentation (family letter, civilian medical records, military medical records, other) was significant ($p = 9.15E\text{-}59$). The Korea War (51.8%) and Cabanatuan (22%) projects had a higher percentage of family letters (51.8%) compared to the Tarawa (5%) and Ships (0%) projects due to the military requesting identifying information for the identification of POWs. Letters from family tend to be more vague than medical records, saying broken leg rather than femur. Tarawa had the highest number of cases where antemortem trauma was noted in medical records (93%), specifically military medical records. Antemortem trauma in medical records is either self-reported at the time of enlistment or occurred during service and was documented by the military. Four cases overall had non-military medical records.

Antemortem trauma is used in modern identifications as well, utilizing both medical records and family informents.[3-7] In a modern decedent population, 34% were found to have antemortem trauma, like the DPAA sample. The same study found that the ribs and femora were the most common elements with trauma.[6] Another study found that nasals were the most often damaged, the same as the DPAA sample.[4] One study found that elements of the hands and feet are the least likely to be recovered.[7] Radiographs are the biggest difference between modern and the DPAA populations as they are more available in modern cases.[3,4] Radiographs can have limitations as the antemortem records may be in an orientation that does not allow for comparison.[5] Like the DPAA sample, the biggest issue in the utilization of antemortem trauma is the lack of records as well as the fact that authorities must have a belief to be able to make a comparison.[8-10]

In conclusion, antemortem trauma was utilized in few identifications in this study. This is due to a lack of records and a lack of elements with recorded trauma being present. To better utilize this, if possible, the element with antemortem trauma should be sampled or brought into the individual by anthropological methods. The DPAA can also continue to make sure that they recover as complete of individuals as possible in the field to make sure these elements are brought in. Further research includes surveys with medical examiners to get a better idea of the use of antemortem trauma records in modern identifications and to learn the best way of obtaining information on antemortem trauma.

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Identification; Trauma; Unknown

A74 Integrating Forensic Archaeology and Historic Battlefield Protocols at Historic Conflict-Related Aircraft Crashscapes: A World War II B-17 Case Study

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Learning Objectives: After attending this presentation, attendees will be cognizant of innovative modified approaches toward the recovery of probative materials from historic conflict-related aircraft crashscapes. Current excavation models depend on the correlation between metal and probative materials, which is inherently problematic, if convenient. A case study involving a complex World War II-era B-17 crashscape in Germany is presented to illustrate the increasingly complicated recovery efforts associated with aging historic losses.

Impact Statement: This presentation will impact the forensic science community by invoking practitioners to consider the myriad factors that complicate recovery efforts of decedents from historic aircraft crashscapes. It will provide insight that may be relevant for any practitioner undertaking similar or related field efforts and offer guidance on tackling complex historical recovery sites. The integration of modified protocols commonly used at present-day aircraft crashscapes with historical battlefield archaeology methods offers an effective way to approach these types of historic conflict-related scenarios, characterizing scenes through multiple and robust lines of evidence prior to emphasizing Phase III recovery.

Abstract Text: Recovery efforts of historic conflict-related aircraft crash sites, including those mitigated by the Defense POW/MIA Accounting Agency and their partners, require unique blends of historical archaeology and forensic investigation towards the recovery of probative materials. Aircraft crashes are extremely variable regarding circumstances surrounding the incident, trajectory of the aircraft crash and subsequent explosions, incident environment, and taphonomic mechanisms between the incident and archaeological recovery of the site. Traditionally, practitioners have employed methods based on assumptions about correlations between decedents and diagnostic wreckage, emphasizing evidence-free horizontal excavation margins. Additionally, the spatial extent excavated, reflecting the Law of Diminishing Returns, is frequently upheld as the primary indicator of successful recovery efforts, barring recovery of human remains or other probative materials. This traditional wisdom is typically applied to recovery designs regardless of the incident circumstances. However, crashscapes are often encountered where decedents or probative materials do not align with diagnostic wreckage (e.g., the pilot may not be found with materials from the cockpit); the horizontal boundaries of the site extend far beyond the immediate crash crater (e.g., incidents with oblique trajectories and/or late ejection attempts); and valuable investigative techniques do not always require intrusive excavation efforts.

This case study of a B-17 aircraft crashscape details the incorporation of innovative methods that adapt and expand beyond standard operating procedures. Multiple lines of inquiry are recommended, prioritizing non- or minimally invasive means of evaluating site boundaries and maximizing spatial assessment of potential recovery targets before focusing on evidence-free margins or extent excavated. Suggestions are provided for alternative measures of recovery progress beyond horizontal area excavated. Forensic investigative and historical archaeological techniques were modified over three field seasons based on excavation results, enhanced understandings of the historical event, site environmental context and history, previous recoveries by other parties, and observed patterning of aircraft wreckage within and beyond the associated crash crater. Previous investigations and excavations by other parties focused on witness interviews, wide-interval pedestrian and metal detection surveys, limited subsurface testing, and block excavation based on interpreted site boundaries from metal detection surveys. The crash crater boundaries were not delineated.

Phase-III recovery efforts were initiated based on this previous work, commencing with block excavation units (400+ m^2 since 2022). However, the complicated nature of the crashscape was quickly revealed, with the need for more intensive site delineation and characterization to develop targeted recovery strategies. This was achieved through a ground-penetrating radar survey (4,760+ m^2 in ~100 hours), delineating horizontal and vertical crater boundaries; close-interval pedestrian (7,140+ m^2 in ~16 hours), and metal detection (800+ m^2 in ~60 hours) surveys, highlighting wreckage distribution patterns; subsurface profile interpretation within, on, and outside the crash crater boundaries; and investigative excavated transects (400 m^2 in ~200 hours), identifying locations for targeted units. These approaches reflect modified forensic and commercial aircraft crashscape recovery protocols and battlefield investigation guidance.[1-6] Work occurred during three field school training programs, averaging 14 students over approximately six weeks per session.

While some may argue these operations are time consuming or detract from the mission, this case demonstrates these approaches can be implemented effectively and offer benefits beyond their initial time requirements toward extensive and robust recovery efforts, which themselves can extend over multiple field seasons with similarly sized teams. Through these efforts, the crash crater boundaries were identified and confirmed, and systematic surveys revealed new areas worth investigating and excavating, all while continuing block excavation units to reach incident-sterile contexts. Practitioners must remain open to flexible and innovative iterative strategies that align with the historical, environmental, and recovery contexts of complicated historical recovery sites to effectively recover such sites.

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Anthropology; Forensic Archaeology; Aircraft Crash

A75 An Anthropological Response to Mass Disaster: Lessons Learned From the October 7th Attack

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Learning Objectives: Following this presentation, attendees will learn the methods used by a lone, small anthropology lab to handle the largest mass casualty event in Israel's history. Attendees will understand: (1) the unprecedented increase in the number of cases, (2) the added challenge of limited contextual information from the field: and (3) the unceasing pressure from various authorities to identify the dead as quickly as possible.

Impact Statement: The mass casualty event resulting from the events of October 7, 2023, produced a large number of casualties and resulted in remains in many differing states of preservation. The idea of an "anthropological examination" changed from what it is during a normal workload, focusing instead on Minimum Number of Individuals (MNI), which formed the basis for a DNA sampling strategy. In addition, since lab anthropologists were too busy with the ongoing increase in workload to participate in the extensive recovery operations, differentiation between various burnt materials, human and non-human bone, based only on small fragments of burnt bone, required developing criteria that had not necessarily been used in the lab before. The attendees will learn about the experiences and struggles of the new head of the forensic anthropology lab, who took over the position on October 8, 2023.

Abstract Text: The State of Israel woke on October 7, 2023, to an unprecedented attack on many of the southern towns by the terror organization Hamas. Over the course of this attack, more than 1,200 civilians, first responders, and soldiers were killed and 250 men, women, and children ranging from 7 months to 85 years old were taken hostage. This is the largest mass casualty event in the entire history of the State of Israel. Even before the true extent of the attack was understood, plans were already being made at the National Center of Forensic Medicine on how to deal with the fallout.

With only one forensic center in the country, and a sudden and unexpected event nearly doubling of the number of annual deaths, the choices made for the management of the October 7th massacre were paramount in ensuring the successful identification of remains through any available means. This was particularly important considering the importance in bringing the deceased to burial in as short of a time frame as possible, as mandated by the religious practices of both Judaism and Islam, which together are practiced by approximately 94% of the total population.

This presentation will discuss the structure of the anthropology laboratory at the National Institute for Forensic Medicine in the context of other national authorities all assisting in the identification of the dead.

The current head of forensic anthropology, and the only person in the country then working in the field, took over the position on October 8, 2023, something that had been planned for a number of months, and was immediately thrust into the highest-pressure situation that the anthropology lab has ever faced. The number of cases increased dramatically within a compressed timeframe (a 411% increase of the average annual caseload). Collection of remains occurred in an active war zone with continuing rocket strikes and was undertaken by various groups with differing degrees of training, which resulted in limited contextual information accompanying remains recovered and brought to the lab. There was also unceasing pressure from various authorities to identify remains as soon as possible. The sheer volume of work that was undertaken by such a small anthropology laboratory necessitated volunteer assistance from highly experienced outside practitioners. In addition, the change required an adjustment in the methodology used in a direction that may not be the most traditional. The focus had to shift from complete biological profiles to MNI and DNA sampling for the fastest possible identification. Other non-traditional methods were used as well, in cases of severely fragmented burnt bone where DNA sampling was not possible due to the degree of calcination.

Mass Fatality; Human Identification; Laboratory Strategy

A76 Ethical Considerations of Cadaver Dog Training, Certification, and Field Use

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Learning Objectives: This presentation will inform attendees about the processes behind the training, certification, and handler use of Human Remains Detection Dogs (HRDD) in investigations requiring the discovery of suspected missing human remains. Additionally, it will underscore the importance of implementing a standardized ethical framework in HRDD practices.

Impact Statement: This presentation will impact the forensic science community by addressing ethical issues inherent in current HRDD practices and highlighting the existing lack of standardization. By doing so, it will catalyze discussions and initiatives aimed at establishing new standards in this field.

Abstract Text: HRDD are canines trained to detect the scent of human decomposition and alert their handlers of its location. Local law enforcement agencies often lack the budget to maintain their own HRD teams, resulting in the common practice of outsourcing this specialty. This presentation addresses the lack of standardization observed in three critical areas - training, handler use, and certification.

Training of HRDDs involves exposing the canines to training aids that mimic the olfactory profile of decomposing human remains. There is significant variability in the methods used for training dogs to detect this scent as well as in the types of training aids employed. This variability arises from limited access to whole cadavers and challenges in storing and maintaining proper training materials. Training aids can include fabric or soil that has been in contact with human remains, small tissue samples, pseudoscents, bones, and pig flesh. Further research is necessary to identify which training aids accurately represent the Volatile Organic Compounds (VOCs) emitted during human decomposition. Additionally, studies are needed to determine if the differences in VOCs of varying stages of decomposition are considerable enough to warrant training aids in all levels of decomposition. Without standardized storage practices, there is a risk of cross-contamination among training aids stored in permeable containers.[1]

Handler practices must be standardized and rigorously reviewed to ensure ethical procedures in the use of HRDDs. Given the variability in training practices, handlers are responsible for acquiring appropriate training aids and thoroughly exposing their canines to human remains. Handlers also play a crucial role in assessing whether their canines can effectively differentiate human remains scents from others. A survey conducted by the National Center for Biotechnology Information (NCBI) reveals that handlers often select their canines based on traits such as playfulness, sociability, and curiosity, rather than olfactory capabilities.[2] Further research is needed to understand the olfactory capabilities of different dog breeds and to identify which characteristics are essential for selecting and training effective HRDDs.

Currently, there is no centralized organization overseeing the training and certification of HRDDs. Existing guidelines from organizations like the Scientific Working Group on Dog and Orthogonal Detector Guidelines (SWGDOG), the National Institute of Standards and Technology (NIST), and the American Standards Board (ASB) lack definitive language and provide ambiguous certification requirements, leaving canine teams to establish their own training and practice protocol.[3,4]

This overview aims to highlight examples of variability in current HRDD training, handler practices, and certification processes, emphasizing the need for standardized protocols in training dogs used in forensic investigations. The variability underscores the necessity for additional research to enhance the scientific basis behind the reliability of training aids and the selection of HRDDs.

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Anthropology; Cadaver Dog; Human Remains

A77 Examining Contributing Factors to Natural Human Mummification at FOREST in Cullowhee, North Carolina

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the effect of seasonality on the speed of mummification in relation to aggregate, humidity, temperature and precipitation. Mummification patterns at Western Carolina University's Forensic Osteology Research Station (FOREST) in Cullowhee, NC, will be discussed.

Impact Statement: This presentation will impact the forensic science community by contributing new information to the field of human taphonomy concerning the circumstances leading to mummification.

Abstract Text: Natural mummification occurs in a diverse range of global locations, including temperate climates.[1] Understanding when mummification occurs in temperate climates will allow forensic specialists to more accurately determine the Postmortem Interval (PMI) of a set of remains found in such locations. Despite being observed frequently, factors that contribute to natural mummification remain poorly documented in current literature. A recent literature review sought to better identify where geographically and what variables contribute to natural mummification.[1] The limited information available suggests factors are largely inconsistent and do not address mummification in temperate climates, highlighting the need for further research. This research contributes new information to the field of human taphonomy concerning the circumstances leading to mummification, specifically the effects of seasonality on the speed of mummification in relation to aggregate humidity, temperature, and precipitation.

This preliminary study utilized factors identified in Vacca 1 to better understand their impact on mummification at Western Carolina University's (WCU) in Cullowhee, NC, where mummification is frequently observed to occur. Using a series of photographs during the decomposition process of 12 donors placed at FOREST in 2022, variables of humidity, precipitation, temperature, and seasonality were assessed for an approximately 16-month period using data from the National Weather Service in order to determine if any of the variables contribute significantly to mummification. The donors were scored using the Connor et al. method, which was determined to be more accurate for scoring decomposition at FOREST by Melerine et al.[2,3] Out of the 12 donors, two did not reach mummification during the study period and were excluded from further analysis. For each donor, the number of days until the first area demonstrated mummification and the number of days until all sections showed mummification were recorded. Variable data were totaled until these days were reached and analyzed using Multivariate Analysis of Variance (MANOVA) to understand what effect the variables had on natural mummification. Inter-observer error analysis was conducted to assess the consistency in scoring using the Conner et al. method.[2] The Coh.en's kappa results showed weak-to-moderate agreement between two observers (0.597-0.712).[4]

A one-way MANOVA analysis showed that the variables of humidity, temperature, and precipitation significantly impacted the date when mummification was first observed and when it occurred on all body segments. To examine which variables were contributing to the first and all sections demonstrating mummification, ANOVA with a Tukey Honestly Significant Difference post-hoc tests were conducted on both data sets. Results indicate that for the date of first appearance of mummification, only temperature and humidity are significant contributors, while temperature, humidity, and precipitation were significant for when all sections of a donor demonstrated mummification.

In order to examine if seasonality was significant in terms of when first and total mummification observed, a two-way MANOVA indicated that seasonality is significant to the days until both total and first mummification, with ANOVA revealing the interactions between all other seasons and fall as significant. By examining histograms of the data, the donors went through the stages of decomposition the fastest in the spring and summer and the slowest in the fall. Examining trends related to seasonality, two donors demonstrated patterns of mummification and rehydration with no consistency in terms of timing of placement.

The results of this study reaffirm the need to further understand factors leading to mummification, specifically in regard to seasonality, and the influence temperature, humidity, and precipitation have in patterns of mummification. Future work on this project will include additional donors, further investigation into observer error rates, and examining how the factors impacting mummification affect the Total Body Desiccation Score (TBDS).

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Taphonomy; Mummification; Seasonality

A78 First Donor of the Forensic Science Research and Training Laboratory (FSRTL): Exploring Unique Geographic Variation in Human Decomposition in the Mid-Atlantic Region

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the local environmental factors associated with the mid-Atlantic region that contribute to the rate of human decomposition. Attendees will also have new insight into the ongoing research efforts and opportunities for collaboration afforded by the FSRTL at George Mason University.

Impact Statement: This presentation will impact the forensic community by providing new insights into the geographic variation of human decomposition. Information gathered from this study and future studies will provide data that can be directly applied to local law enforcement cases by helping to establish a relevant timeline of decomposition

Abstract Text: The purpose of this study is to understand the geographic variation of human decomposition in the mid-Atlantic region through gathering data from the first donor at George Mason University's FSRTL. Information such as decomposition rate, insect activity, and scavenging patterns will be analyzed to provide a baseline for future studies at FSRTL.

Current methods for estimating the Postmortem Interval (PMI) are subject to regional variation. Methods derived from certain regions are inherently biased towards those regions.[1] The literature surrounding decomposition interval methodologies encourages practitioners in other parts of the country to complete their own documentation to better track the changes seen in local environments. Further literature reviews discuss how determining accurate PMI that are focused on specific regions is necessary in investigating cases.[2,3] A misinterpretation of PMI due to a lack of understanding of local environmental factors can mislead a case entirely.[2] Understanding the factors that contribute to the complex postmortem process are therefore imperative.[3]

The first donor of FSRTL was received in May 2024 and was placed above ground partially clothed in a consistently shaded wooded area with a metal cage placed over the top of the donor to minimize scavenger activity. Data collection included qualitative observation, photographic documentation, and decomposition scoring following the scale developed by Megyesi et al. at regular intervals.[1]

Insect activity steadily increased to approximately 25% body coverage for the first five days, progressing to approximately 50-100% on day 8. The progression of insect activity from larva to adult was documented for blowflies, beetles, and gnats. Scavenging activity from vultures was first observed on day 7 and last noticed on day 17. Vulture activity was predominately focused on the left hands and feet with less activity observed on the right. This is possibly related to a chemotherapy port implanted on the right side.

Qualitatively, decomposition progressed differently in each region for the body. The head stayed in the early decomposition phase from day 3 to day 7, entering advanced decomposition on day 8, and reaching skeletonization on day 13. The limbs were in the early stages of decomposition from days 3 to 10, advanced decomposition from days 11 to 16, and skeletonized after day 17. The early stage of decomposition was prolonged in the torso when compared to the head and limbs, lasting from day 2 to 11 and not reaching skeletonization until day 14, likely due to the clothing. Quantitatively, comparisons were made between the actual accumulated degree days and known PMI and the calculated accumulated degree days and PMI.

Similar to previous results, quantitative decomposition scores increase exponentially with increasing known PMI. However, when compared against the published data, distinctive deviations were observed with increasing PMI, indicating greater error in PMI estimation using existing method in later stages of decomposition.[1] The results demonstrate the importance of conducting studies on human decomposition in different regional areas to create accurate methods that can be used by local law enforcement when determining PMI.

Future studies at FSRTL will continue to establish baseline variation in human decomposition to provide information for practitioners.

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Anthropology; Decomposition; Taphonomy

A79 Understanding Human Decomposition in the Great White North: A Review of REST[ES]'s First Five Years and Future Directions

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Learning Objectives: After attending this presentation, attendees will understand the Canadian human decomposition context from an anatomical, chemical, entomological, environmental, forensic, and thanatological perspective.

Impact Statement: This presentation will impact the forensic community by demonstrating the interdisciplinary approach used at a human decomposition facility in a cold, North American climate. It will highlight the wide range of disciplines that benefit from this non-traditional management structure, both within and outside of forensic anthropology.

Abstract Text: The site for Research on Experimental and Social Thanatology, or *Recherche en Sciences Thanatologiques [Expérimentales et Sociales]* (REST[ES]) in French, is a high security outdoor facility primarily dedicated to the physical, chemical, and biological study of human decomposition, with a sociological dimension.[1] As the site is located in southeastern Canada, research conducted at REST[ES] focuses on the study of death and the taphonomic processes that occur in a northern, cold, and forested climate. REST[ES] is currently the only human decomposition facility in Canada and the only bilingual site operating in both French and English globally, thus offering a unique niche to local, national, and international researchers, scientists, practitioners, law enforcement, and other professionals interested in postmortem changes in this particular environment.

As an extension of the Anatomy Laboratory at the *Université du Québec à Trois-Rivières*, REST[ES] benefits from the credibility and public awareness the Department of Anatomy has built with its willed body donation program over the past 30 years. Since the site welcomed its first donor in 2020, REST[ES] has received a total of 26 donors and hosted 21 research projects (as of August 1, 2024) in anatomy, chemistry, entomology, environmental science, forensic science, and thanatology, to name only a few. Research topics have been as diverse as the use of hyperspectral imaging for body detection, the characterization of cadaveric odors, the effect of winter on entomological fauna, the environmental impact of human decomposition in northern latitudes, the restoration of fingerprints in an advanced state of decomposition, and the assessment of postmortem interval through the thanatobiome, among others.[2-4]

Following an administration change in 2023, REST[ES] underwent a major restructuration, with the intent to bring the site closer to the American human decomposition facility model while also embracing the traceology approach recommended in the Sydney Declaration.[5] A substantial provincial grant obtained in 2024 also allowed REST[ES] to further integrate technology into the site's management strategy and optimize data collection. The purpose of this presentation is three-fold: (1) to provide a review of REST[ES]'s first five years as a taphonomy research facility, (2) to introduce the new management structure, and (3) to present future directions planned for the site in upcoming years, namely with regard to professional partnerships, research collaborations, and training opportunities.

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Decomposition; Taphonomy; Canada

A80 Opening the Black Box: Explainability Tools in Postmortem Interval Estimation

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Learning Objectives: After attending this presentation, attendees will learn how Machine Learning (ML) in forensic science can be leveraged to investigate aspects of the decomposition process. Specifically, we will present the use of ML explainability tools to enhance the ethical and responsible use of Artificial Intelligence (AI) in forensic science, particularly in Postmortem Interval (PMI) estimation. These methods support transparency and interpretability when integrating ML into forensic applications.

Impact Statement: This presentation will impact the scientific community by demonstrating how AI can be applied in ethical ways that advance our understanding of the decomposition process.

Abstract Text: Time since death, or PMI, estimation remains an enduring challenge to medicolegal death investigations despite decades of research. Existing methods continue to lack the scientific rigor required within the medicolegal sector due to continued reliance on small sample sizes, the lack of environmental heterogeneity, and inconsistent descriptions and methodologies, which impede our understanding of the decomposition process.[1]

GeoFOR began in 2019 and seeks to inform these longstanding issues by offering data-driven PMI estimations. The geoFOR application serves as a free forensic case entry platform that automates the collection of weather data from the discovery location using the Global Historical Climatology Network (GHCN) and utilizes ML methods to deliver statistically robust PMI predictions directly to users. Cases entered into geoFOR contribute to an ongoing, large, collaborative forensic taphonomy database (n= 3217) used to train and update the ML predictive model.

ML models, though powerful predictive tools, are often "black boxes" due to their complexity. To ensure ethical and fair application of ML, techniques for "opening the black box" must be combined with the use of ML to make these models transparent and interpretable. We leverage a variety of model explainability techniques in conjunction with our ML model to determine how individual variables of the body and surrounding environment contribute to the model's PMI estimation. Our analysis employs permutation importances, SHAP values, decision tree surrogates, and human-in-the-loop interactive tools to extract insights about the ML model of PMI prediction, providing quantitative assessments of how specific variables influence the complex decomposition process.[2-4]

Our results demonstrate the power of these explainability tools in interpreting PMI estimates. Feature importance analysis revealed that "desiccation" is the most critical feature, with a gain value of 205.461. This finding was corroborated by permutation importance analysis, where desiccation showed the highest importance of 0.193, indicating a substantial decrease in model performance when this feature is randomly permuted. SHAP analysis further validated these findings, with desiccation having an average SHAP value of 0.466, the highest among all features.

Interestingly, while advanced decomposition characteristics generally showed high importance, the early decomposition feature "skin discoloration" also emerged as significant, with the fifth-highest permutation importance of 0.032. Among environmental factors, our analysis identified "Precipitation standard deviation days 57-154" as the most important weather covariate, with an average SHAP value of 0.089.

To further enhance interpretability, we employed a decision tree surrogate model. Despite its simplicity, this surrogate achieved an R^2 of 0.675, comparable to our full model. The surrogate tree's structure reinforced our other findings, with desiccation as the top-level feature, followed by "livor mortis unfixed" and deposition site type at the second level.

By providing interpretable, and justifiable PMI estimations, we show how black box ML models can be made simultaneously to yield forensic insights and also to increase their transparency and openness to oversight.

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Decomposition; Postmortem Interval; Machine Learning

A81 A Postmortem Interval Estimation Method for Surface Depositions: A Template Applied in Southeastern Louisiana

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the progression of decomposition in southeastern Louisiana, particularly regarding surface deposition in wooded areas or fields.

Impact Statement: This presentation will impact the forensic community by demonstrating a practical method of Postmortem Interval (PMI) estimation that can act as a template for PMI estimation in any locale.

Abstract Text: Providing estimates of the PMI requires familiarity with regional sequences and patterns of decomposition. The Forensic Taphonomy Education and Research Facility (FTERF) at Louisiana State University provided anthropologists with the opportunity to study the progression of decomposition in southeastern Louisiana. The FTERF is located in the Cfa Köppen-Geiger climate zone (temperate climate, hot summers and no dry season) and eastern temperate forests (Level I ecoregion) on the Mississippi Alluvial Plain and Mississippi Valley Loess Plains (Level III ecoregions).[1-3]

Multiple predictive PMI estimation models have been developed by associating the stages and features of decomposition with accumulated degree days. Alternatively, the current method focuses on the first and last appearance of taphonomic features within a local sequence of decomposition. Predominantly, placement at the FTERF consists of surface deposition in wooded areas or grassy fields (under cages to mitigate scavenging); consequently, the method developed in this study focuses on taphonomic progression in those environments. It is expected that features included in the PMI estimation method would change according to the patterns observed in the ecoregion and depositional context in which it was developed.

For this method, the body was separated into four segments: head/neck, trunk, arms, and legs. For each section, the duration of expression was recorded for color changes, soft tissue changes, and degree of skeletonization by area (i.e., zero, < 50%, or >50% of the body segment was skeletonized). For example, green discoloration was visible on the trunk from day 1 to day 14 of placement at the FTERF. The duration of each taphonomic trait was obtained from photographs recording the progression of decomposition for 12 individuals; observation for each individual spanned the entirety of deposition at the FTERF. The dates of death and placement at the FTERF were never the same day; however, a fresh appearance (i.e., minimal color change) was required for inclusion. Both autopsied and non-autopsied individuals were considered.[4,5] Placement occurred throughout all seasons and the longest placement interval was 241 days.

To test the method, photographs from 15 observation days were culled randomly from the data of 14 individuals not used in method development. A single PMI estimate range was based on observed taphonomic features across all body segments while collectively considering the duration of each trait. Preliminary results indicate that 73% of the observation days fell within the estimate ranges. Additionally, while considerable overlap exists between the ranges of expression, some features can be broadly categorized as occurring in less than one month, between one and three months, and greater than three months. However, for every section except the trunk, a body segment could reach < 50% or >50% skeletonization by six days. Consequently, coloration and soft tissue changes should be given more weight than the degree of skeletonization when estimating PMI in southeastern Louisiana. Future refinement of this method will include increasing sample size, extending the maximum placement interval, examining pattern of skeletonization and weathering (rather than degree of skeletonization only), incorporating insect succession, and developing templates in different seasons, deposition contexts, and ecoregions.

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Taphonomy; Postmortem Interval; Environment

A82 Examining the Effects of Two Freshwater Environments on Decomposition and the Associated Microbial Communities

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Learning Objectives: Research on human decomposition in freshwater environments is limited, forcing researchers and law enforcement to utilize research from saltwater or terrestrial environments, even though there are fundamental differences. A discussion of the factors influencing decomposition in freshwater environments will allow for a greater understanding of the challenges associated with aquatic decomposition research. These factors include submersion and the water environment itself as well as the microbial communities present during decomposition.

Impact Statement: This study serves as a pilot study and proof of concept for future research to better inform the investigative community of a potential means for estimating Postmortem Interval (PMI) in a cold freshwater environment.

Abstract Text: Research on decomposition in aquatic environments is limited, compared to what is known about decomposition on land. Additionally, many of the taphonomy studies that have been conducted in lacustrine or riverine freshwater environments have focused on morphoscopic changes and scavenging. This study sought to determine the effects of two freshwater environments on decomposition by examining the impact of submersion and the water environment itself as well as the microbial communities associated with decomposition.

This research involved the placement of six fetal pigs in three different, but spatially related, decomposition environments. Two pigs were placed in a creek with a notable current; two pigs were placed in a nearby pond created by a beaver dam, and two were placed in a wooded area adjacent to both. Over a one-month period, water samples were collected from near the four pigs in freshwater environments and surface swabs from the two pigs placed on land. Additionally, notes and photographs were taken to document morphoscopic changes to the research subjects; additional data collected were ambient temperature, water temperature, surface temperature (of the specimens on land), rate of the current in the creek, water depth, and scavenger activity. All samples that were collected were submitted for 16s rRNA sequencing and bioinformatics to determine which changes took place over time with respect to the quantity and diversity of microbial communities.

By using pig proxies, this study serves as a pilot study and proof of concept that the researchers intend to build into a study involving human donors from the Body Donation Program under the Northern Michigan University Center for Forensic Anthropology to better inform the investigative community of a potentially novel means for estimating PMI in a cold freshwater environment. Notable differences were observed between the rates of decomposition among the research subjects in the three environments, including their visible progression through decomposition and insect activity. Decomposition rate was found to be highly influenced by environmental factors such as weather, water type, and depth. Changes to the microbial communities over time were also affected by the environment, with notable differences having been observed between what was present prior to specimen placement and which microbes become or remain dominant over time in each sample collection location.

Decomposition; Freshwater; Postmortem Interval

A83 Differences in Tissue Consumption by Maggots

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Learning Objectives: From this presentation, attendees will gain insight into the differential consumption of soft tissue types by maggots during decomposition. They will understand how variations in body composition, particularly muscle and fat content, can influence decomposition rates and potentially affect Postmortem Interval (PMI) estimations. This knowledge will enable forensic professionals to consider body composition as a factor when estimating time since death intervals in forensic casework.

Impact Statement: This presentation will enhance the forensic science community's understanding of the relationship between body composition and decomposition rates, potentially improving the accuracy of PMI estimations. By highlighting the preferential consumption of muscle tissue by maggots, this research emphasizes the need to consider biomass and individual body composition when interpreting decomposition patterns, which could lead to more precise and reliable PMI estimations in death investigations.

Abstract Text: Several different families within the order Diptera, or flies, include insects that consume dead and decaying tissue and thus play an important role in the rate of decomposition and the estimation of the PMI.[1-6] The most common fly families associated with the consumption of dead tissues are Calliphoridae, Sarcophagidae, Muscidae, Piophilidae, Phoridae, and Stratiomyidae.[5] Fly larvae (maggots) are laid quickly after death occurs and utilize dead tissue as a main food source. A single female fly may lay up to 300 eggs, which will hatch and begin the feeding process within 1 to 2 days.[5] Once hatched, maggots will first consume serous fluids followed by soft tissues, which results in removal of the biomass of the remains.[5,6] As maggots are responsible for part of the decomposition process, the association between maggot feeding activity and decomposition rate has been examined closely in relation to its impact on determining time since death in forensic casework. However, it is unclear exactly which types of soft tissues are consumed by maggots, which may ultimately affect the estimation of time since death since individuals have different amounts of soft tissue types on their body.

To determine the soft tissue types maggots consume, four tissue samples were obtained from two willed body donors at the Forensic Anthropology Research Facility (FARF) at Texas State University. The tissue samples included subcutaneous fat, muscle, skin, and a cross section of the three tissues. The same amount and weight of tissue types were collected from each donor at the beginning of the data collection process and placed into separate open glass jars. The jars were placed in an open-air plastic tub and under a metal cage to avoid being scavenged while still allowing for insect activity. The jars were monitored and weighed daily at the beginning of data collection and then moved to every three days once insect activity began to slow. Data collection occurred in the spring of 2022 and 2023.

The data did not meet the criteria for parametric tests and a Kruskal-Wallace test was used on each data set. For each round of data collection, there was a significant difference ($P < 0.001$) in the biomass lost between tissue types, with muscle having the smallest mean rank and the greatest amount of biomass lost, followed by the cross-section sample (that included muscle). The observations made during the data collection process support these results as there was a significant amount of maggot activity on the muscle tissue and comparatively little on the other tissue samples.

The results of this study demonstrate significant differences in maggot feeding preferences among soft tissue types, with muscle tissue consistently showing the greatest biomass loss. These findings have implications for forensic science: individuals with higher muscle mass may exhibit accelerated decomposition rates during stages of maggot activity, while those with higher fat content may decompose more slowly, since maggots do not consume fat tissue. Future studies should expand on these findings by increasing sample sizes and examining seasonal variations, further refining our understanding of the complex relationship between taphonomic agents, body composition, and decomposition rates.

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Decomposition; Soft Tissue; Postmortem Interval

A84 A Homogenous Diet in a Diverse City: Understanding Isotopic Dietary Variation and Its Forensic Implications in San Francisco, California

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Learning Objectives: In this presentation, attendees will understand the variability of dietary isotopic signatures in cultural enclaves in the city of San Francisco. In reviewing current studies frequently demonstrating homogeneity within the country and increased heterogeneity between countries in assessing region of origin, the audience will see if cultural enclaves with roots in various countries around the world reflect isotopic trends in those regions or if the continental supermarket diet permeates cultural variations in increasingly diverse urban contexts.

Impact Statement: This presentation will impress upon the audience the necessity of context when applying dietary isotope methods in forensic science. When addressing region of origin estimates in a global context, regional trends in dietary isotopes may be appropriate; however, they lose their potency and accuracy when assessing region of origin in urban forensic casework.

Abstract Text: Stable isotope analysis as a method has grown in popularity in recent decades. Particularly in forensic anthropology, elements like carbon, nitrogen, and oxygen have demonstrated the ability to inform on the food and water consumed by an individual, which can be used to estimate region of origin. Much of the stable isotope applications first arose in the bioarchaeological literature, wherein the food that was consumed by an individual was somewhat diagnostic of the region of the world the individual was from. Isotope data indicating diets primarily composed of corn or millet versus wheat were indicative of different cultures in the archaeological record and could provide meaningful insight into where those individuals belonged.

The modern United States population exhibits a somewhat homogenized diet, often referred to as the "continental supermarket diet." Additionally, other regions, like Mesoamerica and Southeast Asia, have also been characterized by larger dietary patterns that differ isotopically from the continental supermarket diet. However as forensic anthropologists begin applying these broad categories into specific individualized forensic cases within the United States, the method and assumptions therein may be missing the mark. While there is isotopic evidence to support an increasingly homogenized diet within modern countries, much more data from diverse contexts throughout the United States and abroad is necessary for this pattern to hold.

Community-level isotopic data has the potential to challenge our current methodological assumptions when it comes to interpretation of region of origin by diet. While the United States as a whole has a diagnostic isotope signature, there are many cultural enclaves wherein the diet of community members may look different than what one would expect to see at a country wide level. Specifically, hair samples from the cultural enclaves of North Beach (Italy), Chinatown and the Sunset Chinese Cultural District (China), Calle 24 (Mexico), Japantown (Japan), and SOMA Pilipinas (Philippines) were studied and compared with average country-specific or regional isotopic dietary signatures.[1]

This poster assesses carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) from 47 samples of hair from six different cultural enclaves to assess if dietary homogeneity is true in the city of San Francisco. Mean values of carbon (-17.9‰) fit within the typical range in the United States (-17.6‰ ± 1.2‰) (1). Further, mean nitrogen for the San Francisco population (9.7‰) are captured by the upper bounds of the range of typical values for United States populations (8.9‰ ± 0.9‰).[1] When assessing variance of diet between populations of different enclaves, no statistically significant difference was seen in both carbon ($F(4,42) = 0.9$, $p = 0.46$) and nitrogen ($F(4,42) = 1.1$, $p = 0.38$). In individual comparisons of enclave means compared to source country average values, all San Franciscan enclave nitrogen fit in the typical range for each country of origin. However, those populations from SOMA, Chinatown, and the Sunset Chinese Cultural District fall outside of the typical carbon signatures for the Philippines and China, respectively.

Research within the method is increasingly indicating that socioeconomic and cultural factors play a significant role in the isotopic values in human tissues. Especially as the use of stable isotopes in forensic casework increases, understanding the nuances within populations on which the method is employed and the different factors that can influence our interpretations of data is critical. While results from this study suggest that the larger population level dietary data in the United States holds true, this research also underscores that forensic practitioners must be careful in their interpretation of isotopic data at the individual level.

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Stable Isotope Analysis; Identification; Provenance

A85 On FIRM(S) Footing?: A Review of the Use and Reporting of Stable Isotopic Analysis in Forensic Anthropological Dissertations

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Learning Objectives: Attendees will leave with an understanding of the rigor with which stable isotope best practices are being adhered to and taught within the field of forensic anthropology. They will specifically learn how well these best practices are being implemented in graduate student research, which can be extrapolated to assess the future use of the method as young researchers move forward in their careers. Attendees will come away with a thorough understanding of what best practices are recommended for the use of stable isotopes in forensic anthropology, where the field is doing well in their adherence to these standards, and areas for improvement.

Impact Statement: Stable isotope analysis is becoming a commonly used tool in forensic anthropology, and this presentation will provide important feedback on the effectiveness of its use in the field and the ways in which its use can be improved so as to better serve the purpose of medicolegal investigation and identification.

Abstract Text: Forensic anthropology is a subdiscipline born out of the social sciences that simultaneously requires the rigor of hard sciences. Analytical semi-quantitative methods have become increasingly prevalent within forensic anthropology, including stable isotope analyses. Yet while these methods grow in popularity, practitioners must be wary that the knowledge gap between those generating the data and those interpreting the data does not grow as well. While work has been done to call attention to this need for increased rigor in data reporting in anthropology at large, forensic anthropologists applying stable isotopes should also refer to the Forensic Isotope Ratio Mass Spectrometry (FIRMS) Network for guidance. FIRMS has produced best practice guides for data generation and reporting and interpretation in a medicolegal context and has been in place to address isotope data quality issues for over 20 years.[1,2]

As a preliminary step in understanding adherence to these best practices, this poster assesses theses and dissertations from 2008 to 2023 where stable isotopes were employed in forensic anthropological contexts. Academic products such as dissertations are unique in that academic programs allow for larger amounts of data collection, analysis, and interpretation. In these contexts, students learn how to do research under the guidance of practicing professionals. It stands to reason that in these documents, students will describe the methods for preparing samples and subsequent analyses. Thus, understanding where students are at in their understanding and application of best reporting practices in stable isotope studies is a telling metric as to how the subdiscipline is currently upholding these standards and how we will continue to do in the future.

Thirty-three university repositories were searched for papers from 2008 to present produced from a graduate of the department of anthropology with a focus on forensic anthropology and the inclusion of stable isotope analyses. From these 33 programs, 20 documents were attained. Scoring of these papers occurred according to a rubric that judged the presence and absence of a number of criteria related to sample preparation, as well as reporting of analytical accuracy and precision. These criteria were pulled from both best practice guides developed by FIRMS as well as similar work done in the field of archaeology and best practices developed for archaeologists.

All papers reported raw isotopic values for samples and all reported that the author conducted sample preparation. However, only 80% detailed what steps were taken to prepare tissues, 15% referenced the methodology discussed in an outside publication, and 5% did not detail prep methods at all. In understanding how these papers reported about the analytical side of their study, 95% reported the analytical lab where analyses were conducted; however, only 75% of papers discussed the instrumentation used in those analyses. The reporting of calibration standards was also assessed, finding that only 45% of papers detailed the standards used by analytical labs. Though 25% mentioned that standards were used with no further information, 30% of theses and dissertations did not discuss standards at all. It should also be noted that these criteria assessed presence and absence, variation in which prep methods were used, which instrumentation was employed, and when and how standards and error were disclosed were significant.

Though the sample size is small, the associated degree of variation is cause for deeper investigation. The lack of consistency in reporting, both of sample preparation and of analytical factors, underscore that best practices for data reporting and interpretations in forensics are not being taught. As the push for rigorous methods in the forensic sciences grows, forensic anthropologists must be cognizant of best practices when studying and applying stable isotope analysis in forensic contexts.

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Stable Isotope Analysis; Best Practices; Student Training

A86 The Implications of Quantitative Spectral Analysis of Human Cremains in the Texas State Donated Skeletal Collection (TXST-DSC)

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Learning Objectives: After attending this poster presentation, attendees will have a better understanding of the challenges of identifying burned human remains and the analytical techniques that are being tested to help investigators refine methodology.

Impact Statement: This presentation will impact the forensic community by providing attendees with new tools to use in the future regarding unidentified and/or commingled human remains. The proposed methods open a new realm of research and applied functions for the serious and growing global concern of unidentified, burned, and sometimes commingled human remains.

Abstract Text: Identifying thermally altered or burned remains presents a formidable challenge, as does the identification of commingled remains.[1] When these two complexities are combined, the task of making accurate, efficient, and cost-effective identifications becomes even more difficult and, at times, appears insurmountable. In situations where DNA extraction fails to address issues of commingled remains, often due to the condition of the remains, alternative methods must be explored to initiate the identification process by distinguishing skeletal elements.

Cremains are representative of the most highly thermally altered human remains and occur both as a product of past and present mortuary behavior as well as through accidental and homicidal means. While past studies have been primarily focused on examining intentional cremation produced in the execution of mortuary practices, very limited research has been done on cremains produced in the course of accidental or homicidal means, and no research has been conducted on elemental variation among individual sets of cremains.[1-5]

In this research, both portable X-Ray Fluorescence (p-XRF) and Fourier Transform Infrared Spectroscopy in the Attenuated Total Reflection (FTIR-ATR) mode were used to investigate the characteristics retained in human cremains that may help resolve future cases of commingling and unidentified persons.

A random sample of 13 sets of cremains was analyzed with the age at death ranging from 47 to 91 (mean 64) years old and the temperature of cremation ranging from about 1,100 to 2,300 degrees Fahrenheit. The cremains were sifted through a 35-mesh (500 microns) sieve, and about 5 grams of bone ash were retained for both analyses. Spectral data were produced using both chemometric methods and were analyzed for any individualizing characteristics.

Research on FTIR-ATR use for age estimation is very new and limited, but preliminary results show potential for accurate age estimations.[6,7] In this project, Kruskal-Wallis tests showed a statistically significant result when comparing age with the Crystallinity Index and Carbonate-to-Phosphate ratio in the baseline corrected cremains spectra. Additionally, the Crystallinity Index and the Carbon-to-Phosphate ratio showed some significant potential in Principal Component Analyses (PCAs) and decision trees. Decision trees on the data set created an accuracy of 38% with the Crystallinity Index as an important splitting factor.

Results from the pXRF analysis revealed multiple chemical elements, including Zn, Ti, and K, may be used for decommingling in the future with greater streamlining of analysis. Two individuals clearly separated as outliers from the other samples during a cluster analysis that was conducted. However, the high dimensionality of the data (more features than observations) precluded the pXRF analyses from being able to make broad statistical claims about the usefulness of pXRF on human cremains. A Technical Error of Measurements (TEM) and relative Technical Error of Measurement (rTEM) tests were conducted on the pXRF data and produced unacceptable error rates (>5%) of approximately 12% of the data, which suggests that the pXRF unit being used was producing unreliable readings.

Overall, the results of the FTIR analysis show significant differences in the Crystallinity Index and Carbonate-to-Phosphate ratio but no significant results in other parameters that might correlate with age and sex. Similarly, the pXRF results had some promise, but both methods suffered from a limited and relatively homogenous sample. Future research with a larger, more biologically diverse sample of cremains and a more reliable pXRF unit is indicated.

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Cremains; Identification; Spectroscopy

A87 The Development of a Matrix-Matched Reference Material for Quantitative Elemental Analysis of Bone

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Learning Objectives: This presentation focuses on the synthesis and application of reliable, matrix-matched calibration standards for the quantitative chemical analysis of bone. Attendees will understand the importance of chemical reference materials for forensic investigations of skeletal remains and how ongoing research is making advancements toward this analytical necessity.

Impact Statement: A matrix-matched bone reference material is critical for accurate and reliable chemical measurements of forensic bone samples. The development of calibration standards for bone improves the detection and quantification of trace elements in bones that may be attributed to biological or environmental factors.

Abstract Text: Elemental analysis of bone samples using chemical techniques, such as X-Ray Fluorescence Spectroscopy (XRF) and Laser-Induced Breakdown Spectroscopy (LIBS), is gaining popularity in the fields of forensic anthropology and bioarcheology. Detecting the elemental profile of a bone can help provide contextual understanding. For example, the presence of trace elements such as lead or mercury in bone may indicate toxic exposure within an individual's lifetime. Strontium and barium have historically been used in research as dietary indicators for skeletal remains. The elemental profile of bone has also been studied as a potential tool for sorting commingled remains.[1] Bone's elemental composition may also serve as a potential informer of any diagenetic chemical alterations that occur after death.

Quantifying the concentration of an element within a sample using analytical techniques requires calibration standards. Unfortunately, there is a lack of suitable reference materials that can replicate the chemical and physical matrix of bone samples. Commonly used calibration materials include glass, bone meal, or lab-synthesized hydroxyapatite pellets—all of which fail to fully mimic the physicochemical properties of bone. Therefore, a better bone standard must be created to ensure reliable measurements of elemental concentrations can be made for forensic bone samples.

The objective of this research study is to develop a matrix-matched calibration standard for the quantitative elemental analysis of bone. A fibrillar collagen framework mineralized with hydroxyapatite crystals has been synthesized using a co-precipitation technique. We have shown that this composite material chemically mimics bone through Fourier-Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Laser-Induced Breakdown Spectroscopy (LIBS) spectra. FTIR reveals the amide-based N-H, C=O, C-N, and C-H bonds from collagen, as well as hydroxyl, phosphate, and carbonate signal from hydroxyapatite. XRD confirms the crystalline structure of hydroxyapatite. LIBS spectra of the standard reflect an elemental profile that aligns with that of bone samples. Further, digital microscopy proves the presence of embedded crystals within a porous collagen matrix. Following the creation of a matrix-matched blank, trace elements of interest (Sr, Ba, Fe, Al, etc.) are incorporated at known concentrations into the material. Analytical validation of the elemental concentrations of these reference standards is performed using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) to build preliminary calibration curves. The results of this ongoing research show that critical steps are being made toward the production of a reliable, lab-synthesized reference material for bone. The development of such a material will benefit forensic practitioners in anthropology and archaeology through its ability to produce trustworthy quantitative elemental measurements from bone samples.

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Bone; Spectroscopy; Anthropology

A88 An Examination of Volatile Organic Compounds Associated With Human Decomposition Byproducts in Soil

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Learning Objectives: After attending this presentation, attendees will understand the complex issues and limitations associated with collecting, storing, and analyzing soil for the presence of Volatile Organic Compounds (VOCs) associated with human decomposition.

Impact Statement: This presentation will impact the forensic science community by presenting a novel means for testing soil for the presence of human decomposition byproducts and, more importantly, by highlighting the importance of appropriate sampling and analysis methodologies.

Abstract Text: VOCs are known to be released by all living organisms during life and after death. The presence of certain VOCs can serve as veritable signatures for specific types of organisms, for decomposition, and for human decomposition, particularly. This research was inspired by a question frequently asked by the law enforcement community—If I am looking for the remains of a missing person, but I don't know exactly where to look, what sort of non-invasive or minimally invasive detection technique(s) can be used to help narrow the search?

Samples for this research were collected from Cadaveric Decomposition Islands (CDIs) at the Northern Michigan University Forensic Research Outdoor Station (FROST), which remained visible to the naked eye after the donors who had been allowed to decompose in those locations had been recovered from the facility and relocated to the Forensic Anthropology Research Laboratory (FARL) for curation. Sample collection took place in November of 2022 and involved the collection of soil from CDIs that had held human donors at one time between 2018 and 2022. A total of 35 CDIs were sampled for this analysis. Some were as fresh as three days between the removal of the donor remains and collection of samples and as old as four years between donor removal and sample collection. Each soil sample was collected in a conical tube and submitted for gas chromatography/mass spectroscopy headspace analysis. Homogenized soil samples that were collected prior to the placement of any human remains at the FROST facility were analyzed as controls.

There are numerous complexities associated with the collection and storage of materials that are intended to be analyzed for the presence of VOCs. Results from this research were surprising in that even in locations where human decomposition byproducts were plainly visible and were still emitting significant odors detectable by the human nose, none of the CDI samples showed any significant difference from the pre-donor placement controls. This presentation focuses on the potential limitations of techniques used for sample collection, storage, and analysis for this particular approach to detecting VOCs and how these issues can and likely will affect the results and therefore, potentially, their value to the investigative community.

Decomposition; Volatile Organic Compounds; Soil Analysis

A89 Assessing the Micro- and Macroscopic Changes of Chemically Altered Bone

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Learning Objectives: Attendees of this presentation will gain a deeper understanding of the micro- and macroscopic alterations to bone that can be expected in scenarios in which human remains have been exposed to corrosive chemicals found in common household products. This is especially important in cases where perpetrators intend to obscure a decedent's identity through irrevocable harm, subsequently complicating the identification process.

Impact Statement: This presentation will impact the forensic community by describing the micro- and macroscopic changes occurring in skeletonized remains after exposure to common household chemicals. This research holds implications related to identifying taphonomically altered human remains and better understanding their taphonomic history.

Abstract Text: Forensic identifications may be hampered due to exposure to high chemical concentrations. Additionally, perpetrators search for unique ways of obscuring their victims' identities, such as readily accessible household corrrosive substances. Sodium hydroxide (NaOH), and hydrochloric acid (HCl) are known to change both the structural integrity and composition of skeletal elements. Both hydroxyapatite crystals and DNA become chemically modified by the pH of the depositional encironment.[1]

Chemical studies focus on total dissolution times, rather than on discrete changes over time. However, it is well documented that household corrosive substances are used to disfigure victims due to their high availability and apparent effects.[2] Sodium hydroxide (NaOH) is a corrosive base that effectively dissolves soft tissues and produces high temperatures through violent exothermic reactions, but ostensibly fails to alter the structure and color of bone. Hydrochloric acid (HCl, 31.45%) appears to be among the most destructive chemical agents with all biological tissue dissolving within 24 hours.[2] Studies are often difficult to compare, though, given the use of different brands and concentrations for experimentation.[1]

The aim of this study is to compare and analyze the micro- and macroscopic changes occurring in fully skeletonized remains exposed to household chemicals over the course of a maximum of two hours. Furthermore, this study intends to test how bone shape and volume to surface area ratios may affect the degree of alteration.

Hand bones, from three donated individuals, were exposed to three separate treatments: controlled fire, sodium hydroxide, and hydrochloric acid. Eight elements (carpals, metacarpals, and phalanges) were used for each treatment.

The chemical alteration protocol consisted of four intervals (total: 2 hours) with bone pairs being pulled every 30 minutes from their respective solutions (NaOH or HCl). The Power Crystal Lye Drain Opener (100%) was mixed into a 1:1 ratio (~25M) of 100g NaOH:100mL H20 per cup and the 100mL TransChem Muriatic Acid (31.45%) per cup was left undiluted. pH was tested after bone removal at each interval.

Pre- and post-treatment length, width, volume, and surface area data were recorded using the Artec Spider hand-held 3D scanner and bone weight was measured to the closest ±0.01g. Bones were examined and photographed with an Echo Revolve inversion microscope and Olympus SZX16 whole specimen microscope. All statistics were run using *GraphPad Prism 10*.

Bones soaked in NaOH showed changes in coloration and experienced minimal bone breakdown only after 1.5 hours. Changes in solution pH were minimal within the first hour but stagnated for the remainder of the experiment. The elements soaked in HCl showed changes within the first 30 minutes. The bony surface appeared crystalline and exhibited signs of cortical flaking and bone breakdown. The density of the bone appeared to be compromised after 1.5 hours, with a complete hollowing of the tubular bones after 2 hours. The solution pH remained unchanged throughout the experiment.

Surface area and volume displayed strong negative correlations, with bone surface area (and thus attack area) increasing as volume decreases. As expected, the relationship between these factors is not linear, and the results show a variable pattern of alteration depending on the treatment and duration of exposure. These observed trends provide justification for further analysis with larger sample sizes, longer durations, and more forensically significant conditions.. Additional research is currently ongoing to evaluate the possibility of DNA isolation and profiling.

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Taphonomy; Forensic Analysis; Human Remains

A90 Bullet Grazing: Using Laser-Induced Breakdown Spectroscopy to Analyze Metallic Staining on Skeletal Remains

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Learning Objectives: After this presentation, attendees will learn a new approach to evaluating materials that appear to be metallic staining on skeletal remains.

Impact Statement: This presentation will introduce the forensic science community to the detection of metallic intensities within potential metallic staining on skeletal remains using Laser-Induced Breakdown Spectroscopy (LIBS).

Abstract Text: Forensic anthropologists are not only tasked with creating a biological description of the decedent from skeletal remains, but they also provide information on antemortem pathologies and idiosyncratic traits, perimortem trauma, and postmortem change to help narrow down the parameters for positive identification of the decedent on behalf of the medical examiner/coroner. The use of LIBS can bring elemental analysis to forensic anthropological casework, like identifying metallic traces, formalin, and uniting isolated remains from mass fatality or commingled assemblages.[1,2] In recent years, LIBS has grown in popularity and has impressed scientists with its ability to perform spectral chemical analyses of organic and inorganic elements by utilizing a high-powered, minimally distractive pulsed laser that oblates the sample's surface, creating vaporized plasma for quantitative spectral analysis at various wavelengths (nm).[3] Though researchers and forensic laboratories are using LIBS to quantify typical qualitative observations (e.g., soil, ink, and glass discrimination), researchers have not addressed its ability to identify metallic elements from metallic oxidation staining on human remains.

In this case study, closed and redacted forensic anthological case reports and private anatomical donors from Florida Gulf Coast University's Human Identity and Trauma Analysis (HITA) laboratory where LIBS (System 150 Nano Series Pulsed Nd: YAG laser – RMA 0346-00) was used to detect traces of possible increased metallic properties on areas with presumed metallic staining were evaluated for this pilot study. Following the American Society for Testing and Materials (ASTM) and National Institute of Standards and Technology (NIST) standards, these skeletal fragments were examined for color change using the Munsell Soil Color Chart, consistent with the oxidation of copper, a common metal associated with firearm ammunition.[4,5] The LIBS pulsed the midpoint of the surface on the skeletal fragments and produced spectral analyses from 490nm to 730nm. The LIBS application software is linked with the NIST spectral standards library. The instrument identified peaks of Copper (Cu) and Lead (Pb) in the discolored bones—a finding consistent with bullets. The intensities (au) between the stained bone and the controlled sample without staining trended in a positive correlation ($r = 0.805$, $p = 0.015$; $r = 0.995$, $p < 0.001$; respectively) and suggested that there are statical differences between remains that had contact with oxidized metallic fragments. This study revealed that LIBS could detect increased metallic intensities on skeletal elements, bringing quantified analyses to forensic anthropological casework and deepening the identification of perimortem trauma.

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Skeletal Remains; Metallic Staining; LIBS

A91 An Analysis of Color Changes in Thermally Altered Bones Using Spectrophotometery

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Learning Objectives: After attending this presentation, attendees will understand the need for renewed discourse, research, and standardized data collection for characterizing different heat-related color changes observed on burned human remains and bones in a forensic context. In this research we applied a spectrophotometer to burned bone samples to standardize the interpretation and communication of color changes in burned bones. Spectrophotometers can definitively identify colors and their components (density) and using the LAB color system can identify and quantify (ΔE) the difference between colors.

Impact Statement: This presentation will impact the forensic science community by discussing the standardization of assessing thermally altered burned bone color changes. Transitional colors develop superficially from the thermal decomposition of bone's organic tissues within the mineralized inorganic skeletal matrix.

Abstract Text: It is well established that color changes in thermally altered bones from forensic contexts cannot and should not be used to estimate maximum temperature ranges of the fire.[1-6] While color changes in burned bone cannot be reliably linked to temperature, they are indicators of thermal decomposition at the cellular level and thus differences in color are indicative of altered levels of in/organic degradation and destruction. In 2022, Pope, Juarez, and Galloway published a classification system for thermally altered human remains with forms for charting in situ burning damage.[2] This work is an extension of that classification system, providing a more nuanced understanding of bone color changes, and standardizing data collection applications of color changes for charting and data collection.

Ten fragments of burned bone were selected, measurement locations were identified using metric measuring tape with light adhesive backing applied to bone surfaces with distinct color changes. Bones were measured at 0.5cm intervals through the entirety of the color transition from unburned bone through either carbonization or calcination using a Nix Spectro 2 31 channel spectrophotometer. The spectrophotometer identified each data point as a specific color reporting a spectral curve, density readings, and LAB, CMYK, and RGB data. Five bones were retested within each visually distinct burned region (e.g., carbonization, calcination, etc.) five times using the Nix toolkit software quality control to test reproducibility (N=67 points). Regions between bone samples that appeared visually similar (e.g., carbonized, calcined) were also compared using the same methodology (N=25).

N=338 individual scans along the selected demarcated linear paths were made, and N=332 individual colors (ΔE=0) were identified between all samples. Reclassification of five study fragments had mixed combined results with 17.9% error. However, some fragments performed much better on reclassification with 0% error. When bones that appeared visually similar (appeared to have the same color) were compared across specimens, similarity was confirmed in only 20% of cases.

In the initial identification of colors, hundreds of colors were identified by the spectrophotometer, indicating both instrument sensitivity and also the depth of the range of color changes. Bone colors are evidence of the direction of fire and the level of bone alteration. Currently our discussions and descriptions of color are vague and unreliable. The use of a spectrophotometer allows us to standardize the discussion and description of color changes and provides a more nuanced understanding with mineable data for researchers. Repositories of cataloged color spectrums for burned bones can be shared and accessible to larger international libraries. The Nix Spectro 2 has a highly sensitive 2mm aperture, thus exact repositioning is critical for accurate reclassification. The higher-than-expected combined reclassification error for all tested fragments reflected positioning errors rather than instrument error, which was tested via calibration between samples. Future work using the spectrophotometer may provide a range of colors that can be classified as "carbonized" or calcined with shareable libraries providing researchers with detailed spectra to investigate.

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Burned Bone; Color; Spectrophotometer

A92 The Statistical Relationship of Breakaway Spurs and Notches With Saw Class Characteristics in Experimental Dismemberments

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Learning Objectives: Following this presentation, attendees will understand the statistical relationship between bony breakaway spurs and notches and saw class characteristics.

Impact Statement: This presentation will benefit the forensic science community by presenting statistical results from experimental dismemberments highlighting the potential utility and limitations of breakaway spurs and notches in forensic anthropological case interpretations.

Abstract Text: Bony breakaway spurs and notches that result from human dismemberments have been suggested to indicate saw power, with mechanically powered saws reportedly producing more and larger spurs/notches given their weight, torque, and force.[1] Experimental research supporting this relationship, however, is limited and many studies utilize de-fleshed bones and vices to stabilize the bones, creating a potentially different biomechanical environment than what one would expect in a forensic case. This study aimed to statistically investigate the relationship between breakaway spurs/notches and saw characteristics using two samples: a de-fleshed, viced sample and a fleshed free-sawing sample. Complete cuts were assessed from samples created by researchers at Mercyhurst University (n = 182; de-fleshed humeri and femora in vices) and Des Moines University (DMU) (n = 239; fleshed lower limb bones cut using a free-sawing methodology). A total of 33 hand or mechanical reciprocating saws were utilized. Breakaway spur and notch presence/absence were documented for each complete cut. When present, breakaway spur/notch length and width were recorded. These data were compared to sample, saw power, teeth-per-inch, tooth set, tooth shape, and cortical bone thickness using chi-squared tests, non-parametric Analysis of Covariance (ANCOVA), Mann-Whitney U tests, and correlation analyses

After removing outliers, the total sample size was 349 cuts. Chi-squared tests indicated significant differences in the frequency of breakaway spurs and notches between the Mercyhurst and DMU samples. Mercyhurst had breakaway spurs in 77.5% of the sample but only 37.5% displayed notches (some displayed a combination of spur/notch). DMU had spurs in 31.2% of the sample and notches in 52.4% of the sample. There were also significant differences in cortical thickness, spur width, and notch width between the samples ($p < 0.05$). Quade non-parametric ANCOVA results suggest that the group differences in spur/notch measurements remain even after considering cortical thickness as a covariate ($p < 0.05$). It is challenging to interpret the sample differences, as they could be related to fleshed/de-fleshed samples, sawing methodology, or specific saws utilized, and the current study is restricted to the published data from the Mercyhurst project. If analyzing the DMU sample in isolation, chi-squared analyses indicate no significant differences in spur/notch frequency with power, but significant differences in tooth shape (more likely to occur with rip saws). Mann-Whitney U tests indicate significant differences in cortical thickness between breakaway spur presence/absence groups ($p = 0.027$), but not breakaway notches ($p = 0.907$). Interestingly, when spurs/notches were present, significant correlations were found between cortical thickness and spur/notch size, as well as significant effects of power on spur/notch sizes ($p < 0.05$).

These results suggest that tooth shape (rip versus crosscut) influences breakaway spur/notch presence more so than power. If present, power and cortical thickness affects the size of the spur/notch. Interestingly, no significant relationships were found if the Mercyhurst sample was analyzed in isolation. This discrepancy highlights possible differences in the use of fleshed free-sawing and de-fleshed viced samples, which may be explained by the different biomechanical forces at play in the two different cutting circumstances. Additional research is underway to test a DMU de-fleshed and viced sample that is matched by saw and donor to the fleshed sample to control for all variables except sawing methodology.

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Dismemberment; Saw Marks; Kerf

A93 Damage to the Thorax From CPR and Vulture Scavenging: A Case Presentation From the University of South Florida Donated Skeletal Collection

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Learning Objectives: This poster will present the case of a donor from the University of South Florida (USF) Donated Skeleton Collection that presented damage to the rib cage from both chest compressions and postmortem damage caused by vultures. Following this presentation, attendees will be able to use biomechanics to differentiate between injuries occurring in the perimortem interval and those created postmortem by vultures while bone is still wet.

Impact Statement: This case presentation highlights the importance of both biomechanics and forensic taphonomy in forensic investigations. This presentation will impact the forensic community by highlighting how the successful application of biomechanics can be used to differentiate a fractures origin.

Abstract Text: Vultures are prolific scavengers and have great potential to modify not only death scenes, but the physical body as well. Studies at other outdoor research facilities have shown that vulture scavengers can cause fractures to skeletal elements such as the ribs and scapulae.[1,2] Defects created by vultures can present a challenge in determining the timing of the injury depending on the wetness of the bone. In cases where remains are already skeletal, cause and manner of death may be impossible to determine without physical evidence on the skeleton. Decedents that are scavenged may present injuries that have the appearance of occurring perimortem with no other evidence of trauma, which may confuse cause and manner of death. For this reason, the value of taphonomy in forensic investigations cannot be underestimated, as many taphonomic alterations have the potential to be misinterpreted providing different context for the current condition of the remains.

The purpose of this study is to demonstrate how fractures created through perimortem trauma and vulture scavenging can be differentiated using a foundation in biomechanics. A donor was selected from the USF Donated Skeleton Collection and presented with both perimortem trauma to the thorax and extensive vulture scavenging throughout the body. The donor selected for this case study was a 67-year-old, White female whose cause of death was a combination of cardiomyopathy, respiratory arrest, sepsis, and congestive heart failure. In the perimortem interval, cardiopulmonary resuscitation was attempted, which was evident upon intake and placement. Bruising to the chest was observed, and following decomposition, fractures to the sternal body, left second, third, fourth, fourth, fifth, sixth, seventh, and eighth ribs and second, third, fourth, and fifth right ribs were observed. These fractures generally presented as complete fractures to the sternal third of the rib excluding the left third rib, which also had a complete fracture at the angle. The sternal body presented both incomplete and complete fractures. The force loaded upon these elements came from anterior as evidenced by where the bone ultimately failed. This donor was placed on the surface at the USF Facility for Outdoor Research and Training (USF-FORT) for 119 days. While placed at the USF-FORT, this donor was scavenged 11 times, which first occurred 12 days following placement. At analysis, the donor presented several defects throughout the body that were indicative of vulture scavenging, which included punctures to the sacrum, os coxae and the cranium from vulture beaks. An incomplete fracture was observed on the caudal surface of the first rib on the sternal third of the rib. Although this fracture appeared to occur during the perimortem interval, the position of the fracture suggests that its source was different from the other observed fractures. The fracture occurring on the caudal surface is indicative of tension occurring on that surface which is not consistent with the direction of force used during chest compressions. This could be the result of either vultures pulling on the first rib, creating tension on the caudal surface, or the weight of vultures standing upon it following disarticulation. Through the synthesis of biomechanics and forensic taphonomy, the true origin of fractures can be differentiated even in cases where both occur.

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Taphonomy; Trauma; Scavenging

A94 Squirrels in the Desert: Regional Scavenging Patterns

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Learning Objectives: After attending this presentation, attendees will understand the importance of considering environmental context in cases with scavenging activity.

Impact Statement: This presentation will impact the forensic science community by applying ecological data to better understand a less common scavenger.

Abstract Text: Scavenger activity can be heavily influenced by the local environment.[1] When resources are limited, animals are more likely to scavenge remains they discover and may consume more tissue.[1] Marginal environments, where resources are limited, may further emphasize these differences. The Forensic Investigation Research Station (FIRS) at Colorado Mesa University is in an environment that fits this description, a cool semi-arid steppe (BSk).[2] The FIRS facility studies the decomposition of human remains and the influence of the local environment on that process. The facility's surroundings are limited in water and food resources for local scavengers.

Rock squirrels (*Spermophilus variegatus*) are ground squirrels common in the American southwest and Mexico.[3] They are diurnal with active times that shift with seasonal temperature changes.[4] While most of their diet consists of plant material (e.g., berries, seeds, nuts, green vegetation), rock squirrels also eat insects and other animals (both carrion and prey).[3] Generally, rock squirrels are not selective in food choice during the early spring but discriminate more as resource availability increases later in the year.[4]

Over the span of seven days in March 2024, a rock squirrel scavenged soft tissue from the right arm of a 42-year-old female in early decomposition. Scavenging impacted skin and fat from the inner right elbow. There was minimal impact to muscle tissue and bone was not exposed. The squirrel scavenged exclusively in daylight, visiting the body one to three times per day for up to 16 minutes at a time. While rock squirrels do scavenge, this is not a large part of their diet.[3] Generally, rock squirrels target smaller animals (e.g., rodents, rabbits, birds), but given the time of year and limited resources in the surrounding area, scavenging human remains may be expected.[4]

A similar species, the eastern gray squirrel (*Sciurus carolinensis*), also supplements a predominantly plant-based diet with carrion.[5] When it comes to human remains, the eastern gray squirrel generally gnaws bone rather than consuming soft tissue.[5] This difference in scavenging behavior between the two species may relate to a drastic difference in habitat. While rock squirrels tend to live in marginal environments where resources are often limited, eastern gray squirrels tend to live in eastern woodlands, where resources are relatively plentiful. Limited resources may require rock squirrels to opt for a more diversified diet (including human soft tissue) to survive. Conversely, gray squirrels may not need the nutrients provided by moist soft tissue and so generally choose to only supplement their diet by gnawing bone.

Scavenger ecology is essential to understand how a scavenger may affect an investigation. Local environments can significantly impact which species live in an area and their behavioral patterns. Resource limitations, either temporary or persistent, can impact whether animals elect to scavenge, what they choose to scavenge, and how much they consume. Animals in marginal environments are likely to present different scavenging patterns than their counterparts in resource-rich environments. This all contributes to a scavenger's interactions with human remains. More research into the variation in scavenger behavior across environments can better inform on the potential impact of scavengers on a forensic investigation.

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Scavenging; Rock Squirrel; Taphonomy

A95 Recovery Rates of Teeth From Outdoor Surface Contexts in Louisiana

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Learning Objectives: This presentation provides dental recovery rates for outdoor rural and suburban residential contexts in Louisiana and examines variation in recovery rates according to dispersal, who collected the remains, and estimated Postmortem Interval (PMI). After attending this presentation, attendees will better understand the recovery rates of different tooth types and how variables of interest affect their recovery.

Impact Statement: This presentation will impact the forensic science community by providing recovery data for different tooth types from forensic anthropology casework from outdoor contexts in Louisiana and by addressing how dispersal, estimated PMI, and who collects the remains affect recovery rates.

Abstract Text: Teeth are critical in forensic anthropology for identification, estimating sex, age, and population affinity if other skeletal elements are missing, and signifying nutrition, socioeconomic status, and cultural habits. Haglund writes that teeth recovery is affected by taphonomic processes, and that single rooted teeth may be lost postmortem more frequently, or recovered in lower frequencies, than other tooth types.[1] Empirical data on the recovery of different tooth types are not available.

The current study: (1) provides teeth recovery rates for cases from Louisiana; and (2) examines variation in recovery according to dispersal, estimated PMI, and who collected the remains. 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 (N=168) were recovered between 1980–2024 from outdoor surface contexts in rural or suburban residential areas. Tooth data were collated from dental inventories. Other variables, including whether the remains were dispersed or non-dispersed, the minimum and maximum estimated PMIs ($PMI_{e\text{-}min}$, $PMI_{e\text{-}max}$), and who collected the remains, were gleaned from case files.

All Teeth and Tooth Positions (TTP) were recorded as present, Lost Antemortem (AMTL) or Postmortem (POML), or Not Available (NA); AMTL and NA TTP were excluded from analysis. Descriptive statistics were calculated by tooth type for the maxilla, mandible, and full dentition, for the collective sample, and for dispersed and non-dispersed subsamples. Standardized variables were calculated to account for different numbers of tooth types, and inferential statistics were used to assess variation in the potential for TTP recovery (i.e., "availability") and rate of POML, for all subsamples and among other variables of interest. The alpha value for determining significance was .05.

Three thousand seven hundred ninety-four TTP were recorded, of which 744 (~20%) were lost postmortem (%POML). For the collective, maxillary, and mandibular samples, availability (i.e., the potential for recovery) varied among tooth types, but differences were significant only between molars and other tooth types. Overall, canines had the highest availability (n=559/672, 83%), molars the lowest (n=1136/2016, 56%). Conversely, differences in %POML were significant for all categories except between canines and premolars. Generally, incisors had the highest %POML (n=378/1086, 35%), molars the lowest (n=64/1136, 6%).

Between dispersed (D, n=1,981) and non-dispersed (ND, n=1,815) subsamples, significant differences in availability were found only for mandibular molars, canines, and incisors. Again, canines had the highest availability (n=295/364, 81% (D), n=264/308, 86% (ND), molars the lowest (n=584/1092, 54% (D), n=552/924, 60% (ND)). Regarding %POML, significant differences were found for all categories of data except mandibular molars. For both D and ND samples, incisors had the highest %POML (n=286/564, 51%; n=92/522, 18%, respectively), molars the lowest (n=42/584, 7%; n=22/552, 4%, respectively).

Because dispersal affected %POML, the effects of estimated PMI and who collected the remains were assessed separately for D and ND samples. For PMI_{emin}, significant differences in availability were found for the ND mandibular premolars, canines, and incisors, and for PMI_{emax}, the ND collective canines. Regarding %POML, significant differences were found for D collective incisors for PMI_{emin}, and ND mandibular canines for PMI_{emax}. Regarding who collected the remains, no significant differences were found in ND samples for availability or %POML; however, significant differences were found in D samples in availability of mandibular premolars and canines, and in %POML for collective and maxillary samples.

In conclusion, results support the supposition that single rooted teeth are lost more frequently than teeth with more complex roots, a situation exacerbated when remains are dispersed or collected by personnel less familiar with teeth. Alternatively, in this study, estimated PMI appeared to have more of an effect on the *potential* for recovery than the actual recovery.

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Taphonomy; Search and Recovery; Dental Remains

A96 Assessing the Effectiveness of Alternative Light Sources in the Search for Skeletal Remains

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Learning Objectives: The information disseminated through this presentation will reveal the success and feasibility of locating human skeletal remains in wooded environments using various Alternative Light Sources (ALS).

Impact Statement: Comparison of daytime search methods to nighttime searches with ALS resulted in significant differences in recovery rates, suggesting that this research will prove advantageous to those regularly involved in searching for whole and/or fragmentary human skeletal remains.

Abstract Text: Thousands of Americans are reported missing every year due to accidental events, mental health crises, or criminal activities. In many instances, law enforcement, search and rescue groups, dog handlers, and members of the community are confronted with the task of locating deceased individuals. The recognition and recovery of human skeletal remains in outdoor environments is difficult, and often searches in this context are unsuccessful even when evidence points to an individual's last known whereabouts.

Many types of biological evidence fluoresce under incident light, and ALS techniques are often used at the crime scene and in the laboratory to assist in the discovery of latent body fluids. Although the collagen component of bone is known to emit fluorescence at certain wavelengths, this property has yet to be leveraged consistently during searches for human skeletal remains.

A small study conducted by Brown and Christensen showed that nighttime searches with blue (455nm) ALS recovered significantly more bones than daytime searches; however, a wider range of wavelengths should be tested through a larger series of searches to draw distinct conclusions.[1] Still, it is suggested that the application of specific wavelengths to bone creates a distinctive appearance that is not achievable with the naked eye and that nighttime searches with ALS devices produce vastly improved results over those conducted during daylight hours.

A series of 36 mock searches were performed by volunteers of search and rescue agencies from across the Commonwealth of Virginia. Thirty bones of three size classes were placed in 5,000ft^2 zones; each zone had significant groundcover of fallen leaves and/or low vegetation. Bones were randomly scattered and partially concealed by leaf cover. Pairs of searchers, supplied with various ALS devices (from 385-530nm, ultraviolet to green light) and wavelength-appropriate goggles (clear, yellow, orange, or red, were allotted up to one hour to search in their usual pattern. Daytime searches were conducted under the same parameters, without ALS. To protect the health and safety of the volunteers, light sources requiring red goggles were discontinued after two searches as the red barrier goggles significantly impaired the searchers' awareness of environmental obstacles. Results indicate that: (1) nighttime searches with ALS produced a recovery rate more than twice that of daytime searches and were often finished in half the time of those performed in the daytime; and (2) ALS in the purple-blue light range were the most effective in locating skeletal remains of all sizes. The 395-425nm Crime-lite^{Ò} was superior as it both had a wider berth of illumination and elicited the most distinct fluorescent response from the bones compared to ~450nm blue lights (Crime-lite^{Ò}, ForenScope, and Labino AB) and the 385-395nm ultraviolet light (uvBeast). It is suggested that nighttime searches with ALS can be used both as the primary search method for the location and recovery of human skeletal remains and as a secondary method for recovering any bones expected to be present but not found during daylight searches.

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Alternative Light Sources; Human Skeletal Remains; Search

A97 Standard Operating Procedures for Forensic Anthropology at Accredited United States Laboratories: A Preliminary Assessment

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the existing heterogeneity in the standard operating procedures and quality assurance protocols designed specifically for forensic anthropological analysis at laboratories that are housed in ANAB-accredited facilities.

Impact Statement: This presentation will impact the forensic science community by presenting the findings from a small, preliminary assessment of available forensic anthropology standard operating procedures. This presentation will assist in the discussion around the continuous professionalization of forensic anthropology, specifically in how ANSI National Accreditation Board (ANAB) -accredited laboratories work to ensure quality and reliability in their forensic judgments and decisions.

Abstract Text: Recent discussions have included the topics of education, accreditation, certification, and qualification in regard to the growth of forensic anthropology as a discipline. Additional reports and research have highlighted the potential concern for the production of accurate, reliable, and repeatable judgements, including those impacted by irrelevant contextual information producing susceptibility to issues of cognitive bias. In response, forensic anthropologists and associated laboratories are diligently and consistently working to develop and improve standard operating procedures, best practice recommendations, and quality assurance protocols to improve the reliability of forensic decisions.

The aim of this pilot study was to assess both the uniformity and the variability of Standard Operating Procedures (SOPs) at multiple forensic anthropology laboratories. As an initial assessment, this preliminary research focused only on laboratories that were actively accredited under the ANSI National Accreditation Board at the time of data collection. SOPs, quality assurance protocols, and training manuals were either accessed as publicly available documents or were requested directly from the laboratory. These documents were examined overall, individually coded for the presence of various practices, and assessed for a better understanding of the nature of these practices. For example, this research considered the existing types of SOPs, the required proficiency testing, and the cognitive bias mitigation methods.

Preliminary results indicate heterogeneity at several structural levels, including the number of published SOPs and quality assurance manuals as well as the level of detail contained in the individual documents. There is also variation in the specific accreditation of the lab (e.g., **International Organization for Standardization** [ISO] 17020 vs. ISO 17025) and the scope of any additional certificates other than anthropology (e.g., odontology, scene investigation), which can impact the necessity for particular SOPs. Analyzing forensic anthropology SOPs at accredited United States laboratories can assist in discussions around the professionalization of the field and standardization of anthropological analysis. In the future, this data collection procedure can be repeated at other laboratories, including those with a sole practitioner, which can potentially stimulate discussions around improving the reliability of forensic judgements in laboratories that are substantially limited by physical constraints. Additionally, the coding system employed in this research is modeled after a similar analysis in friction ridge examination and was developed in tandem with another study examining SOPs in firearm and toolmark examination. This purposeful communication between disciplines allows for a cross-disciplinary discussion around variability and improvement in forensic laboratory procedures.

Forensic Anthropology; Standard Operating Procedures; Quality Assurance

A98 An Evaluation of the Forensic Anthropology DEIB Peer Engagement Program

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Learning Objectives: After reviewing this research, attendees will appreciate the specific benefits a peer mentoring program provides for those in the field of anthropology, a particular concern for maintaining and growing diverse representation in the field. Recommendations for improving the current program model are presented.

Impact Statement: This research will impact the scientific community by demonstrating how competency and performance can be improved through Peer Engagement (PE) programs. A PE program has the capacity to significantly impact the development of individuals and increase the retention of historically underrepresented groups. In particular, PE can allow each participant to contribute to the development of one another and ensure that all thrive in forensic anthropology by: (1) exposing one another to the diverse spectrum of professional work and research in forensic anthropology, biological anthropology, and related fields; (2) providing a window through which to view professional roles, obligations, and expectations; (3) serving as role models and advocates for one another; (4) offering advice and guidance; and (5) engaging in dynamic conversations regarding professionalism, challenges, and milestones of the field. The insight shared is invaluable to the professional development of the whole. In turn, active engagement also exposes us to new perspectives, experiences, and ideas.

Abstract Text: In recent years, forensic anthropology has faced substantial criticism regarding its support structures (or lack thereof) for early-career professionals, Black, Indigenous, and People Of Color (BIPOC) individuals, and historically marginalized groups. Concurrently, scholarly interest in this field has burgeoned, evidenced by a proliferation of publications across anthropological, forensic, and scientific domains.[1-6] These issues underscore the need to develop means for fostering Diversity, Equity, Inclusion, and Belonging (DEIB) in the field of forensic anthropology.

Addressing these concerns, a group of anthropologists hypothesized that a PE program could provide DEIB benefits to participants and introduced the Forensic Anthropology Diversity, Equity, Inclusion, and Belonging Peer Engagement Program (FA DEIB PEP) in June 2022, operating independently from established professional bodies. Participation in FA DEIB PEP was actively sought through various networks (e.g., the Forensic Anthropology listserv, personal communication, etc.). This initiative is designed as a voluntary platform open to forensic anthropologists at all career stages, from aspiring undergraduates to seasoned practitioners worldwide. The inaugural cohort of FA DEIB PEP comprised 29 participants encompassing a spectrum of roles, including students, full-time practitioners, academic researchers, and part-time consultants in forensic anthropology. Following the program's first year, a brief survey (IRB #STU00219722, Northwestern University) of PE participants was conducted to assess the impact and efficacy of their participation.

Purposive sampling was used to target program participants. Twenty-two individuals participated in the survey, which collected quantitative categorical data and qualitative data—54% of respondents have ten or less years' experience in forensic anthropology, while 45% had greater than ten years' experience. Eighteen percent of respondents were board certified in forensic anthropology, 27% held doctorates, 36% held master's degrees, and 18% held bachelor's degrees. Fifty percent of respondents noted that they held marginalized identities. Participants were asked about their personal experience with the program to date and suggestions for improvement. Quantitative data is analyzed in MS Excel and qualitative data is coded in MS Excel to identify themes.

Findings support the hypothesis that PE can positively impact perceptions of DEIB and benefit the field of forensic anthropology. Thematically, respondents noted benefits such as receiving advice and professional support, expanding one's professional network across regions/ranks/career paths, gaining new perspectives, and exposure to professional opportunities. These affirmative themes support the hypothesis that PE and support have the ability to significantly impact the development of individuals, promote inclusion and diversity, and increase the retention of historically underrepresented groups. Plans to broaden this initiative and improve the current FA DEIB PEP model will be discussed.

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Anthropology; Diversity; Inclusion

A99 The American Academy of Forensic Sciences Ad Hoc Committee on Vicarious Trauma

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Learning Objectives: Attendees will be updated on the current activities of the AAFS Presidential Ad Hoc Committee on Vicarious Trauma and will be invited to join a greater discussion about the potential effect of vicarious trauma on forensic practitioners.

Impact Statement: Vicarious Trauma (VT) potentially impacts forensic practitioners' mental and physical health. This presentation will raise awareness about VT in forensic practice and highlight resources available to Academy members to develop resiliency and healthy coping mechanisms.

Abstract Text: VT is the cumulative effect of exposure to information about traumatic events and experiences. VT was first recognized in the early 1990s by mental health professionals who recognized that they were being affected by the trauma of their patients.[1] Today, it is described as an occupational challenge for those working in victim services, first responders, and emergency room personnel.[2] The work of forensic providers has only recently been considered a source of VT.[3] Each section of the Academy has members who are regularly exposed to traumatic content in distinctly different ways and with differing levels of exposure. To date, there have been no comprehensive studies evaluating how these differing levels of exposure influence VT or how to mitigate its effects. While research continues, AAFS President Laura Fulginiti proactively created an ad hoc committee on vicarious trauma to evaluate resources to assist Academy members in safeguarding their mental health and increasing their resilience. Under AAFS Presidents Ken Williams and Chris "Kip" Thompson, the Academy continues to support this mission. In 2023, Academy members were offered access to one month of free counseling through BetterHelp, a web-based counseling service that connects Academy members with licensed therapists across the country.

The committee is taking a multifaceted approach to addressing VT in the forensic community, starting with increasing awareness with discussions across and within sections. Many members may be unaware of how VT can negatively impact their professional and personal lives. They may even be unaware that they are experiencing it. Through the AAFS newsletter and presentations such as this one, the committee will detail the emotional challenges that forensic professionals can experience and coping mechanisms that can help reduce the risks of exposure.

Academy members will learn how to access resources currently available to them as well as resources that other professions have been able to use successfully. The committee aims to create a survey to identify the different mechanisms by which Academy members are exposed to VT and if their agencies have policies or resources available to address it.

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Vicarious Trauma; Resiliency; Coping Mechanisms

A100 The Development of a New Pedagogy: The Professional Diploma in Forensic Archaeology at the National Forensic Science University, Gandhinagar, Gujarat, India

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Learning Objectives: After attending this presentation, attendees will understand the importance of pedagogical development related to a major component of forensic investigative work, forensic archaeology, in the world's largest democracy, India.

Impact Statement: This presentation will impact the forensic science community by examining the development of an important subdiscipline in forensic anthropology within a South Asian context. This will contribute to the importance of forensic archaeology in a more global context.

Abstract Text: The curriculum related to a professional certification/diploma has been developed and started at the National Forensic Science University (NFSU) in the Gandhinagar campus, Gujarat. This certification has been in the planning stages for the past two years as a cooperative/trilateral agreement between the NFSU, the University of Nebraska-Lincoln, and the United States Department of Defense's Defense POW/MIA Accounting Agency (DPAA). The primary impetus of this program was a small cooperative excavation of a United States World War II-era B-29 bomber crash in the northeastern part of India in the State of Assam. The program is currently open to professionals in forensic, law enforcement agencies, and judiciary officers as well as for students who have graduated or are at the Master's level.

NFSU is an international-level university with the headquarters in capital of Gujarat, Gandhinagar, established by Government of India (GoI) through Act 2020(32 0f 2020) and is classified as an Institution of National Importance under the Ministry of Home Affairs (MHA), GoI. There are currently nine satellite campuses of NFSU in India and one international campus in Jinja, Uganda. Being the world's first and only one-of-its-kind university, the school was established with the vision and mission to meet the acute shortage of forensic experts by imparting education in various branches of forensic and investigative sciences. That said, the expansion of investigative and forensic sciences to include forensic archaeology is an obvious gap that will be filled with the teaching and practical exercises associated with the Professional Diploma in Forensic Archaeology.

This curriculum is composed of five modules that span a six-month period. The first module includes basics of forensic science, different branches of forensic science and understanding the Constitution, criminal justice system, and legal system of India. A module on forensic archaeology includes basics and definitions of forensic archaeology and understanding the criminal investigative aspects in archaeology and medicolegal exhumations. A module on forensic anthropology includes basics of forensic anthropology, comparative identification, the biological profile, and taphonomy. Practical modules include exercises that culminate in a project-based investigation and excavation associated with actual forensic archaeological material, and/or a project with investigating or excavation a World War II-era United States aircraft loss incident.

While India has a long history of archaeological endeavors on both the federal and state levels, the implementation of a forensic archaeological program and training has not been part of any scholarly or practical curricula till now. This Professional Diploma in Forensic Archaeology is the first of its kind in India and has become widely recognized among professional archaeologists and incoming students at the NFSU. The developed curriculum is approved by the Board of Studies of School of Medico-Legal Studies (NFSU), after which it was approved by the Academic Council of NFSU. The program offers 22 credits with 30 hours/week of teaching, laboratory, and tutorial work. The program spans over 6 months with a combination of online and offline classes.

Forensic Archaeology; Missing Persons; International

A101 Applying Multidisciplinary Methods to Forensic Casework in North Carolina

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Learning Objectives: Attendees will learn about the multidisciplinary approach to the investigation of Unidentified Decedents (UIDs) at the North Carolina State University Human Identification and Forensic Analysis Laboratory (NCSUHIFAL). The methods applied include anthropological analysis and population affinity estimation using 3D-ID, investigative genetic genealogy, and isotopic analyses. Several case studies will be presented that highlight this approach.

Impact Statement: This study will impact the forensic science community by providing examples of the framework for applying a multidisciplinary approach to casework as well as discussing the limitations and barriers encountered in the process. The decision-making procedure for implementing this approach will also be addressed.

Abstract Text: As of June 12, 2024, there are 161 unidentified persons cases listed in the National Missing and Unidentified Persons System (NamUs) for the state of North Carolina. A multidisciplinary approach has been adopted by NCSUHIFAL, state medical examiner's offices, and law enforcement to address this crisis.[1] Modern methods of skeletal analyses are employed when developing a biological profile (e.g., biological sex, age at death, stature, and population affinity) for UIDs, radiographic comparisons for identification, and trauma analysis, to assist medical examiner offices. Additional techniques are employed to develop standard operating procedures for North Carolina casework. In 2019, an interinstitutional grant funded the implementation of a holistic approach to identification involving standard forensic anthropological analysis, Investigative Genetic Genealogy (IGG), and isotopic signatures.[1]

More recently, NCSUHIFAL has partnered with the NC State Bureau of Investigation on a grant funded by the Bureau of Justice Assistance under their Missing and Unidentified Human Remains (MUHR) program in 2023 to advance the multidisciplinary approach to reduce the backlog of unidentified cases in North Carolina. Results from 17 cases sent for FGG have resulted in ten positive identifications, of which five also applied isotopic analyses. Here, we present the results of isotopic investigations from three of those cases using geolocation and dietary isotopes, anthropological analyses, including population affinity estimation using 3D-ID , and investigative genetic genealogy.[2] This presentation aims to highlight the multidisciplinary approach and to show the added value of using advanced testing in forensic anthropology casework in North Carolina.

A multidisciplinary approach in the investigation of UIDs applies a variety of scientific methods that have led to successful identifications using anthropological analysis including population affinity estimation using 3D-ID, investigative genetic genealogy, and isotopic analyses. Results from one scientific method can inform other investigative aspects by providing supplementary information about the decedent thus, enhancing the investigation and the probability of a positive identification. However, both population affinity and isotopic analyses rely on reference data with which to compare individual measurements or values. The continued collection and publication of these data are essential to obtain global coverage.

The three case examples selected for this presentation were recovered in Mecklenburg County, NC. Two of the individuals have been identified by the use of multiple methods, including modern methods of skeletal analyses, tooth enamel and bone apatite isotopic analyses for geolocation, bone collagen isotopic analyses for dietary analysis, bomb-pulse dating to confirm the antiquity of the remains and rule out historical origin, and investigative genetic genealogy. The third unidentified case has undergone anthropological and isotopic analyses and is presently undergoing investigative genetic genealogy.

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Investigative Genetic Geneaology; Stable Isotope Analysis; Multidisciplinary

A102 To Scan or to Slice: A Holistic Investigation of One of Florida's Oldest Unsolved Murders

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Learning Objectives: After this presentation, attendees will understand the use of 3D rendering technologies (including Computed Tomography [CT] in conjunction with a Coordinate Measuring Machine [CMM] FARO Design ScanArm and RevEng software) to aid in the retroactive assessment of exhumed human remains associated with two of Florida's oldest unsolved murders.

Impact Statement: This presentation impacts the forensic science community by informing practitioners of the value of 3D scanning and CT in the analysis of forensic cases. By supplementing the slicing capacity of CT scans with the accurate topographical rendition of 3D CMM FARO Design ScanArm and RevEng software scans, these digital demonstrative aids that represented human remains may be useful proxies for re-analysis or subsequent court aids following the release of remains to funeral homes or reinterment of the remains, following permissions from the legal next of kin.

Abstract Text: Existing literature investigating the utility of CT and 3D surface scanning in forensic trauma analysis has found that digital renderings are accurate and effective representations of the human remains, such that they may be effectively utilized to assist with the visualization and interpretation of skeletal trauma.[1,2] This case study presented the double exhumation and forensic analysis of two of Florida's oldest unsolved murders. On December 19, 1959, two adults and their two children were found dead from apparent Gunshot Trauma (GST) in their home. Their remains were autopsied by the District 12 Office of the Medical Examiner and subsequently interred at the Oak Ridge Cemetery in Arcadia, FL. However, new investigative leads reopened this case, and the lack of an original autopsy report necessitated exhumation of the adult individuals.

Post-exhumation, following the medical examiner's autopsy and forensic anthropologist's analysis, the crania belonging to both adults were scanned using the Florida Gulf Coast University (FGCU) Human Identity and Trauma Analysis (HITA) Laboratory FARO Design ScanArm and RevEng software to render 3D topographical scans. During subsequent CT analysis at the District 12 Office of the Medical Examiner, a glass rod was positioned through the perimortem (at or around death) GST entrance and exit defects to highlight the directionality of the projectile. This provided the medical examiner, forensic anthropologist, and law enforcement with the endocranial perspective critical to the 3D preservation of projectile directionality.

Both decedents evinced a single GST entrance, and one decedent lacked an exit defect. More specifically, the forensic anthropological findings (using both gross and radiographic observations) for the first individual consisted of one entrance defect on the right maxilla that traveled through the spheno-occipital synchondrosis and did not exit the cranium. For the second individual, the projectile entered the left parietal, traveled inferiorly and to the right (crossing the mid-axial plane), and exited through the base of the occipital. This case study highlighted instances where the use of technology in modern death investigations helped to fill a gap in knowledge and allowed for a more holistic interpretation of perimortem trauma. While the CT imagery helped to capture the directionality of the projectile with an endocranial perspective, the FARO Design ScanArm precisely captured the perimortem trauma and foramina to aid the scientist's interpretation and presentation of death investigation findings.

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3D Technology; Unsolved; Murder

A103 Three-Dimensional Research on Ancient Skeletons of South Korea With Computed Tomography

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Learning Objectives: With this presentation, attendees will learn the application of Computed Tomography (CT) to archive information on skeletal remains from historic excavations. With radiographical imaging techniques, further research on the ancient skeletons was performed with access to three-dimensional (3D) models.

Impact Statement: The CT scans to create 3D models have improved the organization and study of ancient skeletal information. Forensic anthropologists can analyze these 3D models to inspect visually ancient remains. This computational analysis would have a broader application in research on ancient skeletons while preventing damage to these valuable human remains.

Abstract Text: CT has gained popularity in archaeology and has been called paleoimaging or paleoradiology. The methodology enabled non-invasive and non-destructive research of mummies, human remains, and artifacts. Also, the digital data provided easier access for researchers regardless of time and spatial limitations.

We first reviewed published excavational reports of possessing institutions, residual status, and availability of the Mahan and Baekje region (Gyeonggi-do, Chungcheong-do, and Jeolla-do) in the Three Kingdoms Period (4^{th}~7^{th} century C.E.).

Among 33 reports of archaeological sites, five excavated collections that are in poor condition, from different eras, or impossible to loan were excluded from this research. In addition, as radiological data from three sites had been procured in past studies, the skeletons of 25 sites were loaned to be scanned with Multi-Slice Computed Tomography (MSCT). The excavation sites were distributed as 1 in Seoul, 18 in Chungcheongnam-do, 2 in Jeollabuk-do, and 7 in Jeollanam-do.

Using Mimics 24.0, we created 151 project files containing 2,550 remodeled skeletal models. These 3D models were then used to deduce forensic anthropological information such as sex, age, pathology, and facial approximation.

Skeletal components like the os coxae and the skull could suggest the skeleton's sex. Though the 3D models could not generally present dental erosion of the occlusal surface, forensic odontologists could analyze eruption and loss of dentition to speculate age. If the auricular surface of the ilium or the pubic symphysis is intact, they were assessed to assume the age. In addition, pathological findings due to congenital deformity or aging were recorded, which were not reported in the previously published information.

We expect this study will contribute to comprehensive research on scattered archaeological skeletal data. In former studies, the radiological data were stored by individual institutions, but we performed to make a nationwide scale dataset. Any future researcher would utilize the result as an inventory and as a collection of 3D models to skim through the data remains and plan further research.

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Anthropology; 3D Technology; Computed Tomography

A104 Demographic Timelines: Postmortem Data and Structural Violence in the Southeastern United States

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Learning Objectives: After attending this presentation, attendees will learn how demographic factors can impact the recovery time of missing persons in the Southeastern United States medicolegal system. Furthermore, attendees will develop an understanding of how structural barriers, lack of policy on recovery data, and underreporting can perpetuate and even exacerbate institutionalized violence, which often leads to adverse outcomes for marginalized and minority groups in the Southeastern United States.

Impact Statement: This presentation will impact the forensic science community by showcasing how the intersection of demographics, forensic casework, and institutionalized violence impacts missing persons in the medicolegal system, both negatively and positively. Through this research, medicolegal professionals can implement strategies to combat the systemic disparities that currently exist in the recovery of persons from marginalized groups, particularly those from the Southeastern United States.

Abstract Text: When an individual's remains are found, it is imperative to determine how long the person has been deceased prior to their recovery, also known as the Postmortem Interval (PMI).[1,2] Over the past few decades, forensic anthropological research has made significant strides in developing better methods for decedent identification and increasing the accuracy of time since death/PMI estimations.[2] Even so, little emphasis has been placed on how socioeconomic considerations might be connected, especially regarding recovery times for individuals from varying demographic backgrounds. Ongoing inequities and negative perceptions can impact factors relating to missing and unidentified persons, such as their likelihood of going missing and being recovered safely.[3-5] Specifically, casework associated with marginalized communities has commonly been fraught with difficulties; these challenges stem from structural barriers and biases in government policies, tensions between marginalized groups and law enforcement, and discrepancies in media attention, including underreporting or misrepresenting cases.[6-8] Implementing both an intersectional and biocultural framework, this research aimed to decipher the role that inequality, through instances of social vulnerability and structural violence, plays in recovery time data throughout the Southeastern United States. PMI recovery data from forensic anthropologists and medicolegal labs in Mississippi, Louisiana, and South Carolina were used. These data included recovery times and basic demographic information. Data received from South Carolina included individuals from a single county between 2021 and 2024 (n = 5,448), while Mississippi (n = 175) and Louisiana (n = 117) datasets were based on statewide repositories. Due to the statistical similarities in the datasets, the data from Mississippi and Louisiana were combined (n = 285).

All statistical analyses were performed using RStudio (v. 2021.09.2). Kruskal-Wallis One-Way Analysis of Variance tests were used to assess the possibility of a significant relationship between PMI and the three tested variables (Age, Sex, and Ancestry). Only the age category variable was significant ($p = 0.000182$) with the combined datasets. However, when the ancestry category was analyzed with just Black and White individuals (each n = 137), the results indicated significance ($p = 0.03835$). South Carolina results indicated that age ($p = 9.631e\text{-}9$) and sex ($p = 1.923e\text{-}4$) were significant, while ancestry was not. The combined repositories and South Carolina had more days prior to recovery for middle-aged (35–66 years old) adults and those who identified as White. Sex differed; females from the combined repositories had higher PMI averages, while South Carolina had higher averages for males. Finally, based on location, South Carolina had shorter PMI time spans, averaging a little over a day, while the combined repositories had PMI averages between 1.5–2 years.

Results from South Carolina and the combined repositories from Louisiana and Mississippi suggest a mix of demographic disparities in recovery times for medicolegal cases reported in these Southeastern states. Moreover, the lack of available postmortem recovery data across agencies in the Southeastern United States was evident. Overall, our findings suggested that individuals from marginalized backgrounds are more underreported than what is currently acknowledged. This result emphasizes the negative impact that the intersectionality of various identities can cause and contributes to instances of structural violence and vulnerability against populations that we cannot fully comprehend. Considerations of how systematic violence can impact socioeconomic and sociopolitical factors for a demographic group's recovery times highlight disparities in how politics, law enforcement, and medicolegal personnel assist and conduct casework.

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PMI; Southeastern United States; Structural Violence

A105 Demographic Trends in Resolution of Adult Skeletal Cases in the National Missing and Unidentified Persons System From 2020 to 2024

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Learning Objectives: From this presentation, attendees will learn about trends in the resolution of the National Missing and Unidentified Person System (NamUs) unidentified cases within the past four years. This will include intergroup differences and proxies for resource expenditure.

Impact Statement: This presentation will impact the forensic science community by highlighting areas in which distribution of case resources could be made more equitable and thereby improve case resolution rates. Specific resources (e.g., anthropological analysis) will be highlighted through proxy variables to make these findings actionable.

Abstract Text: The resolution of unidentified skeletal cases is a considerable challenge, with 5,312 open cases in NamUs as of July 2024.[1] NamUs serves as a repository for case information to aid in the resolution of missing and unidentified persons cases. The size and widespread use of this database provide an opportunity to examine patterns in case resolution in order to identify opportunities for improvement.

The study period spanned from September 2020 to July 2024 with monthly data capture of NamUs case entries. Criteria for inclusion were adult age and skeletal condition of remains. For 6,253 total cases captured, 943 new cases were entered, and 941 cases were resolved (i.e., removed from NamUs) within the study period. Out of all combined "Biological Sex" and "Race/Ethnicity" groups with at least 40 resolved cases, White female cases (n = 109) had the longest mean and median resolution time of 25.8 and 30.2 years, respectively, while unknown "Biological Sex"/unsure "Race / Ethnicity" cases (n = 68) had the shortest mean and median resolution time at 6.4 and 1.6 years, respectively. The groups with the largest and smallest resolution rates were White females and Hispanic males at 0.29 and 0.08, respectively. When only cases entered during the study period (i.e., within the past four years) were examined, White females showed the shortest mean and median resolution times (0.74 and 0.53 years, respectively) and a similar resolution rate (0.32) to White (0.36) and Black males (0.33). This suggests that the elevated resolution rate and long resolution times for White females are being driven by the resolution of older cases, which indicates a potential equity gap in resource allocation toward case resolution.

The "Biological Sex" designation was changed for 132 cases while the "Race / Ethnicity" designation was changed for 137 cases during the study period. For both variables, cases that had a designator change had a higher resolution rate relative to those that did not; however, this was only significant for "Biological Sex," with a negligible effect size according to a chi-square test ($p = 0.02$, $V = 0.03$). Designator changes were also associated with a shorter time to resolution. This was significant for "Biological Sex" according to a two-sample t-test, with a moderate effect size (5.5 vs. 13.8 years, $p < 0.001$, $d = 0.61$). Case counts were too low to examine group-specific trends for designator changes. Changes in case designators throughout the study period can be interpreted as a proxy for additional resource expenditure, meaning that a positive signal for additional resource expenditure is associated with a higher resolution rate and shorter resolution time. Since these are skeletal cases, the most likely resource for updating demographic designators is anthropological analysis.

In conclusion, metrics of case resolution in NamUs indicate demographic patterning and a positive impact from additional resource expenditure. These results suggest the utility of anthropology in resolving unidentified persons cases. While there are many reasons why some demographic groups might show higher resolution rates than others, these findings warrant further investigation of resource distribution equity in unidentified persons cases.

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Anthropology; Skeletal Remains; Unidentified Persons

A106 Comparing Antemortem Trauma Prevalence and Reporting Frequency in Unidentified Migrants Compared to a Non-Migrant Sample: A Pilot Study for Identification Purposes

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Learning Objectives: Attendees will learn how prevalence of antemortem trauma differs between a migrant and non-migrant sample. Attendees will also understand how antemortem trauma is reported in missing persons' reports compared to the prevalence of occurrence.

Impact Statement: The purpose of this study is to determine if there is a difference in the frequency in antemortem trauma for unidentified migrants and non-migrants and to investigate if this trauma is equally reported in missing persons reports for migrants last known to be crossing the United States-Mexico border and its implications for identification. This presentation will ascertain if antemortem trauma is an individualizing characteristic that could be used for identification purposes.

Abstract Text: With the rise in migrant deaths along the United States-Mexico border, primary identification methods are not always available.[1] Under these circumstances, it is necessary to explore alternative ways to achieve identification, such as through antemortem trauma analysis. Between 2001 and 2006, 72% of identified cases of undocumented migrants on the United States-Mexico border in Arizona utilized visual identification methods, among others, to identify individuals.[2] It has been noted that skeletal pathological features might provide valuable information for the identification process, but further research is needed.[3] The goal of this research is to investigate whether there is significant antemortem trauma in presumed migrants found along the United States-Mexico border and the implications of using such data to aid in migrant identification.

The National Missing and Unidentified Persons System (NamUs) was used to gather antemortem trauma data on individuals who were last seen alive in Texas crossing the United States-Mexico border. The hypothesis is that there is an association between antemortem trauma in these reference samples. The null hypothesis is that there is no association between antemortem trauma and these reference samples. The frequency of antemortem trauma between the unidentified (Operation Identification [OpID]) and the missing (NamUs individuals) was analyzed by comparing the frequency of antemortem trauma in the OpID remains compared to the amount reported in the NamUs missing person reports.

Antemortem trauma is not as evident in comparison to a non-migrant population. This indicates that antemortem trauma, although present, is not present at the level needed to be useful for positive identifications in a migrant population. Although the data are statistically significant, the correlation is weak. Antemortem trauma analysis was documented in migrant remains from the OpID program (n=297). The frequency of trauma was compared to individuals in the Texas State Donated Skeletal Collection (TXSTDSC) (n=50). The long bones are examined for signs of antemortem trauma. Both the left and right elements were analyzed to rule out human variation, such as if an individual has non-standard skeletal traits that could be confused with antemortem trauma. This data was compared using a Chi-Squared test in SPSS.

Antemortem trauma in OpID is more evident than in NamUs reports, indicating it is underreported in NamUs (or missing persons reports). Although this research showed that alternatives to DNA are worth exploring, DNA is still needed for positive identification. DNA samples must be collected from these migrants, and the policies that hinder DNA collection and data sharing must be reviewed.

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Migrants; Antemortem; Trauma

A107 Gendered Language Use in Forensic Science Publications (2003–2023)

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Learning Objectives: After attending this presentation, attendees will understand how patterns in gendered language use in AAFS presentations and flagship forensic science journals have changed over the past 20 years, as well as how proper use of gendered language in publications affects decedent identification and case resolution pragmatically.

Impact Statement: This presentation will impact the forensic science community by enabling practitioners across subfields to better align their case reports and scientific publications with timely and accurate gendered language, supported by the most recent *Diagnostic and Statistical Manual of Mental Disorders*.[1] Such shifts can positively impact identification of decedents.

Abstract Text: This research project explores if and how gendered language use has changed in forensic science publications as practitioners gain greater cultural awareness about gender diversity in forensic populations. More specifically, we address and analyze: (1) trends in gender-focused research and gendered language in the forensic sciences over the past 20 years; (2) subfield specific differences in gender-related research; and (3) how forensic scientists' framing of gender, specifically gender identity and gender expression, aligns with the mental health community.

Currently, there is an epidemic of violence, including homicide, committed against transgender and gender-nonconforming individuals, increasing the likelihood that individuals with these identities become a part of forensic casework.[2,3] Therefore, gender-centered research is essential to improving forensic identification methodologies. The language practitioners use to communicate their findings in reports is also equally important as this information must be translated and matched to language utilized in government system (e.g., NamUs), medical records, and social terminology.

This dataset was derived from the American Academy of Forensic Sciences Conference *Proceedings* (2003–2023), the *Journal of Forensic Science* (2006–2023), *Forensic Science International* (2003–2023), and *Forensic Science Synergy* (2019–2023). The following keywords were searched in all publications: transgender, gender, gender queer, queer, FTM, MTF, nonbinary, cisgender, transsexual, sexuality, transvestite, trans, gender identity disorder, gender identity, gender dysphoria, gender dysmorphia, gender variant, gender nonconformity/nonconforming, transsexualism, gender identity disorder, LGBTQ, and homosexuality. The search terms were necessarily broad to explore how forensic scientists write about and conceptualize gender in their research publications. Variations of terms (e.g., "transvestic" instead of "transvestite") were also searched to provide a comprehensive data set. Patterns in gendered language use were compared against the changing standards and classified conditions specified in the *DSM-5-TR* (2022).

The *DSM-5-TR* (2022) names the psychiatric disorders regarding gender as gender dysphoria and transvestic disorder, which falls under their Paraphilic Disorders section as a fetishistic disorder. Forensic science journals seem to have followed suit with the use of the term "gender dysphoria," as the oldest recorded use of it in our dataset was in 2015, and it is still used in forensic science journals presently. However, our results demonstrate that continued use of other, more derogatory terminology in forensic science is not in line with the most recent *DSM-5-TR* or social inclusivity. For instance, the term "transsexual" has generally fallen out of use in the forensic field, though it persists in a recent AAFS *Proceeding* (2018) and a *JFS* article (2021). Incorrect use and variation of gendered language can affect how individuals are entered into databases, negatively impacting the prospect of correctly identifying unknown decedents.[4] Additionally, the use of harmful or derogatory language does not afford non-gender conforming individuals dignity in death. The results of this study indicate that forensic scientists have taken an increased interest in identification of transgender decedents in recent publications and presentations, perhaps because of the increased rates of fatal violence in this population, although the term has been used throughout the past 20 years of publications.

Based on our findings, we suggest strategies to better align the research foci of forensic scientists interested in gender identity, expression, and identification of transgender and gender non-conforming decedents with the *DSM-5-TR*. Furthermore, we propose standardized and regularly updated gender-inclusive language for applied casework.

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Gender; Transgender; Human Identification

A108 Technology You Can Stand On: Skeletal Changes in Human Remains With Lower Extremity Orthopedic Prostheses

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Learning Objectives: After attending this presentation, attendees will have gained an understanding of antemortem skeletal changes observable in human remains with orthopedic prostheses in the lower extremity.

Impact Statement: This presentation will impact the forensic science community by providing practitioners with a pattern of expected skeletal changes related to orthopedic implants in the lower extremity and aid in their ability to discern these changes from those related to other pathological or senescent processes.

Abstract Text: Existing research on the clinical effects of orthopedic device implantation in the lower extremities has identified issues related to alterations in gait, biomechanical loading, leg length discrepancies between antimeres, and, further, how these alterations influence patient functional outcomes.[1,2] From a forensic anthropological context, research has established the utility of using orthopedic prostheses to aid in human identification via radiographic comparison and serial number tracking.[3,4] Notably absent from current clinical and forensic anthropological literature, however, is an investigation into the grossly observable pathological effects of orthopedic implants arising from these established postoperative complications that affect biomechanical functioning in weight-bearing bones. Comporting with American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 134 for analyzing pathological conditions and anomalies, a comprehensive study into the pathological effects of orthopedic implants on non-implant bearing bones is warranted to inform forensic anthropologists of those changes that may be attributed to the implant and help to inform assessments of human identification.[5,6]

In this pilot study, we investigated the propensity of orthopedic implants in the lower extremity to influence the manifestation of grossly observable skeletal pathologies in weight-bearing bones. Further, we supplemented existing leg length discrepancy studies by examining the effect that orthopedic implants have on the severity of unilateral length differences between antimeres as assessed in dry bone.

We compared 14 human remains donations with orthopedic implant(s) in the lower extremity (i.e., total hip arthroplasty, hip hemiarthroplasty, total knee arthroplasty, and internal fixation) to a control sample of 14 implant-free human remains donations of a matched age-at-death and biological sex distribution culled from the Florida Gulf Coast University Human Identity and Trauma Analysis and the University of Tennessee National Forensic Academy Donated Skeletal Collections. We macroscopically evaluated the lower extremities, calcanei and tali, and the lumbar spine for the presence of antemortem fracturing, aberrant facets, Schmorl's nodes, osteochondritis dissecans, heterotopic ossification, and osteoarthritis. Further, we metrically assessed the femora, tibiae, and fibulae to evaluate the severity of leg length discrepancies between the samples. The data revealed a significant difference ($p < 0.05$) between the manifestation of severe limb length inequalities (>1cm), where those remains with an implant had more severe differences between antimeres. Further, those decedents with an implant comprised 65.55% of the total number of antemortem fractures among the samples and produced a higher total pathological frequency across L1 (56%), L2 (78%), L3 (68%), L5 (52%), patellae (57%), tibiae (69%), fibulae (57%), calcanei (79%), and tali (56%)—notably excluding the femora and L4. Conversely, the data revealed a significant difference ($p< 0.05$) between observations of osteochondritis dissecans between the samples, where the implant-free decedents evinced more. This pilot research demonstrates the potential for a pattern of observable skeletal changes that may be attributable to orthopedic hardware implantation in the lower extremity to be identified and used as a guide supporting the ANSI/ASB Standard 134 for analysis of pathological conditions and anomalies in forensic anthropology.

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Identification; Antemortem; Anthropology

A109 Evaluating Cortical Bone Structural Variation in Association With Activity Level

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the practical application of high-resolution X-ray computed tomography for forensic case work.

Impact Statement: This presentation will impact the forensic science community by introducing a new method for assessing activity levels of unknown descendants. Additionally, the results of this project may introduce potential new lines of evidence for forensic practitioners to assist with narrowing down a missing persons list.

Abstract Text: Bone is an adaptive tissue that can change in mass and architecture throughout the course of life due to loading regime through modeling and remodeling processes.[1,2] Because of this, a relationship exists between bone mass, size, geometry, and mechanical usage.[3-5] Numerous studies have highlighted the effect of mechanical strain on cortical bone properties; however, few studies have used known activity patterns to investigate the effect of mechanical loading on a more individual level.

The purpose of this study is to explore the relationship between cortical bone structural variation and activity patterns, specifically occupation, to answer the following questions: (1) are there differences in cortical bone structural variation between individuals with manual and non-manual labor jobs; and, (2) if differences are observed, can these differences be used to provide new lines of evidence for forensic practitioners to aid in the identification of unknown decedents?

High-Resolution X-Ray Computed Tomography (HRXCT) scans of the left and right humerus were obtained for 79 individuals from the Texas State University Donated Skeletal Collection (TXSTDSC). Cross-sectional slices were then analyzed from the midshaft and proximal 65% of the humeral shafts using BoneJ. The "Slice Geometry" tool was used to obtain cross-sectional area (CSA) and maximum (I_{max}) and minimum (I_{min}) bending moments. Torsional rigidity (J) was calculated by adding I_{max} and I_{min}. Prior to analysis, all Cross-Sectional Geometric (CSG) properties were standardized by body size. For the analysis, the handedness of each individual was noted so that the dominant and non-dominant arms were compared, rather than all lefts compared to all rights. Depending on the results of the appropriate tests for distribution and variance, either a parametric Analysis Of Variance (ANOVA) or a non-parametric Kruskal-Wallis test was used to compare the CSG properties of the humeri between individuals with documented manual and non-manual labor jobs.

The results showed that there was a significant difference in the non-dominant arm between manual and non-manual labor workers in CSA ($p=0.0377$), I_{min} ($p=0.0120$), and J ($p=0.0187$) at the proximal 65% slice, with the manual labor workers having larger averages for all three variables. Additionally, there was also a significant difference in I_{max} ($p=0.0157$) and J ($p=0.0215$) in the non-dominant arm midshaft slice. The results of the comparisons between the dominant arm show there were no significant differences between manual labor workers and non-manual labor workers in the midshaft slice, although manual labor workers had larger CSG properties for all variables. The results from the proximal 65% slice of the dominant arm show that was a significant difference between I_{min} ($p=0.0282$) and J ($p=0.0459$), with the manual labor workers having larger CSG properties for all variables, not just those that were significant.

The results of this project indicate the practical use of cortical bone cross-sectional geometric properties and HRXCT to provide potential new lines of evidence for forensic case work. Individuals with manual labor jobs consistently had higher CSG properties in the humeral shafts when compared to individuals with non-manual labor jobs. These results may be explained by the higher levels of mechanical loading on the humeri of individuals with a manual labor job. Additionally, while some forensic practitioners have begun to evaluate musculoskeletal markers to incorporate potential activity levels into casework, these methods can sometimes lack standardization and statistical interpretation to support the claims. The utilization of radiologic imaging technologies to visualize cortical bone may be used to standardize methods for assessing activity levels of unknown decedents and may also help in a forensic context when there is evidence lacking from the remains of the scene.

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High-Resolution X-ray Computed Tomography; Activity Level; Forensic Anthropology

A110 The Utility of Low-Resolution Imaging in the Assessment of a Decedent's Activity Level and Body Mass

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WITHDRAWN

A111 A Preliminary Comparison of Fracture Characteristics Between Fleshed and Defleshed Human Tibiae

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Learning Objectives: After attending this presentation, participants will gain insight into the role of soft tissue and the presence of the fibula in tibial fracture characteristics resulting from blunt trauma.

Impact Statement: This presentation will impact the forensic science community by providing quantitative analyses that explore how fracture characteristics vary between paired tibiae loaded in the same loading conditions when one tibiae is defleshed and the other is still within a fleshed leg.

Abstract Text: Previous studies have demonstrated variation in injury outcomes, such as the number of fractures and fracture classifications, in human tibiae subjected to identical experimental conditions (6m/s, lateral-medial direction, 4-point bending).[1] However, these studies exclusively used defleshed tibiae to control for variations beyond the bone itself, which may not fully represent injury outcomes in real-world leg impacts. Previous research suggests that while the response to loading is delayed in fleshed specimens, the overall biomechanical response is similar to that of defleshed specimens.[2] Despite the reported similar biomechanical responses, the variation in injury outcomes between fleshed and defleshed specimens remains uninvestigated. The objective of this research is to analyze fracture characteristics in paired tibiae to identify variation between fleshed and defleshed specimens that were experimentally loaded under the same dynamic loading conditions.

Human tibiae and their corresponding antimere legs (11 pairs) were experimentally loaded at 6m/s in a lateral-medial direction using a 4-point bending scenario. Left specimens were fleshed legs (n=11), while right specimens were defleshed tibiae (n=11), and all were kept biomechanically fresh using saline-soaked gauze. The fleshed legs were dissected from postmortem human subjects at the tibiofemoral and tibiotalar joints. Soft tissue was removed at the proximal and distal ends to allow for fixation of the legs, but the flesh remained intact along the diaphysis, including the impact locations. For the defleshed tibiae, all soft tissue, except for the periosteum, was removed. Detailed specimen preparation protocols are described by Harden.[3] Specimens were potted at the 20% and 80% sites of the tibia, calculated from the tibia length excluding the medial malleolus, and impacted at the 40% and 60% sites. After post-test imaging, the flesh and soft tissue were removed from the right legs for fracture analysis. Trauma analysis was conducted on the defleshed tibiae and both the tibia and fibula of the legs; however, the focus of this study is on comparisons between the tibiae only. Trauma analysis included the identification of fracture type and group, and the number of fracture types per tibia, following the methods outlined in the AO/OTA Fracture and Dislocation Classification Compendium.[4]

Fracture number (number of classified fractures per tibia) demonstrated a match in 4 out of 11 pairs of fleshed and defleshed tibiae. Of the paired tibiae and legs with the same number of classified fractures (n=4 pairs), three pairs resulted in the same fracture type and two pairs resulted in the same fracture type and group. If the fleshed and defleshed tibiae had the same number of fractures, they tended to result in the same fracture classifications, indicating similar fracture characteristics between fleshed and defleshed specimens. However, these trends need to be further evaluated in a larger sample size, which is currently being addressed by the National Institute of Justice (15PNIJ-23-GG-04219-SLFO, Quantitative Assessment of Fracture Characteristics in Blunt-Force Skeletal Trauma for Forensic Interpretations).[5]

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Trauma Analysis; Trauma Interpretation; Experimental Research

A112 A Preliminary Evaluation of Reliability in Long Bone Fracture Classification Methods Among Observers

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Learning Objectives: After attending this presentation, attendees will gain familiarity with trends of reliability of long bone fracture classification schemes among observers of varying experience levels.

Impact Statement: This presentation will impact the forensic science community by demonstrating the need to re-evaluate the utility and reliability of methodologies for long bone fracture classification.

Abstract Text: Fracture classification is a standard component of trauma analysis. The Standard for Analyzing Skeletal Trauma in Forensic Anthropology (Academy Standards Board [ASB] 147) states that trauma should be documented using peer-reviewed methods; however, guidance on which methods are applicable is not provided.[1] Further, limited methods exist to classify long bone fractures resulting from blunt mechanisms. Most classification systems stem from clinical practice, and prior work demonstrates variability in observer reliability of classifications.[2-4] However, reliability trends for long bone fracture classifications in forensic science are unknown. The objective of this research is to assess reliability of fracture classifications utilizing different systems.

Human tibiae (n=30) were experimentally loaded to failure under repeatable boundary conditions (6m/s, lateral to medial) as part of a larger ongoing research project. Resulting fractures were analyzed on three occasions by four observers of varying experience (novice, advanced, proficient, and expert). For each analysis, observers analyzed all bones independently, recorded the total number of classified fractures, and provided a classification for each fracture. For analysis one, no method was provided, and observers were instructed to employ their preferred terminology and/or method. For analysis two, observers were given the AO/OTA Fracture and Dislocation Compendium and instructed to strictly follow the method's guidelines.[5] For analysis three, observers were provided a preliminary novel classification system to follow. Reliability for number of fractures was assessed via Fleiss' Kappa. Since observers reported varying fracture counts resulting in uneven classification observations, overall percent agreement for fracture classifications was calculated as instances when the same number of fractures and classifications were reported per tibia.

Inter-observer reliability for number of fractures within methods ranged from poor (AO/OTA method [κ=0.079]) to fair (no method [κ=0.250], novel method [κ=0.219]). Overall trends between observers demonstrated that the novice observer tended to classify the greatest number of fractures while the expert observer tended to classify the least. Percent agreement for classifications among observers in analysis one was 3%. Since no method was provided, low agreement could be attributed to the lack of standardized terminology. For analysis two (AO/OTA), percent agreement for fracture type was 27% and only 10% for fracture group. This indicates higher inter-observer agreement for broad classifications (e.g., simple fractures) and lower agreement for specific classifications (e.g., oblique fractures). Analysis three (novel method) had the most fractures classified overall; however, percent agreement of initial classifications (complete or incomplete fractures) was 13% and decreased to 6% for specific classifications.

Reliability in number of fractures between methods ranged from poor (expert [κ=0.013], novice [κ=0.196]), to fair (advanced [κ= 0.230]), to moderate (proficient [κ=0.420]). When comparing classifications within observers, the advanced observer was the most consistent between methods (36%) compared to the proficient (16%), expert (13%), and novice observers (3%). Generally, more experienced observers exhibited greater variation in classification terminology, possibly due to differing interpretations of classification criteria provided.

Overall, low agreement evidenced in this study is likely due to unclear criteria and method variation for identifying and classifying fractures at varying experience levels. Even when agreement in number of fractures increased, observers frequently classified fractures differently. These disparities suggest that differential classifications for the same fracture are possible, potentially impeding documentation and interpretation of trauma in forensic casework. Improvements to fracture classification systems are warranted, with an emphasis on establishing explicit, well-defined classification criteria to better facilitate observer agreement. Findings from this investigation will be used to further develop the novel method for long bone fracture classifications resulting from blunt impact scenarios.

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Trauma; Method Agreement; Blunt Force

A113 Investigating Differences in Methodological Approaches to Scoring Fracture Angle, Outline, and Surface

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Learning Objectives: The goal of this presentation is to present data comparing results obtained when different methodological approaches are applied to score fracture characteristics commonly reported in bone taphonomy studies (fracture angle, outline, and surface). Attendees will learn how methodological choices influence scoring of fracture characteristics and, potentially, interpretations of fracture timing.

Impact Statement: This presentation impacts the forensic science community by critically evaluating how methodological choices may influence the documentation and interpretation of skeletal trauma.

Abstract Text: Fracture characteristics are expected to change as bone transitions from wet to dry over the postmortem interval. Various studies have investigated similar fracture characteristics (especially fracture angle, outline, and surface) to draw conclusions about this transition.[1-4] Because each study presents its own protocols for scoring fracture characteristics, results may not be comparable due to methodological differences. It is currently unknown how these differences may impact the results of bone taphonomy studies and, potentially, interpretations of fracture timing based on these characteristics.

The objectives of this study were to: (1) evaluate method agreement on fracture angle, outline, and surface scores; and (2) investigate whether different methods produce different distributions of scores.

Materials included 40 deer femora exposed to outdoor conditions for different intervals of time. Experimental blunt force impacts were delivered to five bones every two weeks from 0 to 14 weeks post-exposure. Four methods were applied to score fracture angle, outline, and surface expressions for each femur.[1-4] The most distal and proximal fragments of each bone were scored using all methods. One method (Green and Schultz) generated an overall bone score based on a combination of proximal and distal fragment scores.[1] Proximal and distal scores were considered separately for all other methods.[2-4]

Fracture angle, outline, and surface expressions were initially scored according to the original publications. To facilitate comparison between methods, these scores were converted to scores of "wet," "intermediate," or "dry" as presented in the original publications. Fleiss' kappa tests were used to assess method agreement for converted scores. Pairwise chi-square tests were used to investigate associations between methods and scores.

Method agreement was statistically significant ($p < 0.05$) for fracture angle, outline, and surface. Kappa values for fracture angle ($\kappa = 0.591$ proximal; $\kappa = 0.876$ distal) indicate moderate to near perfect agreement, suggesting fracture angle is scored fairly similarly across methods. Kappa values for fracture outline ($\kappa = 0.454$ proximal; $\kappa = 0.692$ distal) indicate moderate to substantial agreement in how this feature is scored across methods. Very low kappa values ($\kappa = 0.0594$ proximal, $\kappa = 0.0458$ distal) indicate considerable variability in how fracture surface is scored across methods.

The results indicate certain methods produce significantly different fracture angle, outline, and surface scores. Wheatley fracture surface scores differed from all other methods ($p < 2.2e\text{-}16$).[3] This method did not use an "intermediate" surface score, and therefore generated more "wet" and "dry" scores compared to other methods. Green and Schultz fracture angle and fracture outline scores differed from proximal fragment scores generated using all other methods ($p < 0.0003$).[1] More fracture angles and fracture outlines were scored as "intermediate" than "wet" compared to other methods. This suggests observation location influences fracture angle and outline scores.

The results of this study confirm that fracture angle, outline, and surface scores vary when evaluated using different methods. Researchers should be aware that studies reporting on similar fracture characteristics may not be directly comparable due to different scoring protocols. While the methods reviewed in this study were largely intended to explore fracture transition timelines and not to diagnose fracture timing, practitioners should be aware that different methods may produce different scores for certain fracture characteristics. If scores are taken at face value, methodological differences in scoring protocols could influence interpretations of fracture timing. Finally, the results highlight a need for clear, detailed, and widely accepted protocols for documenting skeletal trauma and taphonomy in research and casework. The next phase of research will assess intra-observer and inter-observer agreement to investigate which methods yield the most consistent and repeatable results and identify needs for clarification among scoring protocols.

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Forensic Anthropology; Trauma Analysis; Methodology

A114 Taphonomic Change in Fracture Characteristics of Porcine Long Bones in the Sonoran Desert During the Summer

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Learning Objectives: After attending this presentation, attendees will better understand the characteristics used to differentiate perimortem fractures from postmortem damage and how the timeline at which bone becomes "dry" in the Arizona Sonoran Desert compares to other climates.

Impact Statement: This presentation is the first part of a multi-year project aimed at establishing an observed timeline of when bone transitions from "fresh" to "dry" in the postmortem interval and when fracture classification changes from perimortem to postmortem in the unique, extreme environment of the Sonoran Desert. The results from this and following studies will be tested in a medical examiner's sample to test the application of experimental methods in non-human models to real cases.

Abstract Text: Estimating the timing at which a fracture occurred is a critical aspect of skeletal analysis in forensic anthropology. Characteristics have been defined that are considered indicative of "fresh" and "dry" bone, or perimortem and postmortem timing. In general, fresh bones are expected to fracture with U- or V-shaped outlines, have smooth fracture surface morphology, and exhibit oblique or acute fracture angles. Conversely, dry bones typically fracture along transverse lines, with jagged surface morphology and right fracture angles.[1-4] As the drying of bone is a gradual process, the transition of fracture characteristics from those indicating fresh bone, or a perimortem break, to those indicating dry bone, or postmortem damage, occurs on a continuum.[5] A pilot study, conducted in the fall and winter, demonstrated a transition to postmortem characteristics up to one month sooner than previously studied climates, with"'intermediate" characteristics appearing in week 4, a majority of postmortem characteristics by week 8, and observation of all postmortem characteristics by week 12.

The Sonoran Desert, in the southwestern United States stretching through southern Arizona and California and into Mexico, presents a unique climate—in the summer months, extremely high temperatures compounded with low humidity can produce rapid initial decomposition that may lead to degradation of bone occurring faster than in summer months.[5] To observe the effects of the extreme summer in the Sonoran Desert on skeletal remains, 26 porcine (S*us scrofa domesticus*) femora were fractured using a custom drop apparatus. Two femora served as controls and were fractured after collection from a butcher shop without being frozen. The remaining 24 femora were placed in a fenced environment in direct sun exposure in Glendale, AZ, and were collected and fractured at one-week intervals. The drop apparatus applied a force of 567kg/cm^2. Temperature, humidity, and precipitation data were collected from local weather stations. Fracture angle, outline, and surface morphology were scored, and an overall determination of perimortem or postmortem was made for each bone. Two bones did not break on the first impact and so were excluded from the results.

During this study, daily high temperatures ranged from 67–116°F, while lows ranged from 50–93°F. Humidity ranged from 4% to 40% and 0.01 inches of rain was measured. Characteristics scored as "intermediate" were present from the control week through week 12. In this unique environment, perimortem or fresh characteristics cease to be observed a minimum of four weeks earlier than previously tested environments.[1-3] Perimortem characteristics were not observed past week 9. The overall determination of "perimortem" occurs through week 7, at which point the fractures are then scored overall as"'postmortem," although intermediate characteristics are still present. Differences in color between the cortical surface and the fracture surface were noted in the first week, and the cortical surfaces from this point onward were stained red and dark brown. Postmortem characteristics were first observed in week 7, with fracture morphology becoming jagged. Similar to other studies, there was not a complete transition to postmortem characteristics; however, determination of timing based on overall appearance classified the fractures as postmortem. The hot summer in the Sonoran Desert produces a more rapid transition—as much as 11 weeks earlier—from perimortem to postmortem fracture characteristics than has been documented in other climates.[1]

This project represents the first portion of a project encompassing deposition in all four seasons in order to account for the variation throughout seasons. Research further investigating the influence of the environment over the taphonomic change of bones and fracture characteristics is necessary as is testing of how these results translate to real cases.

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Trauma; Taphonomy; Postmortem Interval

A115 Distribution Patterns of Trauma by Event Type: Examining the Differences Between Trauma Distributions of Decedents in Different Traumatic Events

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Learning Objectives: This presentation will illustrate how trauma is distributed in different event types, including falls, blast events, aircraft crashes, and motor vehicle/pedestrian impacts. The results further examine the potential for these distributions to predict the correct event type using random forest modeling.

Impact Statement: While preliminary and limited in scope, this research shows that trauma distributions may have enough differences between event types to potentially create future predictive models using trauma distribution to determine the likelihood of different traumatic events.

Abstract Text: Skeletal trauma distributions by event types have been preliminarily studied, but the trauma distributions have rarely been comparatively studied. Furthermore, there are few studies which use rigorous statistical methods to compare trauma distributions and examine them in comparison to other event types.

In order to examine skeletal trauma distributions by different event types more thoroughly, four different event types were examined for skeletal trauma distribution. These four types were selected in order to be compared to skeletal blast trauma, an event type with little civilian data. In addition to blast events, falls from height, aircraft crashes, and motor vehicle pedestrian impact data were collected. Analysis was conducted using chi square analysis, comparing trauma presence and absence at the zone, element, region, and axial versus appendicular levels. These data were collected from deidentified decedent case files from medical examiner's offices and coroner's offices from across the United States. In addition, blast trauma and aircraft crash trauma data from the Defense POW/MIA Accounting Agency (DPAA) were included to provide a comparison from non-civilian contexts. Hard tissue trauma events were recorded by bone element, limb, and specific bone location using osteological zones described in CORA, a database designed and built by DPAA. Case-specific data were recorded, including a description of the cause of the event, basic demographic information, and a general inventory of the remains to indicate whether the absence of trauma is due to a lack of preservation or a lack of injury.

The data collected includes 227 individuals from blast events, 70 individuals from aircraft crash events, 42 falls from height, and 50 motor vehicle/pedestrian collisions. At all different levels of trauma specificity, there were significant differences in trauma frequencies. The aircraft crashes and blast events were most distinct from the other types of trauma, and falls and motor vehicle pedestrian impacts were most similar. However, there were distinct areas of trauma distribution for each event type. While the data are limited to only a few event types, these trauma distribution data are being used to generate a predictive model of trauma event type using random forest modeling, which will provide investigators with important trauma analysis comparisons to help assess trauma causes.

Skeletal Remains; Trauma; Blunt Force

A116 Is It All the Fault of a Fist? Forensic Reconstruction in a Case of "Imperfect" Skull Fractures

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Learning Objectives: The study of skull fractures is very complex in the forensic field. Traumatic fractures of the head have always been of significant interest in the courtroom, especially if caused by the action of other individuals. Osteogenesis Imperfecta is a bone pathology that causes fragility of the bone tissue, even at the cranial level, although this is poorly documented.

Impact Statement: The aim of our work is to present a unique case of traumatic head fractures caused by a punch. The victim was suffering from imperfect osteogenesis. Forensic analysis together with the radiological study of fractures is essential to highlight some salient data and arrive at a definitive diagnosis.

Abstract Text: The analysis of skull fractures represents an important field of forensic medicine. In some cases, the study of fractures, their origin, and propagation can be difficult.[1] Performing an accurate reconstruction of the injury, with the aid of radiological and digital techniques, is fundamental to have a complete picture of the fractures themselves and, therefore, of the dynamics behind them.[2]

The bones of the skull can be fractured by direct traumatic action, represented by the impact of a moving body against the head, or of this one against an obstacle, or by an indirect traumatic action (i.e., by recoil) transmitted by the spinal column due to a fall on the feet or on the trunk, or again by anteroposterior or later-lateral compression, producing in that case two bilateral symmetrical fracture complexes.

A punch thrown at a subject's face is unlikely to produce fracture lesions by direct action. More frequent is the presence of skull fractures symmetrically opposite to the point of application of the punch, for example in the case where the subject suffers a consequent head injury by crashing on the asphalt. What if a subject has a first lesion complex in the right maxilla and a second lesion complex, globe fracture, in the ipsilateral parietal bone? How can this typical fracture pattern be explained? And again, is the second lesion complex a direct consequence of the first lesion complex? If the subject is suffering from Osteogenesis Imperfecta (OI), can this pathology give cranial "manifestations"?[3] How does one answer these and other questions in a courtroom?

This paper originates from the analysis of a court case that came to the attention of the authors. A subject (Y1) lands a punch on the right hemilateral side of the face of another subject (Y2) suffering from OI in clinical stability. Y2 falls backward onto the asphalt, helpless, first impacting on the buttocks and then supine. The sequence of events is documented by, albeit unclear, images extrapolated from a surveillance camera. After the incident, Y2 was promptly subjected to diagnostic and therapeutic treatment.

Initial X-ray images revealed: (1) a first "molded" or small surface fracture complex, consisting of multiple decomposed bone plugs, in the right maxillary sinus, involving the floor of the ipsilateral orbit (anterior margin);and (2) a second C-shaped fracture complex, with anterior concavity, on the right parietal bone, the upper branch of which culminated at the frontal bone, and the lower branch culminated at the inferior lacerated fissure. Can a fist cause this particular, anomalous injury? Can a fist result in two fracture complexes of such magnitude, in the same haemilate of the skull? Can OI, although clinically stable, be a concomitant factor in the genesis of this fracture pattern?[4,5] For an in-depth analysis of the case, a Computed Tomography (CT) reconstruction of the skull was used, which revealed new peculiar findings: small, symmetrical, Wormian bones in the context of the lambdoid suture, which are characterized by fusion of the outer and inner plateau and absence of diploes.[6-9]

In the literature, there are no documented cases of cranial involvement in subjects with OI, let alone cases of cranial fracture trauma of similar magnitude and morphology. Therefore, carefully examining this atypical fracture pattern, on the basis of the fundamental feedback provided by the radiological reconstruction and the patient's clinical data, the authors believe that the bone lesion entity highlighted in the presented case may have been favored by a particular fragility of the cranial plate induced by OI, albeit in a state of clinical stability.

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Skull Fractures; Osteogenesis Imperfecta; Radiological Reconstruction

A117 Characterizing Entrance Wounds From Gunshot Trauma in Tubular Bones: Comparing Handgun and Rifle Ammunition

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the characteristics that are typically associated with entrance wounds from different types of gunshot trauma in tubular bones.

Impact Statement: This presentation will have a significant impact on the forensic community by presenting valuable data on the characteristics of entrance wounds in tubular bones that are useful for trajectory interpretation.

Abstract Text: Understanding and accurately analyzing gunshot wounds is a difficult task for forensic anthropologists. This is due to the wide variation in firearms, projectile characteristics, and the range of velocities involved, as well as intrinsic characteristics of the impacted bone.[1] The interpretation of entrance and exit wounds resulting from gunshot impacts in tubular bones is currently challenging, as existing knowledge primarily focuses on the wounds in the skull. There have been some experimental studies focused on gunshot wounds in long bones, but such studies remain scarce, with small sample sizes and little consistency between methodologies.[2,3]

Typically, trajectory analysis is performed by identifying entrance and exit wounds based on matching qualitative characteristics (e.g., shape of the defect, beveling, regularity of the defect edges) reported in the literature. However, there are still no systematic approaches aimed at describing the characteristics associated with entrance wounds in long bones that could support forensic investigations. This pilot study particularly focusses on the characteristics of entrance wounds in tubular bones resulting from the impact of 9mm and .223 Remington projectiles.

Deer metapodials (n=20) were donated by a local meat processor in upstate New York and were used as a proxy for human long bones. To replicate wounds that may be observed in casework, the bones were impacted by an expert shooter by using a handgun-type ammunition at 5 meters (9mm non-expandible projectile, n=8), and a rifle type at 20 meters (.223 Remington, n=12). The intact metapodials with soft tissue were vertically held by an industrial clamp in a wooden stand at 110cm from the ground, so that they were placed perpendicular to the weapon and were impacted at the mid-diaphysis region on the medial surface. A plastic tarp was positioned around the stand to collect all bone fragments ejected during the shots. After the shooting, the metapodials were dissected manually, and any remaining tissue was removed using detergent-enzymatic maceration in warm water. The fragments were then realigned using a dissolvable glue, and their qualitative (fracture and defect morphology for both entrance and exit defects) and quantitative fracture characteristics (size and number of fragments) were evaluated.

The .223 Remington caused more fragmentation, which resulted in fragments that were both larger and narrower than those produced by the 9mm. While extreme fragmentation prevented full reconstruction of some bones, both sample groups exhibited circular and semicircular defects with external beveling and cortical chipping. Additionally, both sample groups showed multiple radiating fractures emanating from the entrance wound, resulting in a triangular fragment that was only observed to be associated with the entrance defect. Further, the triangular fragment was usually larger in samples impacted by the 9mm projectile since these impacts had fewer radiating fractures compared to those produced by the .223 Remington.

Preliminarily, this study demonstrates that external beveling and cortical chipping are consistent characteristics of entrance wounds, regardless of the type of projectile. The appearance of a triangular fragment is also linked to the point of impact in long bones, and it could indicate the trajectory when incomplete bones are being analyzed. This triangular fragment, when used in conjunction with other fragment size metrics, may be useful in differentiating between types of projectiles. Further pattern analysis should be performed on more suitable proxies for human long bones to account for the range of variations in bone defects from gunshot trauma.

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Gunshot Trauma; Long Bones; Entrance Wounds

A118 Exploring Inter-Observer Agreement in Assessments of Projectile Defects

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Learning Objectives: Attendees will learn about inter-observer agreement and reliability in assessments of experimentally produced projectile trauma. Potential implications for documenting skeletal trauma in research and casework will also be discussed.

Impact Statement: This presentation impacts the forensic science community by critically evaluating how skeletal trauma assessments may vary among analysts. This line of research may help identify training needs and/or inform development of guidelines for documenting trauma in research and casework.

Abstract Text: Visual and written documentation are required components of skeletal trauma analysis.[1] Current standards require written documentation of defect location and characteristics but offer minimal guidance about the characteristics to document.[1] Research investigating which types of characteristics are most and least reliably documented among analysts may help inform the development of practical guidelines for documenting skeletal trauma.

The objectives of this study were to: (1) determine whether there are statistically significant differences in trauma assessments made by different observers, and (2) identify which variables exhibit the highest levels of inter-observer agreement and reliability.

Four analysts (one PhD, one MA, and two BAs) independently assessed 39 porcine scapulae with experimental gunshot defects. The two analysts with graduate degrees had prior experience evaluating projectile defects for the investigated variables while the two analysts with undergraduate degrees did not. All four analysts evaluated the presence and number of cone cracks, radial cracks, circumferential cracks, and crack branches in all 39 scapulae. Experienced analysts measured entrance (enTFL), exit (exTFL), and total (TFL) fracture lengths and minimum and maximum entrance and exit defect dimensions in all 39 scapulae. Inexperienced analysts measured a subsample of 12 scapulae. Fleiss' kappa was used to evaluate inter-observer agreement on the presence of features. Intra-Class Correlation (ICC) was used to assess inter-observer reliability for fracture counts, lengths, and defect dimensions. Results with p-values less than 0.05 were considered statistically significant. Reported κ and ICC values are significant unless noted.

There were significant differences among observers in the presence of cone cracks, circumferential cracks, and crack branches, and in the number of radial cracks, circumferential cracks, and crack branches. Differences were primarily between experienced and inexperienced analysts. Inexperienced analysts tended to identify fewer features than experienced analysts.

Perfect agreement was observed on radial crack presence. An ICC value of 0.54 suggests a moderate level of inter-observer reliability in radial crack counts. There was moderate agreement on the presence ($\kappa = 0.45$) and number of crack branches (ICC = 0.53) and fair agreement on the presence of circumferential cracks ($\kappa = 0.20$). The presence of cone cracks showed poor agreement ($\kappa = 0.07$, $p = 0.30$).

In the subsample measured by all analysts (n=12), only TFL and exFL differed significantly by observer, with inexperienced analysts measuring shorter lengths than experienced analysts. Inter-observer reliability was high for enTFL (ICC = 0.91), while analysts measured exTFL (ICC = 0.49) and TFL (ICC = 0.43) with greater variability. Among defect dimension measurements, reliability was good for minimum exit measurements (ICC = 0.87) and moderate for maximum entrance (ICC = 0.54) and maximum exit measurements (ICC = 0.54). Minimum entrance measurements (ICC = 0.23) exhibited considerable variability, indicating poor reliability.

In the full sample measured by experienced analysts (n=39), fracture length ICC values ranged from 0.94 to 0.96, indicating excellent inter-observer reliability. ICC values were higher for minimum exit (ICC = 0.84) and minimum entrance (ICC = 0.73) than maximum entrance (ICC = 0.46) and maximum exit (ICC = 0.54) measurements, indicating greater inter-observer reliability for minimum than maximum dimensions.

This study explored inter-observer agreement and reliability across various trauma assessments. Analysts identified and counted radial cracks and crack branches more consistently than circumferential cracks or cone cracks. Among experienced analysts, fracture length and minimum defect dimensions exhibited the highest inter-observer reliability, suggesting these measurements may be particularly desirable in trauma documentation. Notably, the results indicate experience influenced trauma assessments. Standardized guidance and increased training may improve reliability of trauma documentation in research and casework. Further research is needed to investigate intero-bserver reliability among a larger pool of analysts and in different trauma types.

Reference:

1. Academy Standards Board (ASB) 2024. *ANSI/ASB Standard 147: Standard for Analyzing Skeletal Trauma in Forensic Anthropology*, 1st Edition. https://www.aafs.org/sites/default/files/media/documents/147_Std_e1.pdf (Accessed 7/26/2024).

Trauma Analysis; Projectile Trauma; Inter-Observer

A119 The Validation of 3D Photogrammetry Models to Document Cranial Trauma

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Learning Objectives: After attending this presentation, attendees will understand a practical application of photogrammetry, specifically in documenting cranial fractures, as well as the accuracy of this method when investigating skeletal cranial trauma.

Impact Statement: This presentation will impact the forensic science community by providing a method to investigate and three-dimensionally document skeletal trauma in a concise and ethical manner. Possible future outcomes of this research include accurate three-dimensional models of skeletal remains that can be used in place of physical remains when necessary.

Abstract Text: Three-dimensional imaging is an ideal way to document skeletal trauma for independent evaluation by other forensic practitioners, which allows the physical remains to be released to the decedent's loved ones in a timely manner. This study investigated validating the accuracy of photogrammetry on fractured human crania. Photogrammetry is a method that consists of taking a series of photographs at various heights and angles to then compile into a three-dimensional model using a software program. The program used to create the models is called "Reality Capture." This program uses the photos to match points on the object and stitch the photographs together to render a three-dimensional model. This approach is both cost effective and convenient for ease of access and portability as it only requires a high-resolution camera. This makes it preferable to other three-dimensional imaging technologies of Computed Tomography (CT) scans and 3D laser scanning, which require specialized and expensive equipment.

While some studies have tested the use of photogrammetry reconstructions on cranial measurements, none have explored its utility in skeletal trauma analysis. Ten previously fractured crania were photographed and modeled using Reality Capture. The ten fractured crania were photographed 180 times from three different angles and heights as well as positioned three different ways: Anterior, Inferior, and Superior views. This setup was utilized to have a sphere-like pathway of the camera to result in many overlapping photographs to create a measurable three-dimensional model. The same three fractures were measured on both the physical crania and the digital model, using a sliding caliper and the measuring tool on Reality Capture. A paired t-test was conducted to compare the measurements between the physical hand measurements and the Reality Capture measurements. The p-value came out to be 0.05119, suggesting there is no statistically significant difference between these measurements. The mean difference was 0.8416, indicating that the program, on average, overestimates the measurements by 0.8416mm. To have the shorter and longer measurements on the same factor scale, a relative error test was conducted. The average relative error was 0.0274, which indicates that Reality Capture, on average, overestimates values by 2%.

Photogrammetry; Cranial Trauma; Forensic Anthropology

A120 Enhancing Forensic Investigations: Leveraging Transfer Learning for Cut Mark Classification on Skeletal Remains

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Learning Objectives: After attending this presentation, attendees will understand the challenges of classifying cut marks in forensic investigations, the principles of using Artificial Intelligence (AI) and Convolutional Neural Networks (CNN) for image classification, and the specific application of transfer learning in this context.

Impact Statement: This presentation will impact the forensic science community by introducing a novel approach to cut mark classification, potentially increasing the reliability of forensic investigations. Furthermore, this research offers practitioners a cutting-edge method that can be easily integrated into already established workflow.

Abstract Text: Sharp force tools, and most commonly knives, are one of the weapons most frequently used in homicides in the United Kingdom.[1] Recent research has demonstrated that the ability to reliably classify cut marks using traditional methods is greatly experience-dependent and can impact forensic investigation.[2,3] This pilot study explores the potential of AI to assist in classifying cut marks produced by knives, leveraging computer vision to analyze and process two-dimensional images. Neural Networks (NN) are usually the preferred choice for image classification as these machine-learning structures can detect complex patterns.[4]

Traditional NN models, which require large amounts of training data, are impractical in the forensic field due to the difficulty of obtaining sufficient cut mark images. To overcome this limitation, the present project employs transfer learning, a method where pre-trained algorithms are fine-tuned using a new specific dataset to solve the desired classification task.[4]

The pilot study aims to assess the effectiveness of transfer learning for classifying knife trauma produced by serrated or non-serrated knives and by single- or double-beveled blades.

For this purpose, the authors have produced a new dataset of cut mark images. The dataset, which includes 130 vertical and 130 profile images of cut marks, has been made by photographing 130 cut marks from different angles. The nine knives that have been used to generate cuts on sus scrofa defleshed ribs are: Knife 1—non-serrated single-beveled; Knives 2,3,and 4—non-serrated double-beveled; and Knives 5,6,7,8, and 9—serrated single-beveled.

Photographic images of the cut marks have been acquired using a stereomicroscope with an integrated camera at various magnification levels to mimic real investigative scenarios. Photographs have been taken vertically and at a 90° angle (profile) following the methodology proposed by Steiger and Borrini.[3] This kind of photograph is consistent with the documentation routinely provided by forensic practitioners, which could be easily integrated into this process in the future.

The cut marks images have been pre-processed following model-related requirements, and data augmentation procedures have been applied to generate additional training data. Two classifiers have been trained and fine-tuned with the vertical images dataset to distinguish between serrated and non-serrated knives and single- and double-beveled knives, while a second pair has been trained and tested using the profile pictures.

The implementation of AI and transfer learning in the classification of cut marks is a promising advancement in forensic science. This preliminary study serves as a starting point for further research and development to provide forensic practitioners with a machine learning method for cut mark analysis. Furthermore, the creation of the dataset serves as a preliminary version of a larger, standardized dataset for trauma classification.

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AI; Machine Learning; Anthropology

A121 Cutting Edge Comparisons: Analyzing Saw Kerf Variables in Bones Versus Casts

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the differences in observing and measuring saw kerf variables from bony surfaces and their casts.

Impact Statement: This presentation will impact the forensic science community by providing a comparison of saw kerf data collected from bony specimens and their casts to contribute to best practices in forensic anthropological cases of dismemberment.

Abstract Text: In medicolegal cases involving sharp force defects, forensic anthropologists often create casts of defects. A cast is an impression of a defect surface that can be useful in situations where defect surfaces are not easily observable (e.g., incomplete cuts) and creates a permanent record of the defect. While previous research has looked at the use of casts to determine tool class for knives, its use in saw mark analysis has been largely anecdotal and based on knife research. The goal of this research is to compare saw kerf variable scoring and measurement from complete saw cuts in bone and their casts to assess inter-media error rates and provide recommendations for forensic saw mark analyses.

The research sample includes 258 complete cut surfaces created during a previous research project using 27 different saws with varying class characteristics.[1] Nineteen anatomically gifted human humeri and femora were macerated, then sawed while secured with a vice. Each of the cut surfaces was cast. Two kerf variables were selected for this study—tooth hop and pull-out striae. All surfaces were assessed under a stereoscopic microscope. The number and length of tooth hop chains and measurements between consecutive peaks and troughs as well as the number of pull-out striae groups, number of striations within each group, and measurements between each striation were recorded for all cut surfaces. Simple comparisons of kerf variables identified on cut surfaces were calculated using Excel. A McNemar test was used to assess differences in kerf variable identification between media, and Wilcoxon signed rank tests assed differences between the number of tooth hop chains or pull-out striae. Tooth hop chains found on both media were isolated and measured. Relative and absolute differences and relative Technical Error of Measurement (rTEM) were calculated, and a Wilcoxon test was used to assess the difference between the measurements.

The presence/absence of tooth hop was consistently identified on 66.28% of specimens. Tooth hop was identified on 14.34% of the bone specimens and 47.29% of the cast specimens, with a significant difference ($p < 0.001$) between the two media. Of the 36 times tooth hop was identified from both media, the number of chains identified matched in only 8.33% of the specimens; 2.78% had more chains identified on the boney surface as compared to 88.89% on the casts. On average, the casts had four times the number of chains identified. There was no statistical difference in measurements between the media ($p=0.503$), with average relative and absolute differences at -0.034mm and 0.092mm, respectively. rTEM was 14%. There were no systematic trends in measurements deviations, suggesting this is normal measurement error rather than systematic errors caused by the media. Pull-out striae presence/absence matched in 86.43% of specimens. Presence was noted in only 2.71% of boney specimens as compared to 13.95% of casts, with a statistical difference ($p < 0.001$). No significant measurement differences were found.

Both kerf variables were identified more often on the cast surface compared to the boney surfaces with no significant differences in measurements between the media. The use of casts to identify and measure tooth hop and pull-out striae on cut surfaces is recommended as these features are more identifiable in casts, likely because of the cast coloration and lack of glare.

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Sharp Force; Dismemberment; Mold

A122 Testing Bias in Cut Mark Analysis: An Exploratory Study

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Learning Objectives: After attending this presentation, attendees will have an opportunity to evaluate the accuracy of cut mark analysis among different levels of expertise and understand how the reliability of the observers changes when exposed to biasing stimuli.

Impact Statement: This presentation will impact the forensic science community by analyzing the implications of human errors when adopting morphological observation in real-life forensic cases involving knives as weapons of choice.

Abstract Text: Forensic anthropology plays a critical role in the criminal justice system and the overall security of our society; consequently, examiners' potential lack of reliability, objectivity, and independence can have far-reaching implications beyond the discipline itself.

In the past 15 years, there has been increased interest in studying the factors and stimuli that negatively affect forensic work, primarily cognitive bias, which influences experts' judgments. This is even more important when considering methods based on visual comparative analyses that are widely used in forensic anthropology, while having the highest potential of being influenced by bias. Therefore, the authors used cut-mark assessment classification standards as background for the present research that investigates susceptibility to cognitive bias.[1]

Four different types of knives (single- edged, double-edged, serrated, and non-serrated) were used to create 17 cut marks on pig ribs (*Sus scrof*a); the cuts were photographed under a stereomicroscope (38x magnification) with a tangential light source. Photographs were taken vertically and at a 90° angle (profile). Additional photographs of two cut marks per knife type stained with synthetic blood were also taken. Similarly, photos of each knife were taken, with the addition of some without a metric reference and some with synthetic blood stains on the blade and handle.

An online questionnaire containing photographs of cut marks and knives was created. The questions, randomly mixed, were divided into three categories: (1) 11 control questions; (2) 7 confirmation bias-evoking questions; and (3) 11 contextual bias-evoking questions. In the latter category, the context was provided through photographs of blood-stained cut marks and/or blades and pictures without metric scales. Each questionnaire page contained a single query and always provided the viewer with the classification standards.

The survey involved 70 forensic anthropologists of differing educational levels (23 BSc students, 23 MSc students, 6 PhD students, and 18 practitioners) who were tested for their susceptibility to bias.

The results show that the classification standards used offer an accurate basis for analyzing cut marks, with a statistically significant difference in accuracy among educational levels. The findings also demonstrated the statistically significant differences between educational levels and their susceptibility to contextual bias. While statistically significant differences between educational levels were not observed for confirmation bias, statistically significant decreases in the accuracy of answers were detected when comparing control and confirmation bias questions. This was especially true for the cases of PhD students and forensic practitioners, which implies they were more susceptible to confirmation bias than undergraduate and master's students.

The authors suggest that the biasing stimuli employed in this study, such as leading questions and specific contextual clues, need to be mitigated in the everyday work of forensic anthropologists. Effective procedures, such as blinding and the involvement of independent experts, should be employed to mitigate the effects of cognitive bias. In the future, the development of machine learning and AI-assisted software for the observation of cut marks could also improve the reliability of cut mark and blade classification.

Reference:

1. Steiger GS, Borrini M. A proposal for cut marks classification using machine learning: Serrated vs. non-serrated, single vs. double-beveled knives. *J Forensic Sci*. 2024;00:1–13.

Trauma; Anthropology; Evidence Evaluation

A123 Assessing Cranial Macromorphoscopic Traits Used in the Forensic Estimation of Population Affinity

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Learning Objectives: This presentation will provide a brief overview of cranial Macromorphoscopic (MMS) trait analysis in forensic anthropology. This research assesses the utility of a number of new MMS traits and explores the relationships between their various manifestations within a sample.

Impact Statement: This study will provide attendees with: (1) standard definitions for five underexplored cranial MMS traits, as well as a description of the character states for each; (2) a report of the frequency distribution of each trait within eight diverse samples, and; (3) the correlations between the traits and the samples. After attending this presentation, listeners will be well informed on the use of MMS trait analysis and provided with more robust methods for cranial non-metric assessment.

Abstract Text: MMS traits are minute variations in bone, feature, or suture morphology which contribute to overall cranial morphology.[1,2] These traits have been applied to methods for the forensic estimation of population affinity as well as biodistance studies more recently. Population affinity estimates are one component of the biological profile (along with sex, age, and stature) that forensic anthropologists generate for medicolegal death investigators and law enforcement. These profiles are used to help narrow the pool of potential individuals compared with the unidentified skeletal remains. Research has shown that MMS trait analysis can accurately classify unknown individuals into one of the reference groups for a given sample.[1-4]

To further explore cranial MMS traits and assess their potential to correctly classify population affinity for an unidentified decedent, this research tests the utility of five traits for the estimation of population affinity using data from individuals curated by the National Museum of Natural History (n = 720), previously collected by one of the authors.[1] Female and male adults from eight populations (African: n=30, American Black: n=150, American White: n=169, Chinese: n=59, German: n=8, Dutch: n=7, Japanese: n=15, and Native American: n=282) were included to assess any differentiation in trait manifestation by sex and/or population affinity. The cranial MMS traits assessed in this study include: Depression at Nasion, Trace Metopic Suture, Transverse Palatine Suture, Venal Etching, and Sub-nasal Grooves.

Initial data analysis was conducted using a variety of summary and descriptive statistics as well as base-level statistical analyses, including correlation and covariance and Kruskal-Wallis tests to determine significant influence of demographic data on trait expression. Classification models (random forest) were used to assess the classificatory power of these traits.

Results indicate the degree of manifestation of each MMS trait is significantly influenced by both sex and population, excluding the Trace Metopic Suture and the Transverse Palatine Sture by sex. A cluster dendrogram demonstrated significant association in the expression of Venal Etching and Sub-nasal Grooves and Depression at Nasion and the Transverse Palatine Suture. A random forest model correctly classified ~48% of the sample; Transverse Palatine Suture shape was the most important variable in that model.

The five MMS traits assessed in this study have been around for decades, but little or no frequency distribution data has appeared to document their manifestation. These traits are useful in population affinity estimates, but more importantly, they are also useful for exploring aspects of modern human variation.

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Macromorphoscopic Traits; Population Affinity; Human Variation

A124 Exploring Cranial Macromorphoscopic Variation in a Modern American Indian Sample From New Mexico

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Learning Objectives: After attending this presentation, attendees will be introduced to a new reference dataset of cranial morphological variables for a modern American Indian (AI) sample from New Mexico (NM).

Impact Statement: This presentation will impact the forensic science community by providing a new reference sample of cranial Macromorphoscopic (MMS) data for AIs and exploring patterns of variation within the data and compared to other population groups encountered in NM casework.

Abstract Text: Skeletal morphology can be used to examine human variation and assess population affinity in forensic anthropology using several different methods. Method performance is highest when populations present in forensic casework are represented in reference databanks used for methodological development. Cranial MMS traits are highly heritable, reflecting neutral traits under selection, and can be used to estimate population affinity in forensic anthropology successfully, serving as a proxy for genetic relatedness.[1] Currently, cranial MMS data for contemporary AIs are absent from reference databanks, leading to uncertainty when using these data in population affinity estimates. Here we present cranial MMS data from modern AI, identify patterns in the data, and compare biological distance among AIs and other samples.

Cranial MMS data were collected from Computed Tomography (CT) data housed in the New Mexico Decedent Image Database (NMDID) for individuals with American Indian (n=1022), Hispanic (n= 404); American White (n=47), American Black (n=270), and broadly Asian (n=95) affinities. These scans were collected during postmortem examination at the New Mexico Office of the Medical Investigator from 2010–2017.[2] Data for 12 cranial MMS traits were collected following available protocol for CT data based on original instructions for dry bone.[3,4] Initial Data Analysis (IDA) identified patterns among skeletal data for the AI sample and biological distance analysis examined relationships among samples.

IDA showed weak positive correlations between Nasal Aperture Shape (NAS) and Nasal Aperture Width (NAW; 0.13), NAW and Interorbital Breadth (IOB; 0.19), and Anterior Nasal Spine (ANS) and Inferior Nasal Aperture (INA; 0.13). Multiple correspondence analysis shows group separation was most significant with nasal derived traits (NAS, NAW, ANS) and Palate Shape (PS). The AI sample exhibited a wider NAW, minimal to no projection of ANS, a more bell- and bowed-shape NAS, and a more hyperbolic PS. Biodistance analysis using Smith's Mean Measure of Divergence indicated significant distances among all group pairs at the $p = 0.05$ level. The White sample was the furthest from the AI sample and plotted away from all other groups. The AI, Black, Hispanic, and Asian groups plotted in a cluster, but the distance matrix separated the AI sample slightly from the other three. A hierarchical plot separates the White sample at the first node, then Asian/Black and Hispanic/American Indian.

These patterns reveal that the AI sample is different from other samples encountered in forensic casework, and that the AI sample is the most like the Hispanic sample, aligning with the migration history of these two groups. Cranial MMS traits indicate group separation using midfacial traits of the nasal and palate area. Further exploration of the AI sample will include classification modeling to assess utility in casework in NM and the United States Southwest.

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Forensic Anthropology; Population Affinity; Computed Tomography

A125 Patterns of Covariation in Cranial Metric and Macromorphoscopic Variables

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Learning Objectives: After attending this presentation, attendees will better understand the relationships between cranial Macromorphoscopic (MMS) traits, Interlandmark Distances (ILDs), and demographic variables from three samples.

Impact Statement: This presentation will impact the forensic science community by demonstrating the consistent patterning in relationships across cranial phenotypic variables regardless of geographic origin or temporal variation. This knowledge impacts how and when forensic anthropologists should mobilize cranial variables to estimate aspects of the biological profile.

Abstract Text: Previous research has quantitatively demonstrated that, except for traits assessing analogous nasal and interorbital breadths, craniometric measurements (ILDs) and MMS traits provide different information about cranial phenotypic variation in contemporary United States samples.[1] The current study quantifies patterns and associations among ILDs, MMS traits, biological sex, and country of origin in three temporally and/or geographically distinct samples. Data were obtained for 385 adult individuals for two 21st-century samples and one early 20th-century sample. These include the Subadult Virtual Anthropology Database (SVAD; n=240), a cemetery sample from Manila (n=75), and the Forensic Anthropology Databank (FDB; n=70). The SVAD and FDB samples were downsampled in a previous project to balance the biological sexes, and with the inclusion of the Manila sample, the total sample is slightly skewed to more males (n=201) than females (n=184).[1] Twelve MMS traits were scored and 22 ILDs were derived from either volumetric models of the cranium reconstructed from computed tomography scans (SVAD) or collected directly from skeletal remains (FDB and Manila). Unsupervised techniques, like Mixed Factor Analysis (MFA) and Factor Analysis of Mixed Data (FAMD), were employed to analyze both variable types using structured grouping (MFA) and variable level exploration (FAMD).

MFA analyses indicate the first four dimensions account for 40% of total variance. Dimension 1 captures the relationships between country of origin and the MMS traits. Dimension 2 captures the relationship between ILDs and sex. Dimension 3 captures the temporal and geographic variation across samples. Dimension 4 captures shared variation among ILDs and MMS. FAMD analyses indicate that country of origin is most closely associated with mid-face MMS traits and splanchnocranium ILDs. Additionally, biological sex is most closely associated with all ILDs and the more metrically assessed MMS traits, such as post-zygomatic tubercle and malar tubercle. Country of origin and biological sex are separated in every analysis across the first and/or second dimensions, demonstrating differential impacts of these demographic variables on cranial phenotype. While these multivariate relationships consistently differentiate between biological sex and between country of origin, the patterns among variables are consistent across all samples whether explored independently or in tandem.

These results support long-standing interpretations concerning the relationships between variable traits and population demographics mobilized in forensic anthropological casework. Furthermore, the analyses presented herein reinforce the intricate, multi-faceted nature of the cranial phenotype and provide empirically grounded results to guide practical applications. Incorporating advanced statistical methodologies and computational approaches capable of concurrently assessing categorical and metric data may better capture cranial variation and provide stronger estimates for the biological profile components in forensic casework.

Reference:

1. Stull KS, New BT, Corron LK, Auchter LE, Spradley MK, Wolfe CA, Chu EY, Hefner JT. 2024. Exploring Mutual and Exclusive Biological Information in Cranial Metric and Morphological Variables. *Forensic Anthropology*.

Craniometrics; Macromorphoscopic Traits; Factor Analysis

A126 Exploring Population Affinity Analysis for Forensic Anthropology Casework in Latin America

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Learning Objectives: After attending this presentation, attendees will better understand the relationship between cranial morphology and genetics. Using these data, we investigate variation among three regional samples from Latin American in a biological distance framework.

Impact Statement: This presentation will impact the forensic science community by providing biological distance estimates among paired skeletal data and genetic microsatellite loci to discuss the use of craniometric and cranial Macromorphoscopic (MMS) data in the estimation of population affinity on a regional scale.

Abstract Text: Variation in skeletal morphology has been used to estimate population affinity within the United States for decades, largely using craniometric and cranial MMS trait data. This is possible due to the correspondence between skeletal morphology and the genetic structure among population groups; however, this relationship is poorly understood among Latin American populations, which is problematic when unknown individuals cannot be identified through traditional means (i.e., DNA analysis, medical radiography). Herein we focus on the biological profile for identification. We investigate skeletal and genetic variation in three Latin American samples to understand the potential for population affinity modeling in the region. Given different skeletal features identify different aspects of variation, we explore relationships among matched craniometric and cranial MMS data, and test if these skeletal features can serve as proxies for genetic variation in population affinity modeling within Latin America.[1]

Paired data (craniometric and cranial MMS) were collected from Mérida, Mexico (n=111), Guatemala City, Guatemala (n=60), and Medellín, Colombia (n=158). Craniometric data were collected using a Microscribe digitizer and 3Skull software.[2] Cranial MMS data were collected using MMS v1.61.[3] In total, 24 Interlandmark Distances (ILDs) and 10 MMS traits were used in analysis. A set of microsatellite loci (n = 305) was selected from the Pemberton Genetic Dataset as population-genotype comparative data.[4] Initial Data Analysis (IDA) identified patterns among skeletal data; biological and genetic distance analysis assessed similarity and dissimilarity among groups and data types.

Results indicate metric and cranial MMS data separate the samples using different aspects of cranial morphology. The most influential ILDs for group separation include MAB, MDH, and several vault length and breadth measures, while cranial MMS traits (ANS, INA) were highly variable in character state among groups tested. When compared to genetic data, patterns of skeletal variation for both data types reflected those observed in the genetic samples. The craniometric and genetic data produced a strong correlation coefficient of 0.65 ($p < 0.05$), while the cranial MMS and genetic data produced a remarkably strong correlation coefficient of 0.93 ($p < 0.05$), suggesting a highly significant association between cranial MMS and genetic variation. These findings underscore the potential utility of cranial MMS as a proxy for genetic relatedness. Further research identifying the biological mechanisms underlying this correlation is warranted.

Results from this study show that craniometric and cranial MMS variables can be used separately or together to examine variation on smaller, regional scales. This study indicates that population affinity shows promise in Latin America and can be used to identify possible migrants or non-citizens in forensic casework.

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Forensic Anthropology; Cranial Morphology; Biological Distance

A127 AI-Assisted Technological Advancements for the Collection of Cranial Landmark Data and Interlandmark Distances From Medical Imaging Software

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Learning Objectives: This presentation introduces a novel module for collecting craniometric landmark data using 3D surface renderings of Computed Tomography (CT) scans in the medical research imaging software Amira. Developed with Artificial Intelligence (AI), this module integrates with the Amira interface and is fully customizable to meet researchers' needs. Attendees will be introduced to the module and gain insights into data collection capabilities and the extensive information it can provide.

Impact Statement: The presentation will guide attendees through the complete process of using the craniometrics module, demonstrating how to leverage Amira's functionalities. This module exemplifies the potential of combining AI and CT scans in forensic applications, and represents an innovative approach to collecting craniometric data in virtual osteology.

Abstract Text: Virtual anthropology databases provide substantial benefits for research, offering large-scale, diverse cohorts that can be used to drive methodological advancements in research and allow for testing of theoretical models that may otherwise not be possible. As technology evolves, it is crucial to develop methods that utilize these new forms of imaging and visualization effectively.

Our interdisciplinary team developed a craniometric data collection module within the Amira software to enhance, improve, and expedite large scale data collection for cranial landmark data in virtual anthropology. This module's capabilities include 3D visualization of CT scans, creating reference data to be used as a standard for data collection, registering sample data to the reference data, AI-driven landmark plotting, and obtaining an output file containing the x, y, and z coordinate data of all plotted landmarks and the interlandmark distances. Using Amira's general functionalities, researchers can also plot floating landmarks and endocranial landmarks, enhancing the flexibility and comprehensiveness of data collection.

One of the most significant advantages of the Craniometrics Module is its complete customizability. Researchers can tailor the specific landmarks and the number of landmarks collected as well as the skull registration process, based on their research questions and goals. This customization ensures that the data collection process aligns precisely with the unique requirements of each study. The ease of data collection provided by this module is a marked improvement over other virtual craniometric data collection procedures. Here we demonstrate the module and cranial landmark data collection.

In its current form, the module allows researchers to load input files, register the sample skull, and plot 106 cranial landmarks (both ectocranial and endocranial) in approximately 40 minutes. Previous pilot studies requiring the collection of only 64 cranial landmarks using different methods took over an hour, not including the preprocessing time for CT scans. Since its implementation in April 2023, data on over 650 individuals have been collected to be added to the Forensic Databank, increasing reference sample sizes for underrepresented groups such as American Indians.

The Craniometrics Module represents a significant advancement in the field of forensic anthropology. By leveraging the latest in AI and medical imaging technology, it offers a faster and easier method for collecting craniometric data. Its customizability and integration with Amira's powerful tools make it an invaluable resource for anthropological research. As technology continues to advance, the Craniometrics Module sets a new standard for data collection in virtual anthropology, demonstrating the potential of combining AI and CT scans to push the boundaries of forensic science.

Craniometrics; Virtual Anthropology; Computed Tomography

A128 An Evaluation of the Accuracy and Reliability of MorphoPASSE for Skeletal Sex Estimation Using the University of Tennessee Donated Collection

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Learning Objectives: After attending this presentation, attendees will better understand the accuracy, reliability, and limitations of the MorphoPASSE trait descriptions and associated random forest modeling approach to skeletal sex estimation. Attendees will also have a better understanding of the impact of previous experience using traditional sex estimation methods on the implementation of MorphoPASSE.

Impact Statement: This presentation will impact the forensic science community by informing practitioners of MorphoPASSE's ability to yield accurate sex estimates despite variation in skeletal trait scores among raters. This presentation will also draw attention to how ambiguous trait scores and by extension probability values for sex membership impact the accuracy and reliability of this program. Finally, this presentation will provide compelling evidence to support previous assertions that all practitioners regardless of experience level should calibrate using MorphoPASSE-specific trait descriptions prior to using this new program in forensic casework.

Abstract Text: Skeletal sex estimation is a component of the biological profile that contributes probative information during identification. Traditional methods consider pelvic and cranial non-metric traits separately, preventing the generation of a singular estimate of biological sex that exploits multiple aspects of human skeletal variation.[1,2] To address this issue, Klales introduced MorphoPASSE, which is an online open-access program that uses revised traditional non-metric trait descriptions and random forest modeling to statistically integrate these traits from both the pelvis and cranium.[3] To date, MorphoPASSE has only been independently validated once using cranial CT data, though practitioners have begun to incorporate this software and its revised trait descriptions into routine casework.[4] To address this issue, we evaluated the accuracy and reliability of MorphoPASSE using the intended methods of observation (visual and tactile) of both cranial and pelvic non-metric traits collected from 262 donors from the University of Tennessee Donated Skeletal Collection.

Donors were analyzed blind by six independent raters categorized as either experts or non-experts based on terminal degree held and years of experience applying traditional sex estimation methods. Donors were randomly selected from a list of donors not included in MorphoPASSE program development or its reference sample. Experts (n=2) analyzed traits for all donors included in this study, while non-experts (n=3) and one undergraduate with no previous experience analyzed a subset of 57 donors. Trait scores for each donor were run through MorphoPASSE using the contemporary United States reference sample with separate analyses for known and unknown ancestry, and male and female probability values were recorded.

An 80% classification threshold was chosen in consultation with current literature to assign donors to estimated sex categories of female, male, or undetermined.[5-7] Accuracy levels based on this threshold exceeded 85% for both experts and non-experts across analyses when compared with known sex. When donors classified as undetermined were excluded, accuracy levels improved, exceeding 98%. Cohen's kappa values comparing known with estimated sex indicated near perfect agreement at the $p < 0.001$ level for expert and non-expert raters.

Intra-class Correlation Coefficients (ICC) were used to evaluate inter-rater reliability in trait scores collected by expert and non-expert raters. Inter-rater reliability was good to excellent for experts (ICC >0.831) and non-experts (ICC >0.819) across all skeletal traits. Intra-rater reliability, which was evaluated for experts only using a subset of 36 donors, was also good to excellent with ICCs that met or exceeded 0.802 excepting the mental eminence.

A series of Wilcoxon signed-rank tests, which were used to compare probability values produced by MorphoPASSE among raters, identified small ($r < 0.300$) significant differences in these values between the two experts at the $p < 0.001$ level. Another set of these tests was used to compare probability values generated for two separate observations of the same 36 donors for experts only as an additional measure of intra-rater reliability. A moderate ($0.300 < r < 0.500$) significant difference was detected for one expert when ancestry was known ($p = 0.045$).

These findings suggest that, despite some variation in trait scores and generated probabilities among raters, MorphoPASSE produces accurate sex estimates at an 80% classification threshold, especially when undetermined donors are excluded. Importantly, accuracy and inter-rater reliability do not appear to scale with experience level once raters have at least two years of previous experience. It is strongly recommended that practitioners calibrate prior to implementing MorphoPASSE in casework regardless of previous experience and that the program be subjected to further validation across geographic regions and temporal periods. Moreover, further research and clarification is needed to understand the impact of ambiguous skeletal variation and associated trait scores on the accuracy and reliability of the MorphoPASSE random forest model.

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Biological Profile; Sex Assessment; Method Validation

A129 The Application of Decision Trees and Random Forest Modeling for Combining Morphological and Metric Variables of the Skull for Osteological Sex Estimation

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Learning Objectives: This presentation will discuss the application of decision trees and Random Forest (RF) modeling for estimating osteological sex from a combination of morphological and metric variables of the skull. After attending this presentation, attendees will have a better understanding of the benefits of combining morphological and metric variables into one classification model for osteological sex estimation.

Impact Statement: This presentation will impact the forensic science community by demonstrating the applicability of decision trees and RF modeling for combining morphological and metric data for estimating osteological sex in forensic anthropology casework.

Abstract Text: Numerous recent publications have demonstrated the utility of decision trees and RF modeling for osteological sex estimation of the skull, yet none of these studies have incorporated both metrics and morphological traits into their models. Combining traits and measurements into one model has the potential to increase osteological sex classification accuracies for the skull, as well as allow the analyst to make their sex estimate utilizing one robust model, rather than making a decision based on multiple models. The main objectives of this research were as follows: (1) to assess intra-observer error for the selected morphological and metric variables, (2) generate multiple decision trees and RF models that incorporate various combinations of morphological and metric variables for estimating osteological sex, and (3) demonstrate the applicability of the models for use in forensic casework.

Twenty-one metric and 21 morphological variables of the skull were chosen from popular osteological sex estimation methods. A total of 220 males and 183 females were sampled from the University of Tennessee, Knoxville (UTK) Donated Skeletal Collection, the Texas State University Donated Skeletal Collection, the Southeast Texas Applied Forensic Science Facility Collection, and the Hamann-Todd Human Osteological Collection. Statistical analyses were conducted utilizing the R Language. Intra-observer error was assessed from 43 individuals from the UTK Collection to determine which variables to incorporate into the models. Intra-observer error for the morphological data was assessed using Krippendorff's Alpha, while the relative technical error of measurement was used for the metric data. Models were generated for the pooled sample (n = 403) as well as for African Americans (n = 191) and European Americans (n = 212) separately. Prior to generating the models, each dataset was split into a training sample (80% of the data) and a Holdout Validation (HV) testing sample (20%). Decision trees and RF models were generated for the skull, cranium, and mandible utilizing the training samples. In addition to the combined variable models, separate morphological and metric models were generated for comparison. The error of each tree and RF model was assessed using the HV validation sample. Additionally, the RF models were assessed utilizing the Out-Of-Bag (OOB) error.

Twenty-six of the morphological and metric variables achieved an acceptable level of intra-observer error and were incorporated into the models. The highest performing decision trees and RF models incorporated combined variables from the entire skull and achieved the same HV accuracy rates for both model types: 98.7% (pooled), 97.0% (AA), and 100% (EA). The OOB accuracies for the RF models were slightly lower compared to the HV accuracies: 93.0% (pooled), 89.9% (AA), and 94.2% (EA). The combined variable models for the cranium achieved similar accuracies to the skull models, while the models that included mandibular variables only produced the lowest accuracy rates overall. Further, the pooled sample models were tested utilizing the separate HV samples for African Americans and European Americans. In this additional test, the pooled sample models achieved higher accuracies for European Americans compared to African Americans. Overall, most of the models achieved classification rates over 80%, with most skull and cranium models achieving accuracies over 90%. Combining data types resulted in higher accuracies compared to the separate morphological and metric models as well as compared to popular osteological sex estimation methods for the skull. Future research should evaluate the accuracy of the present study's models on different population affinities as well as continue exploring the development of population-inclusive models. Additionally, future research should explore employing decision trees and RF modeling to generate osteological sex classification models that combine morphological and metric variables from multiple regions of the skeleton.

Sex Estimation; Biological profile; Machine Learning

A130 The Application of Non-Metric Sex Estimation Methods to Virtual 3D Cranial Models

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Learning Objectives: After attending this presentation, attendees will have a better understanding of how non-metric sex estimation methods may be applied to virtual 3D cranial models in comparison to real human skulls.

Impact Statement: This presentation will impact the forensic science community by presenting the findings from a preliminary study comparing the decision-making between method application on human crania against their 3D virtual counterpart created from CT scans. These findings help to highlight the necessary research and training needed to continue development of virtual forensic anthropology.

Abstract Text: The possibilities of employing radiological data as a proxy for real human remains are increasingly being considered as the development and validity of forensic anthropological methods are being explored.[1,2] However, published research has not yet fully examined the application of existing sex estimation methods originally developed on human skeletal remains on 3D skeletal models. This study examined whether the commonly employed non-metric sex estimation assessments can be comparably applied to human skeletal remains and their virtual 3D bone model counterparts created from computed tomography.

Eight human skulls were selected from the archaeological assemblage housed at the University College London (UCL) Institute of Archaeology. The skulls were scanned with an O-arm Surgical Imaging System from Medtronic (cone beam computed tomography) and converted to three-dimensional models using 3D Slicer. Participants with osteological experience were asked to score non-metric sex indicative traits on real human skulls using the Walker method, make final sex estimations, and score their confidence.[3] Participants were asked to repeat this process on the virtual 3D cranial models approximately two weeks later.

This presentation discusses the results of the trait scores, the sex estimations, and the participants' confidence in the different sources. Weighted kappa values were consistent with other recent literature, ranging from fair to moderate for trait scores (0.242-0.528), and poor to moderate for sex estimation in five groups (0.027-0.600).[4] Participants who reported previous virtual osteological experience were consistently higher than the other participants, with kappa scores in the moderate range for five sex estimation groups (0.455-0.600) and even higher for three sex estimation groups (0.600-0.857). Additionally, participants overall were more confident in their scores and estimation on the real crania than on the 3D models.

Further discussion addresses the key factors surrounding the comparable application of methods as well as the variables that impacted this experiment. These include the difficulties in the direct transfer of traditional osteological knowledge to virtual bone models, the importance of specific training and experience, and the possible limitations of DICOM data from CT scans.

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Forensic Anthropology; 3D Models; Sex Estimation

A131 Evaluating Subadult Age Estimation Methods From Long Bones Using a Contemporary Japanese Sample

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Learning Objectives: After the presentation, attendees will gain a better understanding of how current subadult age estimation methods for long bones perform when validated using a Japanese sample.

Impact Statement: This presentation will impact the forensic science community by demonstrating how skeletal variation can impact subadult age estimation. In addition, this presentation supports ongoing efforts to diversify skeletal reference material for validation of current methods by encouraging population-specific or globally inclusive methods.

Abstract Text: Age estimation using long bones is a well-established aspect of the subadult biological profile.[1-5] These methods rely on the strong relationship between age and long bone length and, therefore, size. However, most methods are primarily developed by, trained and tested on, and used for the skeletal remains of individuals of Western European descent.[6] The lack of appropriate skeletal reference materials for a variety of population histories may lead to biased (e.g., consistent over- or under-) estimates due to differences in growth patterns and proportions across the globe.[7,8] Technological advances have supported the increase of diverse skeletal reference material available for research by using alternative methods to dry bone such as Postmortem Computed Tomography (PMCT) or radiographic images.[9] The goal of this project is two-fold: (1) to evaluate the performance of currently available methods of subadult age estimation, and (2) to investigate whether differences between geographically disparate groups generate patterned age estimates in a contemporary Japanese sample.

Long bone lengths and breadths of 64 individuals (F=27, M=37) aged between birth and 16 years were measured from reconstructed skeletal models processed from full-body PMCT scans at the Education and Research Center of Legal Medicine at Chiba University in Japan. Known age was compared to point age estimates generated from long bone measurements using five common subadult age estimation methods: Cardoso and colleagues, Facchini and Veschi, López-Costas and colleagues, Rissech and colleagues, and Stull and colleagues.[1-5] Root Mean Squared Error (RMSE) was used to evaluate the average precision of each method. Bland-Altmann plots were used to evaluate the patterning of point estimates compared to known age. The accuracy of each method was evaluated as the number of times for which the known age fell within the 95% interval provided by a method.

RMSE values ranged from 0.97–2.79 years. Bland-Altmann plots demonstrate a general overestimation of age by all methods, with no systematic difference in error by sex. However, estimates generated by Stull and colleagues show point estimate bias around the age of seven, whereas the other methods show bias starting at age three.[5] Even considering these overestimation biases of point estimates, accuracies using methods from Cardoso and colleagues and Stull and colleagues ranged from 82.0%–96.7%.[1,5] As the global reach of forensic practitioners increases, methods for forensic identification will increasingly rely on technological advances, such as forensic imaging, to diversify skeletal reference material for use in method creation, validation, and application. Findings from this project demonstrate that while current methods of subadult age estimation can still be used to accurately estimate age for Japanese subadults, the patterned bias of point estimates warrant the consideration of population-specific methods, especially for individuals of Japanese descent who may be generally smaller than age-matched individuals from Western European descent.

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Age Estimation; Subadult; Method Validation

A132 Variables Impacting the Application of Radiographic Age Estimation by Pulp/Tooth Ratios

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Learning Objectives: After attending this presentation, attendees will have a better understanding of how to apply radiographic dental age estimation methods on teeth from adult individuals and the factors that contribute to error in measurement within and between observers. Attendees will know the necessary parameters required to apply the method.

Impact Statement: This presentation will impact the forensic science community by identifying two main sources of error in a major dental age estimation method. It will highlight what factors need to be addressed prior to applying radiographic dental age estimation methods to ensure accurate results.

Abstract Text: The Cameriere et al. method of age estimation by pulp/tooth ratio has been popularly applied in many contexts.[1] As age estimation methods are limited for adults, especially in fragmentary cases, this is a valuable method in the forensic toolkit. However, practical conditions may deviate from the ideal scenarios put forth in previous publications. Based on observed scenarios within a forensic sample at the Defense POW/MIA Accounting Agency, this exploratory research tests a variety of experimental conditions to assess the potential impact of two common variables on the practical application of the method: superimposition of alveolar bone and tooth rotation.

Two types of periapical radiographs were measured to test the impact of image quality on inter-observer agreement. Measurements were recorded on periapical radiographs of 16 canines articulated in maxillary or mandibular alveolar bone, and on 27 extracted canines with no superimposed tissues. On the radiographs with alveolar superimposition, there was an average difference of 0.009 (ranging from 0.0009 to 0.03) in the pulp/tooth ratios between observers, constituting an average difference of 4.6 years in estimated age. For the radiographs without superimposition, the average difference in ratios was 0.007 (ranging from < 0.0001 to 0.02), with an average difference in estimated age of 3.5 years. While both radiograph conditions are viable for the method to be applied, radiographs without superimposition were associated with reduced observer difference. However, the difference in absolute error between radiograph types is not significant ($p = 0.24$). A comparison of the outlines recorded by each observer for the articulated canines demonstrated areas of particular concern in the pulp shape and tooth root perimeter.

The second variable tested was the effect of tooth rotation on the measurements. Radiographs of nine individual canines were captured in three positions: (1) standard labial/lingual orientation, (2) 45 degrees of rotation (representing a degree of rotation observed in multiple radiographs), and (3) 90 degrees of rotation (representing the maximum possible impact, but not a realistic scenario). When the tooth was rotated 45 degrees, the average impact on the pulp/tooth ratio was +0.05 (reducing the estimated age by roughly 25 years). When rotated 90 degrees, the average impact was +0.08 (reducing the age by roughly 40 years). Although the degree of rotation tested may be more extreme than is commonly encountered, this exploratory study indicates the severe reduction in estimated age that tooth rotation can create. These results demonstrate a significant impact on the age estimate and confirm that radiographs not taken in the correct orientation must be excluded from use.

The results of this exploratory research suggest caution is warranted in applying Cameriere et al. on radiographs of teeth that are not extracted, although the method can still be reasonably applied. It is important for observers to be able to identify possible rotation in the image, as this will significantly affect the age estimate and the method should not be applied.

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Age Estimation; Dental Materials; Radiology

A133 A Multifactorial Method of Age Estimation in the Living From Computed Tomography (CT) Scans

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Learning Objectives: Attendees will be provided with an overview of a method of age estimation in the living that combines data from multiple regions of the skeleton. They will gain insight into the benefits of a Bayesian approach and probability as a way of presenting age estimates of living people in this specific type of case report.

Impact Statement: This presentation will contribute to the literature focused on improving statistical approaches to estimating the age of living people from skeletal and dental biomarkers.

Abstract Text: Age estimation in the living is undertaken in countries worldwide to inform legal decisions where the subjects' age is a factor. Scenarios where an age estimate may be sought range from immigration and criminal culpability cases, to sporting events, and international adoption.[1] A person having no verifiable proof of their age may occur for a large number of reasons but, commonly, there may be low rates of birth registration in their nation of origin, or there may be armed conflict resulting in documents having been destroyed.[2-5] Age estimation from multiple regions of the skeleton is preferable; however, conclusions from multiple age estimates are often presented as a discussion of variable estimates and error ranges.[6] This paper presents a method that utilizes a multivariate cumulative probit model for combining estimates from multiple regions to estimate age and give a probability that an individual has attained majority age.

A sample of 507 Computed Tomography (CT) scans were obtained from the New Mexico Decedent Image Database for individuals aged 10 years to 26 years. Numerous skeletal indicators were scored for each individual in the sample. The medial clavicular epiphysis was scored after Schmeling et al. and Schulz et al., with the enhancements described by Kellinghaus et al.[7-9] The hamulus was assessed after Shapland and Lewis.[10] The distal radius and ulna were staged using Tanner-Whitehouse 3.[11] The distal femur, proximal fibula, and proximal tibia were assessed using written stage descriptions by Kellinghaus et al. for the clavicle.[9] The left mandibular third molars were examined using Demirjian, Goldstein, and Tanner's method.[12]

The data were split into testing (n= 99; females= 50) and training (n= 408, females= 183) datasets. The training data were used to inform the model, and the testing was used to examine the models' performance. A multivariate cumulative probit model was used to generate posterior density regions and probabilities that an individual was above the age of 18.

Of the female testing sample, 58.00% of the individuals' known ages were within the 50% prediction interval with the 95% Highest Posterior Density Regions (HPDR) falling between 44.37% and 70.85% across the tested models. The model was less likely to accurately predict male age, with only 38.78% of known ages falling within the 50% prediction interval. The 95% HPDR was 26.14% to 52.53% across different models. The model overestimated the older female sample, but younger females and both older and younger male groups were estimated without significant bias. Further analysis to be presented will include the number of males and females who were correctly classified as minors by the model.

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Age Estimation in the Living; Bayesian Statistics; Computed Tomography

A134 Applying Deep Learning Methods to Adult Age Estimation of Pelvic Joints

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Learning Objectives: After attending this presentation, attendees will have a better understanding of how deep learning image classification methods can be applied to skeletal adult age estimation of the pelvic joints.

Impact Statement: This presentation will impact the forensic science community by addressing a gap in the current methodological approach to older adult skeletal age estimation. In particular, this investigation provides the first application of a Convolutional Neural Network (CNN) to multiple pelvic joints surfaces that can automate an age classification into middle (35–65 years) or older (66–102 years) adults from a digital image.

Abstract Text: Deep learning models are a type of artificial intelligence designed to mimic the neural pathways of human decision making for analyzing visual imagery, without the subjectivity of human biases. The architecture of a deep learning image classification model works by recognizing patterns of features through the analysis of high volumes of images and then classifying those images to a particular class or group. Researchers have used various deep learning models for the assessment of living individuals whose age can be a matter of legal concern (e.g., asylum-seeking minors or legal status for criminal proceedings), as well as for sex estimation, postmortem interval assessments, and radiograph identification; however, little research has been conducted using deep learning models with digital images for adult skeletal age estimation that focuses on multiple skeletal features.[1-7]

The average global life expectancy has risen over the past several decades from 66 to 73 years with many individuals living past 100 years.[8] The inability to provide more narrow age range intervals to distinguish older adults (i.e., those over 50 years) is a deficiency with many of the adult qualitative age estimation methods. Therefore, the objective of this investigation is to develop and validate a method for separating middle-aged adults (35 to 65 years) from older adults (66 to 102 years) with deep learning models. For this purpose, 2,085 digital images were collected from three pelvic joint surfaces (695 per joint surface), including the pubic symphysis and the auricular surfaces of the ilium and sacrum. High resolution images were obtained from three collections, the William M. Bass Donated Skeletal Collection, the John A. Williams Documented Human Skeletal Collection, and the Texas State University Donated Skeletal Collection using a Cannon 5DSR digital camera with a 100mm macro lens.

This investigation employed adapting the ResNet50 CNN to create three training models for each of the pelvic joint surfaces: the pubic symphysis (CNN_PS), the auricular surface of the ilium (CNN_IAS), and the auricular surface of the sacrum (CNN_SAS). Each model had ten layers with over three million training parameters and underwent 20 Epochs to classify an image into one of two categories: (1) middle aged (35–65 years) (2) older aged adults (66–102 years). The sample was divided into a training set (80%) and a validation set (20%), with an additional ten randomly selected images from the middle and older age categories to test the final models.

The results of the initial models are promising. The CNN_PS model was the best performing with a testing precision of 95%, a validation accuracy of 96%, and a test accuracy of 100% for both age categories. The CNN_SAS model performed second best with a training precision of 93%, a validation accuracy of 93%, and a testing accuracy of 100% for the middle age category and 80% for the older age category. The CNN_IAS model had the poorest performance with a training precision of 90%, a validation accuracy of 93%, and a testing accuracy of 60% for the middle age category, and 100% for the older age category.

This investigation suggests that deep learning models may be successfully used in forensic anthropology for adult age estimation. Specifically, they may be used to automate the distinction between middle and older adult skeletal remains. Additionally, the results suggest certain joint surfaces may be more suitable for aging older adults (i.e., the pubic symphysis and auricular surface of the ilium). Further work, with a larger sample, is needed to refine the model to provide more narrow age estimate intervals for adult remains from pelvic joint surfaces.

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Forensic Anthropology; Age Estimation; Deep Learning

A135 A Comparison of Regression Analysis and Mathematical Methods for Stature Estimation From Upper Limb Anthropometric Measurements in the Modern South Indian Population

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WITHDRAWN

A136 Forensic Anthropology in China: History and Current Status

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Learning Objectives: Participants can learn about the history, research progress, and current status of forensic anthropology in China. Given the large number of Chinese people around the world, forensic anthropology research based on the Chinese may provide more targeted and specific information for forensic anthropology cases worldwide.

Impact Statement: China's forensic anthropology has not been well known by peers around the world. Many studies are published in Chinese in Chinese journals. This presentation can provide forensic science peers with more information about the progress of China's forensic anthropology.

Abstract Text: Forensic anthropology aims to search, recover, and examine skeletonized human remains in a forensic context, contributing knowledge of osteology and physical anthropology to the emerging needs of forensic science. However, in most countries except the United States, forensic anthropology work is still considered the responsibility of forensic pathologists. In China, the earliest record of handling skeletonized cases is in a book written during the Song Dynasty called *The Washing Away of Wrongs* in approximately 1247AD. However, there have been no further advances in this discipline, either in methods or techniques.

In 1956, the first internal textbook for training forensic specialists only briefly introduced the methods of bone examination, including species identification, commingled bone identification, sex identification, age estimation, height estimation, bone trauma, and bone tissue analysis, which basically covered the contents of forensic anthropology. In the 1980s, *Forensic Osteology* was the earliest Chinese monograph on the identification of skeletons. Since then, forensic anthropology courses have been offered in a few colleges, but the subject and the personnel are still blank. Currently, only very few institutions have collections of modern Chinese skeletal specimens that contain clear antemortem information.

The Institute of Forensic Sciences, Ministry of Public Security in Beijing owns a collection of more than 200 modern Chinese skeletons. Human osteoarchaeologists and bioarchaeologists often participate in the handling of skeletonized cases, but they only provide some professional consulting, which cannot be used as standard evidence. In the past 30 years, a series of specialized methods and formulas for the Chinese have been developed based on native Chinese skeletal collections and hospital clinical cases. Additionally, in the past ten years, new methods based on Computed Tomography (CT) images and larger sample sizes have also been developed and more internationally published. However, most of these studies were published in Chinese journals. There is no doubt that these studies will provide more potential support for worldwide forensic anthropology cases involving Chinese or Asian bones.

Anthropology; China; Skeletal Remains

A137 The Progress in Standards Development in Forensic Anthropology: Updates From the OSAC and ASB

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Learning Objectives: After viewing this presentation, attendees will be updated on the status of proposed 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 Practice Recommendations (BPRs) in forensic anthropology, including publication by the ASB and placement on the OSAC registry. This presentation will further highlight the need for the forensic anthropology community to engage in the development of these consensus standards.

Abstract Text: The OSAC was established in 2014 to facilitate the development of national standards for forensic science. The Forensic Anthropology Subcommittee (FACS) is one of four under the Medicine Scientific Area Committee and includes representation from academia, medical examiners' offices, government labs, and legal, quality assurance, and human factors. Each OSAC subcommittee is charged with drafting proposed standards, which are then submitted to a Standards Developing Organization (SDO) for further development and publication. The subcommittee submits draft standards to the ASB, established in 2015 as an SDO accredited by the American National Standards Institute, to publish forensic science standards. The Anthropology Consensus Body (CB) of the ASB includes representation from various interest groups, including academia, governmental and non-governmental users, general interest, and producers. Once a standard is published, the FACS reviews it for technical merit, makes a recommendation for its placement on the OSAC registry, and submits it to the Forensic Science Standards Board (FSSB) for final approval.

This presentation will describe the differences between standards and BPRs; summarize recent standards development activities by the OSAC FACS and the ASB Anthropology CB, including ASB standards currently on the registry; ASB standards eligible for the OSAC registry; proposed standards that are currently open for public comment or in comment adjudication at the ASB; and OSAC-proposed standards in progress. As of August 2024, ASB has published 11 standards and 1 best practice. The best practice is currently listed on the OSAC registry and all of the standards are eligible for the registry (several of which are in review by the FSSB). Three best practices are being drafted by the ASB, five proposed standards are currently under development and nearing completion by the OSAC FAC Subcommittee, one OSAC proposed standard was submitted to the ASB for development as a joint venture, and two published ASB standards are in revision for second editions.

Standards; ASB; OSAC

A138 The Roaring 20s: A 20-Year Review of a Forensic Anthropologist's Casework That Spans Pre-and Post-ABFA Certification

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Learning Objectives: After this presentation, attendees will understand the degree and distribution of a Forensic Anthropologist's (FA's) caseload pre- and post- American Board of Forensic Anthropology (ABFA) certification with a focus on the digital and physical case archival methodologies that facilitated this review.

Impact Statement: This presentation discusses with the forensic science community the physical and digital case archival methods used by forensic anthropologists and exposes the forensic science community to a Florida-based FA's caseload before and after ABFA certification. Positioned in Southwest Florida with consultation purview that spans the state and beyond, this research has implications for the forensic anthropological and forensic science communities as it pertains to method and database development as well as data security considerations.

Abstract Text: Published research on forensic anthropological databases emphasized method use and accuracy and highlighted the importance of forensic anthropological analyses in medical examiners' and coroners' offices.[1,2] However, the manners in which FAs archive case-related information and the distribution of their respective caseloads is largely unknown. Nevertheless, findings from past research found that FAs were underutilized in medical examiner contexts, following results from survey data that summed an average of 12.33 FA skeletal analysis reports and two FA field reports annually.[3]

For this study, we culled from a Florida-based board-certified FA with over 1,800 cases during a 20-year period (2004–2023) that were archived physically and digitally using a cloud-based, CJIS-certified IRIS platform. Though used the most by law enforcement agencies, the IRIS software can be used in the FA context to initiate case consultation, track case progress, and evaluate trends in the forensic analyst's caseload, among other benefits to the FA laboratory. This study revealed the FA received approximately 91 case consultations annually. This quantity increased from 77 cases prior to ABFA board-certification to 133 cases per year following ABFA board-certification. In the grouped sample, 10% of cases represented non-human elements, 14% involved field recovery components, and 22% utilized Antemortem-Postmortem (AMPM) comparisons to help establish positive identification in conjunction with the medical examiner.[4]

Overall, 13% of cases involved analysis of juvenile individuals. Fifty-five percent of cases involved both identification and trauma analyses with 30% of cases being trauma only and 15% identification only, as requested by the medical examiner. Fourteen percent of cases were related to establishing forensic significance of human remains. Since 2005, the quantity of cases and distribution of case trends varied dynamically (1 in 2004 to 212 in 2023), thus highlighting the sporadic nature of FA casework. This research supplemented existing literature regarding FA caseloads while exploring novel approaches to case consultation, documentation, and archival methods.

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Anthropology; Forensic Archival Methods; Digital Database

A139 A Multidisciplinary Approach to the Rematriation of Indigenous Remains

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WITHDRAWN

A140 Community Experiences of Forensic Anthropological Work in Human Rights Settings: A Case Study of El Mozote

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Learning Objectives: Forensic anthropology teams working in human rights settings are working not only with the deceased, but also with the living who are healing from traumatic events. Understanding the impact of the Argentine Forensic Anthropology Team (EAAF)'s work on El Mozote survivors and community members is crucial to recognize successful and unsuccessful strategies employed in the past by forensic anthropology teams. Such knowledge can enhance the development of frameworks that ensure survivor communities obtain the desired results from the work of forensic anthropology teams, specifically in human rights contexts.

Impact Statement: Attendees will learn some of the key strategies employed by the EAAF that are associated with community member satisfaction with the exhumation and identification process. Attendees will also learn some traits that may be associated with dissatisfaction. This knowledge will help forensic team coordinators plan forensic responses to ensure that these support rather than further traumatize and marginalize affected communities.

Abstract Text: The El Mozote massacre, in December 1981, was the largest mass killing event reported during the Salvadoran Civil war.[1-4] However, the occurrence of the massacre was denied by the Salvadoran government, and its survivors were ignored for almost a decade. A group of survivors, assisted by the non-governmental organization Tutela Legal, pressured Salvadoran authorities to open an investigation and prosecute perpetrators at the end of the 1980s. Tutela Legal petitioned the EAAF for assistance in conducting a forensic investigation of the El Mozote massacre. In 1992, the EAAF arrived in El Salvador and conducted exhumations intermittently between 1992–2004 in and around El Mozote. This investigation, coupled with other forensic work, clearly demonstrated that the victims were executed by the government soldiers and were not killed in combat action as the government had claimed.[5,6] The work done by the EAAF in El Mozote was pivotal in corroborating that a massacre occurred in 1981 and facilitated the prosecution of the perpetrators.

The aim of this research is to present how surviving communities experience forensic anthropology teams in human rights contexts. Though contributions of forensic anthropology to justice processes are well known in cases of human rights violations, very little is known about how survivors and community members experience this type of work. By considering the case study of El Mozote, this study contributes to the literature focused on survivors' experiences with large-scale forensic investigations.

Data for this study were collected through one-on-one in-person interviews conducted in 2023 in the Morazán region of El Salvador (El Mozote and nearby villages). Interviews were conducted in Spanish using a semi-structured questionnaire, including the following topics: exhumation experiences and long-term impact of exhumations, community engagement strategies used by EAAF's members, and participants' satisfaction with the work done by the EAAF. All interviews were voice recorded, transcribed in Spanish, then translated to English. A total of 20 interviewees participated, all of whom were alive at the time of the massacre in 1981 and were old enough in 1992–2004 to remember the EAAF's work.

Overall, all participants relayed mixed emotions during exhumations—joy because they would be able to properly bury their loved ones, and sadness due to reliving the memories of how their loved ones tragically died in the massacre. Survivors also reported feeling a sense of acknowledgment during the exhumations, which corroborated their narrative of the event. Participants reported that the EAAF team members were well integrated into the community throughout their stays in El Mozote. Specific actions that the team took to engage with the community included: introductions with stakeholders prior to onset of exhumation, regular updates to families about progress of the work, welcoming families to attend and participate in the exhumations themselves, and informal social interaction with community members. Lastly, participants provided positive feedback on the work conducted by the EAAF, with very few suggestions for improvement. All participants who had interacted with EAAF members described them as professional, polite, and very approachable. Interviewees affirmed the credibility of the EAAF versus that of other teams, potentially due to the team's efforts to engage with the community in combination with their status as an independent, international organization.

Given the positive appraisal of the EAAF given by the survivors at El Mozote, this case study can be seen as a precedent on how forensic anthropology teams should engage with the communities they serve. More research should be conducted to understand how survivors experience forensic investigations in both the short and long term in order to continue to improve working processes.

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Anthropology; Massacres; Human Rights

A141 Rated or Ratioed? Using Open-Source Software to Investigate Public Perception of Human Taphonomy Facilities on Social Media Platforms

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Learning Objectives: By attending this presentation, attendees will learn about the role of social media in the dissemination of forensic science topics and how social media can be interrogated to find meta-data regarding public opinion about, in this case, human taphonomy facilities or "body farms." They will see how social media engagement can be measured to assess positive and negative attitudes toward controversial subjects, and how they can change over time. They will also see how opinions expressed on X and Reddit may differ from those expressed on professional social platforms such as LinkedIn.

Impact Statement: This presentation will impact the forensic science community by revealing the potential for analysis of social media as a source and record of ever-fluctuating public sentiment toward a variety of forensic science topics and how attitudes toward taphonomy and human taphonomy facilities have evolved over the timescale studied.

Abstract Text: Social media, in all its forms, is inescapable today. It is a powerful medium for communication of entertainment, news, gossip, and criticism, used by 62.6% of the global population.[1] Much has been published about the use of online digital evidence, including social media, in forensic investigations.[2,3] However, the role of social media in the dissemination and communication of forensic science is yet to be fully identified, and few have recognized the hitherto untapped potential for interrogating social media to measure and analyze public perception of forensic science. The billions of posts, uploads, tweets and retweets, and comments made every day are a rich source of public sentiment about endless topics, including potentially controversial ones such as human taphonomy facilities.[4]

Human Taphonomy Facilities (HTFs), or "body farms," are outdoor laboratories where donated human cadavers are used to understand how human decomposition progresses in a variety of conditions. There are currently 11 facilities globally. Their work is, by its nature, controversial. There are objections on scientific grounds, mainly based on concerns about scientific rigor and reproducibility, as well as ethical, moral, and religious opposition.[5-7]

Here, we explore how forensic science topics of interest to the public, such as HTFs, are presented and represented on social media and mainstream media to determine whether the reporting is skewed toward positive, negative, or neutral sentiment. We examine whether we can quantitively assess how HTFs are perceived and received by the public (as represented by social media users) by collating social media posts from X (formerly Twitter) and Reddit. These platforms were chosen as they are widely used, can be anonymous, and therefore lend themselves to freer expressions of opinion with fewer repercussions for users. Open-source web scraper tools were used to collate posts from these platforms using keywords such as "human taphonomy facility" and "body farm." Open-source sentiment analyzers were then used to categorize posts into those expressing either negative or positive emotion. Data including the content of the post, location of the post, date/time of the post, whether it was original or retweeted, and the presence of URLs or emojis was recorded. In addition, we scrutinized the professional platform LinkedIn to ascertain how professionals perceive and express their opinion about HTFs, in comparison to "lay people" on X and Reddit. We also compared opinions expressed via social media to opinions expressed more formally through academic questionnaires.

Preliminary results suggest that the opinion of "lay people," as expressed on X or Reddit, has grown more positive toward HTFs over time. Comments analyzed were recovered from 2010–2024 in English and were mostly of United States/Canadian/United Kingdom origin. Comments on X have expressed surprise and fascination, such as, "*It's gross, but really fascinating, too*," to disgust and repulsion, such as, "*Dead bodies? But why? I feel so scared*," and "*This is very horrible and terrifying scene*." The trend of rising acceptance of HTFs is less visible in LinkedIn posts and comments, which suggest professionals are more circumspect about HTFs or less willing to express their true feelings on the platform. Our findings could be useful to campaigners wanting to recognize and address specific public or practitioner concerns or could be used to evidence public objection to HTFs.

In conclusion, our results demonstrate how social media reflects attitudes of our peers and the public, compared to more traditional academic surveys and how it is a hitherto under-utilized, yet powerful, tool to gauge opinion about forensic topics. We use the example of HTFs to illustrate the potential for social media meta-analysis as a valuable tool for academic and practitioner forensic scientists to scrutinize and quantify the zeitgeist.

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Taphonomy; Social Media; Public Opinion

A142 An Exploratory Study of Human Skeleton Sales on E-Commerce Platforms and Social Media

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Learning Objectives: Attendees will gain insight into the current state of the human skeletal trade online. Attendees will learn the kinds of human bones available for sale, the reason individuals are buying/selling human remains, and the methodology sellers use to effectively violate prohibitory policies.

Impact Statement: The world of online skeletal trade exists primarily through the exploitation and dehumanization of already marginalized people. By publicizing the harmful culture of the commodification of the human skeleton, forensic science practitioners can begin to contribute to conversations about ethical human remains procurement and combat this through public outreach and education. This presentation brings awareness and context to forensic anthropologists who may encounter these kinds of remains.

Abstract Text: The sale of human skeletal remains is, for the most part, unregulated. The Native American Graves Repatriation Act applies to Native American remains located in Federal or Federally funded agencies, and only a few states have laws regarding the sale of human remains. It is only in recent years that some e-commerce websites have begun to regulate the sale of human remains. The general lack of legal guidelines has contributed to a lucrative business in which human remains are sold through online platforms. Concurrently, a greater focus has been placed on ethical considerations concerning the origins of skeletal collections in anthropology and anatomy. Many institutions are critically examining the procurement and provenance of their osteological collections. Therefore, an evaluation of modern sales practices would contribute to the important conversations already occurring in the field today.

This study sought to find if posts advertising the sale of human bones continued to exist regardless of new policies. Etsy, eBay, Craigslist, Facebook Marketplace, and Instagram were searched utilizing keywords to discover posts attempting to sell human skeletal remains. Over 12 weeks, 403 posts of this nature were found, the majority advertising the sale of skulls (51%) or appendicular elements (23%). Most of the posts were in some way targeted toward educational or teaching purposes (89%) and oddities collection (70%). Of the posts discovered, 23 were in direct violation of website policies. In some of the posts, sellers circumvented policies through deliberate means such as misspelling or mislabeling items. Other posts were direct policy violations that were not detected nor removed by the online platform.

This research confirms that the human skeletal trade remains active on social media and e-commerce websites even if consumers from academia no longer use these means to curate osteological collections. The popularity of oddity collection potentially accounts for the prolific commodification of human skeletal remains, particularly those belonging to groups that have been marginalized and exploited in life. This commodification includes the purposeful comingling of remains in the creation of more desirable products, purposeful damage or disfigurement of remains, disrespectful treatment of individuals or their culture, and more. The rampant commodification of human skeletal remains is potentially fueled by the idea that these remains have been and continue to be "ethically sourced." In addition, there is always the concern that some of these remains are of medicolegal significance. Thus, forensic anthropologists have both a scientific and ethical obligation to be apprised of modern sales practices involving human remains.

Human Remains Sales; E-Commerce; Commodification

A143 Expanding the Forensic Anthropology Database for Assessing Methods Accuracy (FADAMA): Standardizing Case Management Systems, Real-Time Reliability Metrics, and Promoting Training Accessibility

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Learning Objectives: Attendees of this presentation will learn how the enhancements in FADAMA 2.0 can be used for casework application and research needs.

Impact Statement: This presentation will impact the forensic science community by increasing awareness and the ability to use the new case management system in FADAMA that can improve case work tracking and security as well as increase competency through centralized method trainings.

Abstract Text: Forensic anthropologists employ methods from myriad sources (publications, online tools, computer download, etc.) when estimating the biological profile in casework. Published standards governing the estimation of the biological profile exist, yet because of the uniqueness of each case context, these standards call on forensic anthropologists to use the most reliable methods with integrated statistics for the case at hand. However, the practice of forensic anthropology suffers from both a lack of up-to-date understanding of methods performance and a disaggregation of methods tools. These issues are compounded by a fundamental lack of accessible training opportunities in new methods. As a result, studies show that forensic anthropologists continue to use older methods—devoid of validation and/or probabilistic frameworks—in casework, while newer methods are slower to be applied in casework, regardless of typically having more representative sampling and better statistical approaches.[1,2,3] Research has indicated that providing a comprehensive and accurate biological profile can increase the odds of an unidentified decedent case being resolved—thus, sound method selection and application are critical.

This presentation will cover our project's overarching goal to improve and streamline the practice of forensic anthropology by integrating real-time methods performance measures, methods training, and method access in a single web interface, all of which can be considered the first formalized active Case Management System (CMS) for estimations of the biological profile not tied to a single agency/entity. The ideal location for this CMS is the FADAMA, an existing tool developed for centralizing data on resolved cases for research purposes.[4] This presentation highlights, in collaboration with the University of Illinois Urbana-Champaign's National Center for Supercomputing Applications, the enhancements from FADAMA to FADAMA 2.0, which integrate an active case user platform. Three objectives are encompassed within this upgrade: (1) consolidation of forensic anthropology method interfaces into a centralized user-friendly web platform with state-of-the-art HIPAA-grade security in place to protect casework data, (2) development of an automated real-time computational approach for accuracy-informed method selection based on real-world case outcomes, and (3) implementation of training modules associated with frequently used methods for the biological profile estimation. FADAMA 2.0's upgrades directly benefit forensic anthropology by addressing the National Institute of Justice's established forensic science research priorities, including the consolidation of forensic anthropology tools and calculators, and accessible high-quality training on forensic anthropology methods into a single user-friendly program, and interdisciplinary innovation.

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Error Rate; Casework; Biological Profile

A144 Advancements in the Accessibility of AI and Large Language Models (LLM) Tools Can Assist in the Development of State-of-the-Art Models in Forensic Sciences: Examples From Forensic Anthropology

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WITHDRAWN

A145 Establishing a Protocol for Machine Learning Applications in Forensic Anthropology

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WITHDRAWN

A146 Testing the Effectiveness of Virtual Reality Training in Teaching Mass Fatality Response

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Learning Objectives: After attending this presentation, attendees will understand the efficacy of Virtual Reality (VR) as a training tool for mass fatality incident responders.

Impact Statement: This presentation will impact the forensic science community by showcasing how VR technology can be used to enhance mass disaster training programs, ultimately contributing to better-prepared responders and more effective incident management.

Abstract Text: VR is emerging as a tool for training in various areas, including forensic science and mass disaster/fatality response.[1-3] Studies have found that VR can be useful in training first responders and students in dealing with crime scenes, mass casualties, and hazardous materials.[1,3,4] These studies suggest that VR training has improved expertise and confidence in many trainees, although some findings show there can be limited expertise improvement when emergency responders already have high levels of knowledge in the field.[1,3,4] Therefore, VR has significant potential as a training tool for individuals with limited experience in disaster response, such as students and new staff. This project, which is a collaboration between the Forensic Analytical Science program at the University of Otago and the International Committee of the Red Cross (ICRC), tests if training outcomes can be improved by adding VR scenarios to student education on mass fatality response. This represents the first quantitative research of the efficacy of the ICRC's own VR program for training first responders in management of the dead following disasters.

Participants in this study was comprised of students enrolled in a third-year forensic science course (n = 19) at the University of Otago New Zealand (Ethics Reference D23/268). Participants' knowledge of managing the dead following disasters was tested at four points throughout training using a 16-question survey administered via an online learning platform. The questionnaire was given: (1) before any training had commenced, (2) after a lecture teaching ICRC protocols for disaster management, (3) after a lab session using the VR scenario to apply skills learned in the lecture, and (4) two weeks following the complete training to measure knowledge retention.[5]

Preliminary findings indicate that initial knowledge of mass disaster response protocols was limited (average questionnaire score of 63%), but improvements were seen in performance both after attending a lecture outlining ICRC protocols (average score to 77%), and further after VR training (average score 84%).[5] These improvements in scores after training were statistically significant (Kruskal-Wallis, $p = < 0.01$). Results also suggest that knowledge was retained two weeks following the complete training, with similar average scores between the third (84%) and fourth questionnaires (83%). These preliminary results indicate that the use of VR to supplement more traditional pedagogical techniques improves learning outcomes through simulated practical application of knowledge.

This research provides evidence supporting the integration of VR into forensic training programs, emphasizing its effectiveness in preparing responders for real-world mass fatality incidents. This study supports the idea that further maintenance and/or development of VR training scenarios to supplement existing educational programs in disaster response is important.[2] Future research will explore long-term retention, practical application of skills acquired through VR training, and training of more experienced cohorts.

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Virtual Reality; Forensic Training; Mass Disaster Response

A147 Investigating Sample Bias in United States-Based Documented Skeletal Collections

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Learning Objectives: After attending this presentation, attendees will be familiarized with collection-specific biases in United States-based documented skeletal collections.

Impact Statement: This presentation will impact the forensic science community by addressing a significant gap in current understanding of the representativeness of United States-based documented skeletal collections.

Abstract Text: Documented skeletal collections are essential to the field of forensic anthropology as the primary source of skeletal samples for the development and testing of analytical methods. Best practice recommendations include using modern collections to minimize sample bias from more historic collections, which are no longer representative of the modern, living population. However, few studies have directly compared demographic information of individuals across modern collections to assess collection-specific bias. Investigating collection-specific sample bias is essential for employing appropriate mitigation practices during study design and validation.

This study investigates collection-specific sample bias using a sample of individuals with demographic data from six United States-based skeletal collections (n=1,173): Maxwell Museum (MM), Michigan State University (MSU), Texas State University (TSU), Western Carolina University (WCU), Western Michigan University Homer Stryker M.D. School of Medicine (WMed), and University of Tennessee (UTK). Individuals were included in the study sample if at least one demographic parameter was available; the dataset was filtered for each analysis. Factors investigated include sex, population, age, height, birth year, year of death, birth state, state at time of death, Socioeconomic Status (SES), donation type, cause of death, and occupation. All analyses were conducted in R.

Males constituted a majority of the total sample (56.89%), but an Analysis Of Variance (ANOVA) revealed the ratio of females to males significantly differ by collection ($p< 0.01$). Although American White individuals comprise the largest population group in every collection (75-99%), overall population composition still varied significantly between collections ($p< 0.01$); post hoc comparisons using a Tukey Honest Significant Difference (HSD) test revealed these differences are attributed to MSU (adjusted $p< 0.01$), MM, and TSU, as these collections contained a higher proportion of non-White individuals than UTK, WCU, and WMed. Mean adult age differed significantly between collections ($p< 0.01$), which post hoc comparisons using the Tukey HSD test attributed to a lower mean adult age in MSU. Females were younger than males across all collections ($p< 0.01$), but this age difference is most prevalent in MSU (adjusted $p< 0.01$). Mean height did not differ significantly by collection ($p>0.05$) or birth year ($p>0.5$) but did differ by sex ($p< 0.01$).

Although all collections included in this study are considered modern, significant differences in mean birth year ($p< 0.01$) and year of death ($p< 0.01$) were noted. Comparisons using the Tukey HSD test revealed MM had a significantly earlier mean birth year than all other collections (adjusted $p< 0.01$), while MSU had a significantly later mean birth year (adjusted $p< 0.01$) than all other collections, excluding WMed. In accordance with the various geographic locations of the six collections, a series of ANOVAs revealed significant differences in state of birth ($p< 0.01$) and state at time of death ($p< 0.01$). Neither childhood SES ($p>0.05$) nor adult SES ($p>0.05$) differed significantly between collections, as most individuals reported middle SES. Type of donation (e.g., self, next-of-kin) differed significantly between collections ($p< 0.01$) and by population ($p< 0.01$).

Cause of Death (COD) and occupation were analyzed using the "tidytext" package. Cancer was the leading COD at all collections except MSU, whose leading COD was high-velocity projectile trauma—likely attributable to MSU's sole connection to a forensic laboratory, while other collections are associated with medical examiners, medical schools, and/or decomposition facilities. Common occupations were consistent across collections and included teachers, homemakers, and laborers.

Significant differences in demographic factors indicate a level of collection-specific sample bias is present; however, the type of sample bias (e.g., age, geographic origin, etc.) varies by collection. Researchers must account for collection bias during sampling and future research should assess potential ramifications.

Bias; Documented Skeletal Collections; Demographic Data

A148 Biomechanical Testing of Non-Human Analogs Used in Skeletal Trauma Research

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Learning Objectives: After viewing this presentation, attendees will understand that porcine and biofidelic femora are poor analogs for skeletal trauma research. Further, they will appreciate the contributions from biomechanical engineering to skeletal trauma research in forensic anthropology.

Impact Statement: This presentation will benefit the forensic science community by critically examining the utility of porcine and biofidelic femora toward the study of human skeletal trauma and recommend additional research with biomechanical engineers to address the use of human and non-human analogs.

Abstract Text: Forensic anthropologists often contribute to medicolegal investigations by performing skeletal trauma analyses. These analyses are performed using knowledge garnered from decades of experimental research, though much of this work is built on experience and anecdotal information (e.g., case studies). Much of the experimental research comprising this foundation is based on unvalidated non-human analogs such as porcine femora. This study employes a multidisciplinary framework that combines biomechanical concepts and experimental design with forensic anthropological theory and applications to address the validity of porcine and biofidelic analogs in skeletal trauma research.

Porcine (n=21) and biofidelic (n=16) femora were subjected to concentrated 4-point bending, modified 4-point bending, and 3-point bending. Data from human femora (n=7) were also included to use as a comparison.[1] The experimental sample were loaded posterior to anterior at a rate of 600mm/min using an Instron (model 68-TM-50) machine, and all experiments were filmed at 40,000 frames per second. Biomechanical data and fracture patterning were evaluated between femur types and loading conditions.

Both porcine and biofidelic femora did not behave discriminately between loading conditions. With porcine femora, an analysis of variance revealed no statistically significant differences ($p > 0.05$) in mean peak force, deformation at peak force, energy absorbed, or peak moment across loading conditions. With respect to fracture patterning, a Fisher's Exact test showed no relationship between fracture type outcomes and loading condition ($p=0.806$), and 57% of the porcine sample exhibited a notable continuity in overall fracture pattern morphology. In biofidelic tests, only mean peak force was significantly different between loading conditions ($p=0.007$). Statistical analyses were not performed to compare fracture type classifications across loading conditions as 15/16 of the biofidelic sample were classified as transverse fractures.

When comparing porcine and biofidelic femora in concentrated 4-point bending, significant differences were found between deformation at peak force ($p=< 0.001$) and energy absorbed ($p=0.022$). Concentrated 4-point bending tests also showed significant differences between human and biofidelic femora in mean peak force ($p=< 0.001$) and energy absorbed ($p=0.015$).

In concentrated 4-point bending, significant relationships were found between femur type and fracture type when comparing porcine and biofidelic ($p=< 0.001$), and biofidelic and human femora ($p=< 0.0001$). Some similarities were observed between fracture type classification in porcine and human femora ($p=0.216$), but a distinct difference between porcine and human femora with respect to overall fracture pattern morphology.

The culminative results indicate a strong likelihood that neither porcine nor biofidelic femora are an appropriate non-human analog in experimental trauma research. The stark differences observed in both biomechanical and fracture data necessitate questioning the validity of previous research. However, additional research aimed at further investigating these non-human analogs under different loading conditions is warranted. Further research using a multidisciplinary framework is required to critically evaluate the appropriateness of these and other non-human analogs with respect to specific loading conditions.

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Forensic Anthropology; Skeletal Trauma; Non-Human Analogs

A149 Proposing Sustainable and Cruelty-Free Proxies for Bones in Cut Marks Analysis

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Learning Objectives: After attending this presentation, attendees will have an opportunity to evaluate a non-human and a cruelty-free alternative for tests on cut marks.

Impact Statement: This presentation will impact the forensic science community by providing the possibility to use a sustainable and cruelty-free composite material as a non-human proxy on which tests cut marks for forensic and educational purposes.

Abstract Text: In Forensic Anthropology, multiple studies on sharp force trauma have used animal skeletal elements instead of human-donated material. Although *Sus scrofa* is usually considered the closest animal proxy for humans, there is still an ongoing debate about the accuracy of experiments performed on pig bones and the ethical principles behind how they are supplied.[1] Few studies consider using alternative non-animal-based proxies such as polyurethane, plaster, and wax to simulate impressions of sharp force trauma.[2-4] Polyurethane bone substitutes are becoming increasingly common in forensic investigations, although it is an expensive resource and not readily available to researchers.

In a novel approach, this study tests four different non-animal proxies for human bone: paraffine, plaster, beeswax, and a compound for bone reconstructions.[5,6] This unique mixture includes beeswax, paraffin, pine rosin, casting powder, and calcium carbonate (limestone flour).

The proxies have been used to test and examine cut marks according to the following traits: shape of the cut mark (general morphology/entrance/exit); rising of the walls (entrance/exit); feathering; shards; and mounding (entrance/center/exit/general morphology). The test has been performed on serrated knives, as this category exhibits the most characteristics.

The synthetic bone replicas have been molded in five rectangular prisms with a d-shape top (mail-box shape) per proxy: 10 cut marks were executed using a serrated knife on each prism for a total of 200 cuts. The results on each proxy were then compared with an additional 50 cuts on porcine ribs in order to evaluate the similarity between the artificial material and the animal one. The same knife was used at a 90° angle perpendicular to the sample for all the marks, moving the knife forward through the sample and backward.

The traits have been observed under a stereomicroscope with 38x magnification and compared. Intra-observer analysis has been performed and no significant difference between the observations has been detected, (p = >0.05) confirming the reproducibility of the study. Based on the findings, the reconstruction compound and beeswax emerge as the most suitable materials for crafting an alternative to animal bones for use in cut mark analysis and observation.

In the future, the authors propose to replicate the study by comparing the two most suitable proxies with incisions on fleshed porcine ribs to better assess their applicability in forensic contexts. Furthermore, a comparative analysis between the proxy and human bones is planned.

This research presents an opportunity to consider the feasibility of using readily available, sustainable, and ethical composite materials as cruelty-free surrogates for human and animal bones in forensic research and educational applications.

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Anthropology; Environment; Toolmarks

A150 Ethics Around the Creation and Use of 3D-Printed Human Remains in Crime Reconstruction: Toward the Ethical Use of Technology in Forensic Anthropology

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WITHDRAWN

A151 A Water-Cooled Probe Thermocouple System for Accurate Temperature Measurements in Human Donors During Forensic Fire-Death Scenarios

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Learning Objectives: After attending this presentation, attendees will learn about a new thermocouple-based temperature measurement system using off-the-shelf hardware and plumbing components that has produced consistently accurate data with high reliability in forensic fire-death scenarios.

Impact Statement: This presentation will impact the forensic science community by providing details of a thermocouple-based temperature measurement system that offers a unique solution to significant challenges encountered when measuring internal temperatures of human donors exposed to fire. The system has successfully been used to produce 14 separate measurements of internal donor temperatures in two experimental burns.

Abstract Text: Thermocouple measurements are conducted during forensic fire-death scenarios to correlate temperatures with the effects of fire exposure on human tissue and bone.[1-3] However, use of inadequate thermocouples can cause severe errors in temperature data. Thermocouple wire with exposed non-combustible woven-fabric insulation can exhibit severe insulation embrittlement and disintegration in response to fire exposure, which can cause movement of the location of measurement out of the donor and into the fire.[4] This type of wire is also unsuitable for direct implantation into donors because contamination with rendered fats and other bodily fluids will worsen temperature errors caused by insulation overheating and low electrical resistance.[5] Insulation failure of a thermocouple is strongly indicated when its tip remains shielded from fire by a large tissue mass, but the temperature reading shows a rapid increase by hundreds of °C nearly concurrent with fire exposure to portions of the thermocouple outside the donor. Use of mineral-insulated metal-sheathed thermocouple probes greatly reduces the chances of insulation failure, but stiffness and lack of anchoring points will cause difficulty in accommodating movements due to pugilistic posturing and pyre collapse.[6] Stainless steel loses strength at high temperatures, which makes probes more vulnerable to damage from falling debris and refueling of fires.[7]

This system is based on SCAXL-125U-120 probes from Omega Engineering which are 3 meters in length with an outside diameter (OD) of 0.3175 cm and rated to 1150 °C. Probes pass through approximately 2.4 meters of 0.635cm OD copper tubing with brass tee fittings at each end. The length of the exposed probe tip to be inserted into an incision in the donor is adjustable. The tee provides an anchor point to suture the probe assembly to the donor with 24-gauge steel wire. Each probe assembly is supplied with a continuous flow of water by a distribution manifold connected to a garden hose, and compression fittings are used to prevent water leaks. Additional tubing is used to direct discharge water away from the fire area. All components are available through thermocouple vendors and hardware supply stores, and the system can be assembled with basic tools without any need for specialized training or custom fabrication of parts from scratch.

Temperatures were successfully measured in a total of 14 different locations throughout the body during experimental burns of one male and one female donor. Probe assemblies were pre-bent to accommodate pugilistic posturing by rotating with limb movements. Donors were burned on stacks of wooden pallets (3–4 pallets high) with refueling continuing for 2–3 hours, then fires were allowed to burn out and cool undisturbed. None of the probes were dislodged by any movements or disturbances, nor did they leak water into the fire area.

Elimination of errors caused by thermocouple insulation failure has enabled observation of a consistent trend of internal donor temperatures remaining constant for extended durations after fire start before increasing, despite intense fire exposure. Distinct temperature plateaus around 100°C due to the boiling of water in tissues have also been observed. The time at which a probe tip is no longer shielded from fire due to tissue destruction can also be identified from data plots. Additional details of system construction and implementation and examples of temperature measurement results and trends will be discussed.

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Thermocouple; Temperature Measurement; Fire-Death Scenario

B1 Revisiting Guidance on Sampling of Populations for Highly Polymorphic Forensic Loci

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Learning Objectives: After attending this presentation, attendees will understand the relation of several important factors to sample size, different ways of handling previously unobserved alleles, and the effect of using different formulas for evaluating the evidence.

Impact Statement: This presentation impacts the forensic science community by reviewing important concepts for determining sample sizes for population studies and demonstrating its application to modern autosomal Short Tandem Repeat (STR) data.

Abstract Text: When we find that a person of interest cannot be excluded as a potential contributor to an evidentiary DNA profile, we wish to express the strength of this observation. For autosomal STR data, population databases allow us to attach probabilities to DNA evidence by the estimation of genotype frequencies, which rely on accurate sample allele frequencies. In 1992, Chakraborty estimated that, for Variable Number Tandem Repeat (VNTR) loci with mostly rare alleles, a sample of 300 individuals would provide 95% confidence that alleles with frequency of at least 1% are represented.[1] In practice, a sample size of 200 has often been employed for forensic STR population databases, while a minimum requirement for publishing forensic STR sequence population data sets has been set at 50 samples.[2,3]

In the years since Chakraborty's seminal paper, increased knowledge of the human genome has facilitated determination of highly polymorphic STR markers and technology advancements have resulted in a higher level of STR marker multiplexing. This combination of factors has led to the development of expanded and far more discriminating kits. Sequencing techniques have further improved our ability to distinguish between alleles based on variation in underlying base-pair structure. Considering STR markers in current use have a much broader allelic range than some of the earlier characterized loci, it is important to reevaluate existing guidance on sample sizes for STR population databases. Additional important questions include: What inferences can we make when we only have a limited set of samples to work with? Beyond what point is it no longer beneficial to sample more individuals? And how do these sampling decisions or constraints affect the methods we use to account for previously unobserved alleles?

In this presentation we address these questions and discuss the implications of sample size for autosomal STR loci. We start by briefly reviewing some theory to understand the relation between heterozygosity, allele frequency, the number of alleles, and sample size. We demonstrate the effect of both length- and sequence-based STR data on sample size implications and highlight differences between lesser and more polymorphic markers. We also look at population differences and the effect of the underlying allelic distribution on the results. Furthermore, we show how increasing sample sizes by combining different data sets can be helpful in determining the point at which continued sampling may not be a worthwhile effort and what the consequences are of using limited samples.[4] Finally, we discuss different methods for accommodating previously unobserved alleles and the effect of using various evidence evaluation formulas as described in the second NRC report.[5]

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Forensic DNA; STR Profiles; Next Generation Sequencing

B2 Assessing the Added Value of Non-Human DNA Analysis for Forensic Soil Analysis

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Learning Objectives: After attending this presentation, attendees will: (1) understand the wet-laboratory steps for completing DNA metabarcoding of plants, fungi, arthropods and bacteria from surface soils; and (2) the combination(s) of biological taxa most informative for Q-to-K comparisons of surface soils.

Impact Statement: This presentation will impact the forensic science community by assessing whether biological taxa characterized using DNA metabarcoding provides added value as a supplement to traditional inorganic examinations currently performed by forensic geologists.

Abstract Text: Geologic materials, including soil and dust, are ubiquitous and often inadvertently transferred during crime events. Forensic geologists use a range of particle-based analytical approaches to characterize the inorganic fraction of soils, with resulting data primarily used to form subjective interpretations. Geological materials are also increasingly used to help address provenance questions for investigative leads and intelligence purposes. In many cases, such analyses provide sufficient information to conclude there is or is not the possibility the questioned soil originated from the same source as the known. However, there are inevitably cases where the samples being compared lack exclusionary differences or there is too little inorganic material for analysis. In these scenarios, information gleaned from new quantitative methodologies might provide valuable exclusionary differences.

In this study, two types of surface soils representing scenarios that would potentially benefit the most from new quantitative methods were collected from across the state of North Carolina, those with: (1) similar inorganic content but with distinct land use (15 locations), and (2) limited inorganic content but recognizable organic fractions
(15 locations). At each location, triplicate samples (1m apart) were collected from two sites ~100m apart to assess method reproducibility, accuracy, and small-scale variation that might be realistically observed in Q-to-K comparisons (total n, ~180). Each surface soil sample was subjected to DNA metabarcoding to characterize four broad groups of taxa: (1) bacteria via 16S, (2) fungi via ITS1, (3) plants via ITS2 and p6 loop of the *trnL*-UAA intron, and (4) arthropods via *COI*. Subsequent statistical analyses focused on assessing: (1) the appropriate level for analyzing DNA data (i.e., sequence level or family), and (2) the most appropriate combination(s) of taxa for Q-to-K comparisons. Initial findings include: (1) recovery of arthropod data was poor regardless of soil type, (2) biological communities are relatively consistent between triplicate samples, and (3) Principal Coordinates Analysis (PCoA) plots are valuable for visualizing differences in biological communities across large sample cohorts. The results of this proposed study could demonstrate the value of DNA metabarcoding for differentiating soils, providing a supplemental analysis method for samples containing too little inorganic content for conventional examination.

DNA Metabarcoding; Geologic Materials; Non-Human DNA

B3 A Small-Scale Ancestry Informative DIP-STR Marker Set for Biogeographic Ancestry Inference in United States Populations

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Learning Objectives: After attending this presentation, attendees will gain a better grasp of how a set of Deletion-Insertion Polymorphism-Short Tandem Repeat (DIP-STR) markers performs to infer the biogeographic ancestry of individuals.

Impact Statement: The forensic science community will benefit from this presentation since it will give investigators a different kind of intelligence tool for biogeographic ancestry inference.

Abstract Text: DIP-STRs are an alternative class of genetic markers that link high mutation STR with low mutation DIP loci.[1] DIP-STR markers were initially developed for targeting the minor DNA contributor in imbalanced two-person mixtures using specific primers that overlap the DIP (S or L) sequence providing allele-specific amplifications that include the non-shared DIP allele with the major DNA contributor. In the past, these markers have been used as ancestry informative markers [2] to determine an individual's Biogeographic Ancestry (BGA), and they have shown to be useful in differentiating between the main continental regions.[2,3] The purpose of this research was to evaluate the performance of a minimal set of 10 DIP-STR markers in inferring the BGA of individuals belonging to four main population groups from the United States.[4] This research offers researchers a different kind of intelligence tool for biogeographic ancestry inference.

A set of 100 individuals of self-reported African American (US AFR, #25), European American (US EUR, #25), Asian American (US EAS, #25), and Southwest Hispanic (US SWH, #25) origin was selected. The samples were quantified using the Quantifiler Trio Quantification kit (Thermo Fisher Scientific) on a QuantStudio Real-Time PCR system. A first round of Polymerase Chain Reaction (PCR) amplification and separation of DIP markers is necessary in the genotyping workflow to identify the target DIP allele. This is followed by a second round of PCR amplification of DIP-STRs utilizing allele-specific PCR amplifications.[1] Amplicons are separated using the SeqStudio Genetic Analyzer, and GeneMapper ID-X is used to analyze profiles. Population genetic data is evaluated and clustering analysis is performed using STRUCTURE software to characterize and visualize the genetic structure of the populations of interest.

The estimated allele frequencies were determined for each population group to highlight the distribution of the genotyping results and allow for biogeographic estimation of an unknown sample. The results obtained showed that allele diversity was observed among the four population groups across the ten DIP-STR markers. Common alleles were detected for each of the DIP-STR marker along with unique alleles observed only in some of the population groups. STRUCTURE results from ten DIP-STR loci showed that the US AFR, EUR, and EAS population groups can be clustered with AFR, EUR/CAUC, and EAS continental groups. On the other hand, the SWH group appears to be admixed, and this is expected because the US EUR and US SWH groups are genetically close populations. This study highlights how a small-scale, ancestry-informative DIP-STR marker set that combines fast-evolving and slow-evolving polymorphisms can be implemented as an intelligence tool to provide more refined resolution of biogeographic ancestry inference of major continental groups.

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Forensic DNA; Population; Ancestral

B4 Addressing the Challenges of the ISFG Minimum Sequence Ranges When Implementing NGS Technologies for STR Typing

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WITHDRAWN

B5 Assessing the Viability of DNA Yields From Taphonomically Altered Bone and Teeth

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Learning Objectives: Attendees of this presentation will gain a better understanding of how DNA is preserved in scenarios in which human remains have been exposed to thermal alteration or corrosive chemicals contained in common household products. This is especially important in cases where no other means of identification are applicable or when different body fragments must be reassociated.

Impact Statement: This presentation will impact the forensic community by presenting a study on the efficiency of DNA analysis for identifying taphonomically altered human remains exposed to fire or common household chemicals.

Abstract Text: DNA is more resistant to degradation in hard tissues due to the protective effects conferred by the surrounding inorganic matrices of bones and dental tissue.[1] United States laboratories currently favor femora, teeth, and temporal bones as preferred samples for DNA isolation and analysis. However, the recommended elements are not always available for sampling and subsequent genetic profiling. Studies suggest that smaller bony elements, like metacarpals, carpals, and phalanges, may render similar, if not better, DNA yields.[2] These smaller bones offer ease and efficiency to the whole process and are not used in the biological profile in anthropological analyses unlike the cranium and long bones.

Circumstances, such as fatal fires, mass disasters, and exposure to corrosive substances, could hamper the possibility of DNA recovery and characterization, complicating the identification of human remains.[3] Furthermore, perpetrators may use household corrosive substances and fire as an attempt to destroy remains based on their availability and effects. Thermal alteration, sodium hydroxide (NaOH), and hydrochloric acid (HCl) are known to change both the structural integrity and composition of skeletal elements, affecting the quality and/or quantity of DNA available for extraction. Recent studies have explored whether authentic DNA profiles can be generated from human skeletal elements exposed to varying stages of fire-induced destruction. Results show that identification from DNA profiles were reliable and reproducible from both well-preserved and semi-burnt bones.[4,5] The aim of this study was to assess the possibility of DNA isolation and profiling from bones and teeth exposed to fire, sodium hydroxide, and hydrochloric acid, respectively.

Hand bones, from three donated individuals, were exposed to three separate treatments: controlled fire, sodium hydroxide, and hydrochloric acid. Eight elements (carpals, metacarpals, and phalanges) were used for each treatment, selected based on pre-alteration surface area and volume data collected using the Artec Spider hand-held 3D scanner. Additionally, 16 healthy human molars from 16 different individuals were exposed to the chemical treatments.

The fire treatment protocol consisted of elements being placed on a ceramic grill plate on top of an open-air fire pit for 1 hour, and temperatures were recorded every 5 minutes. Readings taken from above ranged from 100ºC to 500ºC.The chemical alteration protocol consisted of four intervals (total: 2 hours) with bone pairs being pulled every 30 minutes from their solutions (NaOH or HCl).

DNA was extracted using a silica-based method and quantified through a human-specific quantitative Polymerase Chain Reaction (qPCR) methodology, providing degradation and inhibitor ratios. In those samples rendering quantifiable DNA, 0.5ng of DNA was used to carry out the STR profile. These profiles were compared to the full genetic profiles taken from the control element

Our results demonstrated, as stated by previous studies, that teeth are better sources to obtain good quantity and quality of DNA compared to bone.[6] In the teeth samples, DNA yields were more affected by the NaOH treatment than HCl. Despite this, the degradation ratios as well as the inhibitors were below the threshold, and the quantity was enough to obtain full STR profiles from all teeth samples. In contrast, the degradation ratios were beyond the threshold in bone samples. Though there were no inhibitors, the quantity of DNA was low, leading to only several incomplete profiles. These differences are due to tooth anatomy. These findings indicated that teeth should be the sample of election to obtain DNA profiles in human remains exposed to fire and corrosive agents, as they may lead to a positive identification. Future research will seek to expand on these results, assessing other corrosive agents, different concentrations and durations, as well as other skeletal elements.

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Forensic DNA; Identification; Taphonomy

B6 It's Common Cents—How to Spend a Million Dollars in Five Months!

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Learning Objectives: The District of Columbia Department of Forensic Sciences (DFS) lost accreditation and suspending DNA casework for approximately two-and-a-half years, accumulating a large backlog. After attending this presentation, the participants will understand a model to quickly eliminate a DNA backlog through the coordination of multiple vendors, adjusted testing strategies and turnaround times, and interagency cooperation.

Impact Statement: The impact of the presentation on the field of forensic sciences is an explanation of an innovative model to quickly reduce a DNA backlog that can be implemented in other jurisdictions with a large number of unanalyzed cases.

Abstract Text: The DFS recently obtained re-accreditation in December of 2023. Prior to the loss of accreditation, the Forensic Biology Unit had eliminated the entire casework backlog. During the span of not being accredited (approximately two-and-a-half years), a backlog of over 1,000 cases had accumulated. This presentation will demonstrate the strategic plan of how the DFS was able to effectively and swiftly reduce the backlog by 90% utilizing multiple vendor laboratories.

Through the use of previously frozen Capacity Enhancement for Backlog Reduction (CEBR) funds, multiple contracts/procurements and a muti-agency collaboration, the backlog reduction project was executed within a period of five months. Success was achieved by temporary assignments of staff to assist with evidence transfers, barcoding, and testing plans. Contracts were formulated specifically for property crimes and less complex offenses that consisted of simplistic testing strategies. Vendor laboratories coordinated with the DFS to determine numbers of cases accepted and turnaround Times (TAT). Coordination also occurred with the Evidence Control Branch, which secures and properly stores the District of Columbia's evidence.

Upon completion of testing at the vendor laboratories, strategies were implemented for the receipt and distribution of the case reports and also for the return of evidence and extracts. Each report was triaged and evaluated for possible Combined DNA Index System (CODIS) entry. Cases deemed suitable for entry were sorted and allocated for ownership reviews based on batching by the vendor laboratories.

As this was a novel project, several important lessons wre learned, including the importance of both intra-agency and inter-agency communication, the need to establish and adhere to procurement timelines, and how to assess the downstream effects of large-scale outsourcing.

Overall, the project was successful and beneficial to both the District of Columbia and to the Forensic Biology Unit. By outsourcing a majority of the backlogged cases, analysts were able to focus on CODIS ownership reviews, training, and other essential tasks. Upon resuming casework, the burden of tackling a sizeable backlog for the unit is no longer present and the agency was able to provide investigative leads in a timely manner.

Backlogs; Outsourcing; Casework

B7 The Molecular Diversity of 23 Y-STR Genetic Markers in an Endogamous Ramgharia Population of Punjab, India

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Learning Objectives: This presentation provides a deeper understanding of how historical events, social practices, and migration patterns have shaped the genetic landscape of the population, offering valuable context for historical and anthropological studies. This presentation enhances knowledge of how genetic markers are distributed within endogamous groups, contributing to the broader field of population genetics and helping to elucidate the genetic basis of cultural and social practices. It also offers practical benefits for forensic investigations and genealogical research by providing detailed genetic profiles and understanding specific challenges in tracing paternal lineages in such communities.

Impact Statement: By presenting detailed data on Y-chromosomal Short Tandem Repeat (Y-STR) markers within a specific endogamous population, forensic scientists gain insights into the genetic diversity and uniqueness of this group. This understanding is crucial for refining databases and improving the accuracy of forensic identifications involving individuals from this population. The research can lead to the creation of more comprehensive and targeted forensic databases that include population-specific genetic profiles. Such databases help with better population coverage and more accurate forensic analysis, reducing the risk of false matches or exclusions. Sharing findings on the genetic diversity of a specific population encourages collaboration between forensic scientists, geneticists, anthropologists, and other researchers. Such interdisciplinary efforts can lead to more comprehensive solutions and advancements in forensic science.

Abstract Text: India is a diverse nation comprising about a 1.2 billion population as reported in Census of India, 2011. Perhaps this nation is famous for its culture and traditions but is also known to exhibit intra- and inter-population variations.[1,2] This is mainly due to existence of different castes, religions, and communities. Among various religions known, Sikhism accounts for the fourth-largest religion founded by Sri Guru Nanak Dev Ji in the late 15th century C.E. There are a few Sikh communities that practice the gotra (clan) system of marriage, namely Ramgharias, Khatris, Jats, and many more. However, the Ramgharia community is highly renowned for its endogamous practices and thereby holds high potential to exhibit remarkable genetic variability.[3]

In forensic investigations and population studies, Y-STR analysis has been shown to be quite useful.[4-8] The present study aimed to evaluate genetic polymorphism in the Ramgharia population of Punjab. Two hundred unrelated healthy male individuals were typed using the Powerplex Y-23 PCR amplification kit.

A total of 170 haplotypes were obtained, among which 146 were found to be unique. Multidimensional Scaling (MDS) Plot was used to study the intra-population relationship between the studied population data and other reported populations of India, which revealed that the Ramgharia population of Punjab lies in a cluster with the Bhil Population of Madhya Pradesh.[9,11] A total of 140 different alleles were reported with allelic frequency ranging from 0.006 to 0.765. The genetic diversity for 23 Y-STRs ranged from 0.375 (DYS391) to 0.836 (DYS635).

This population data may generate a significant source of information for the investigations of forensic relevance in forensic laboratories.[10,12-16] Moreover, this will strengthen the Y-STR Haplotype Reference Database (YHRD) of the Indian population, which would facilitate researches related to anthropology, population origin, evolution, and other related researches in the future. This 23 Y-STR marker data can be used as a reference for determining genetic relatedness among various Indian populations and with the other populations worldwide. Overall, the data obtained will enrich the forensic database of endogamous populations of India.

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DNA; STRs; Retrospective Study

B8 A Comprehensive Drug Analysis of Vaporizer Products From Pennsylvania High Schools

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Learning Objectives: After attending this presentation, attendees will have insight into the diverse and evolving market of vaporizer products and their respective complex chemical composition to provide awareness of the disparities between nicotine and cannabinoid-containing vaporizers.

Impact Statement: This presentation will impact the forensic community by communicating the findings of confiscated vaporizer products from high school students, emphasizing the value of timely monitoring of the evolving and unregulated vape product supply for the benefit of public health and awareness.

Abstract Text: E-cigarette, or vaporizer devices (vapes), are products that can be used to deliver nicotine, cannabinoids, flavorings, chemicals, and other substances. These products are referred to by varying names and synonyms and are manufactured in a variety of shapes, sizes, and device types. The rapid expansion of vaporizers has led to a competitive marketplace in which vape products are advertised with appealing flavors and fashionable, attractive packaging, which can appeal to younger generations despite federal laws that restrict their sale to individuals over 21. In a recent study, 43% of 15- to 20-year-old users obtained their products from retailers like vape shops, gas stations, and convenience stores.[1] Comprehensive surveillance, inventory, chemical testing, and reporting of vape contents can provide insight into composition variability and help inform future testing and regulations.

In 2019, the Center for Forensic Science Research and Education (CFSRE) established a research collaboration to evaluate vape products that were recovered from western Pennsylvania high schools, which has continued to the present. The main objective was to differentiate nicotine from cannabis vape products (or others) through comprehensive drug testing utilizing Gas Chromatography/Mass Spectrometry (GC/MS). Sampling was not random but "for cause," and the sample set may not be reflective of the larger population. In 2023, 416 vape product samples were recovered and documented, and 210 were submitted for analysis.

Of the 210 vape products, 65% of samples contained nicotine and of those, 81% contained glycerin, a common base solvent additive. Additional solvents and flavoring agents were identified based on external database matching, including methyl benzoate, benzoic acid, gamma-decalactone, vanillin, and more. Among middle and high school students reporting current e-cigarette use, 89.4% used flavored products daily. The surveying of vape use can provide insight into the popularity of specific brands and flavors as the tobacco product marketplace continues to diversify. ELFBAR was the second most frequent brand seen in the 2023 nicotine-containing vapes. There were 31 different flavor names advertised within that group (45 samples), which illustrates the versatile flavor profiles on the market. Terpenes found in cannabis vaporizers contribute to scents, flavors, and psychological effects; caryophyllene was the most abundant terpene found in both delta-8 and delta-9 vapes.

In 2023, 29% of tested vape samples contained cannabinoids, such as delta-9-THC, delta-8-THC, CBN, and CBD, and of those 43% were deemed as disposable products in which the device cannot be recharged and is designed to be prefilled and single use. Between Q1 2022 and Q1 2024, disposable products grew their share of vape dollar sales by 92%, and 56% of vape consumers cite ease of use and 44% convenience as their reasons for choosing disposable vaporizers.[2] Previously, the enclosed form of a disposable vaporizer could be an indicator that the vape contained nicotine; in the vapes tested in 2022, there was only one observed disposable vape containing cannabis. The recent market share rise of disposable cannabis-containing vapes has further led to the complexity of distinguishing between a nicotine or cannabis vaporizer device.

Rapid expansion of available products, lack of regulation, and inconsistent terminology associated with vaporizer devices have all led to a general lack of understanding at both consumer and public health levels despite a rapid increase in rate of consumption in recent years. The nuances between nicotine and cannabinoid-containing devices and products create yet another complex dynamic. Comprehensive testing of vapes and reporting of their components is vital to public health stakeholders and responsible decision making by consumers. Each vaporizer device is different, requiring an understanding of their composition and use patterns to educate, reduce harm, and provide recommended regulatory parameters.

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Vaping; Teens; Cannabinoids

B9 Field Testing of Drug-Incorporated Edible Jellies Using Portable Raman-Based Analyzers and Colorimetric Tests

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Learning Objectives: Attendees will learn about the capabilities and limitations of Raman-based hand-held analyzers, including the ThermoFisher TruNarc, Rigaku Progeny ResQ, and Agilent Resolve Hand-Held Analyzer, in field drug detection. They will also explore the importance of traditional color tests and the need for advanced separation techniques and AI integration for improved identification of complex drug mixtures.

Impact Statement: The presentation will deliver a clear understanding of risks associated with the use of modern equipment in drug field testing and the management of such risks. The law enforcement agencies will think over the recent training needs of their field officers to cover the lapses and ensure more ethical, accurate, and just seizures. It will draw the attention of the scientific community and the developers to improve the technologies.

Abstract Text: The rise of technological advancements has transformed the field of forensic sciences, presenting both opportunities and challenges.[1] In this study, a cannabis-based product, known as "weed jelly," was analyzed to determine best practices in drug field testing for such products. Cannabis has a long-standing history of traditional use.[2] Recently, there has been increased interest in cannabis-based products globally, despite legal restrictions in many regions, including Pakistan.[3-5]

For the analysis, Raman-based hand-held analyzers; ThermoFisher TruNarc, Rigaku Progeny ResQ, and Agilent Resolve Hand-Held Analyzer, were used under ambient conditions. For sample excitation, the devices use 785nm, 1,064nm, and 830nm wavelength lasers, respectively.[6-8] Raman shifts displayed in the spectrum ranged from 0-3,000cm^{-1}, 0-2,450cm^{-1}, and 200-2,000cm^{-1}, respectively. Chemical spot testing using a Modified Duquenois-Levine test was conducted as a complementary method.[9] The substance was then dissolved in methanol, filtered, and subjected to Gas Chromatography/Mass Spectrometry (GC/MS) analysis using an SDP TOP GC1290 and MS8100.[10] Retention times were compared with reference standards, and MS spectra were matched with the National Institute of Standards and Technology (NIST) and cientific Working Group for the Analysis of Seized Drugs (SWGDRUG) (version 3.13) MS libraries.[11,12]

The Thermo Fisher TruNarc provided inconclusive results. Rigaku Progeny ResQ detected mixtures such as Starch Protein and DiPT with spectral component contributions of Starch Protein 62% and DiPT 28%; a mixture of Phosphorous Chloride 75% and D-Penicillamine 25%; and a mixture of Cholesterol Steroid 65% and Starch Protein 35%. The Agilent Resolve Hand-Held Analyzer showed an 82% match with milk powder; other probable results were tributyl citrate (81%), totoaba fish powder (81%), plain flour (83%), and glycerin (81%).[12] The Modified Duquenois-Levine test indicated a positive result for cannabinoids.[7] GC/MS analysis identified cannabidiol, delta-9-tetrahydrocannabinol, and cannabinol, with high match quality, alongside benzyl alcohol and phloroglucinol.

Traditional color tests remain effective for specific analytes in mixtures during field drug testing, but Raman-based hand-held analyzers showed limitations while analyzing mixtures without any separation techniques. Baseline correction and the algorithms in calculating probabilities should be revised since the match qualities above 80% are high enough to mislead a field officer, leading to the momentary apprehension of an innocent and release of criminals.[13,14] Meanwhile, for a more appropriate, ethical, and just utilization of these technologies, field officers should be educated enough to understand clearly the capabilities and limitations of these devices, that the results displayed are probabilities and the results should be counterchecked with alternative methods, the well-studied and established.[15]

Moreover, the technologies should remain developing and updating during this transformation. Improved DL & AI in machine learning can enhance data interpretation of mixtures and even synthetic analogs by developing the modules with a deep understanding of deviations in sample spectrum governed by dissolution, adulteration, and even small structural variations such as in the case of synthetic analogs. In multiple overlay spectra, spectra of concern, though challenging, may be focused. In the case of structural analogs, such features can predict the probable parent molecule and the possible differences introduced therein, with real-time data interpretation. Continuous learning and updates will further enhance the sensitivity and specificity, thereby optimizing resources at forensic labs.[16-18]

To further improve the drug field testing devices, enhancing the sensitivity and specificity and expanding libraries for broader substance detection, utilizing advanced spectroscopy techniques like SERS and hyperspectral imaging [19], implementing real-time data sharing and connectivity, developing integrated sample preparation and separation to reduce human error, and promoting collaborative development among stakeholders, can be considered.[19-22]

Recent reports of products similar to this study, being seized in various regions underscore the importance of effective detection methods.[1-3]

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Raman Spectroscopy; Seized Drugs; Artificial Intelligence

B10 Source Attribution of Fentanyl Via Metals Analysis

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Learning Objectives: After reading through the poster, attendees will be able to appreciate how metal data, instead of the usual organic precursors, is used to differentiate between illicitly manufactured fentanyl and pharmaceutical fentanyl using statistical applications.

Impact Statement: The overall goal of this poster will be to develop a model that uses the metal data information from the seized samples to differentiate between pharmaceutical and illicit manufactured fentanyl. This information will help law enforcement to end the illicit production of fentanyl that is killing a lot of drug users in the United States by predicting the source of these controlled substances.

Abstract Text: Fentanyl is a synthetic opioid and is a significant contributor to fatal and non-fatal overdoses in the United States. Even in small doses (2mg), it can be deadly.[1,2] There are two types of fentanyl: pharmaceutical fentanyl and Illicitly Manufactured Fentanyl (IMF), and both are considered synthetic opioids. Scientific research has primarily focused on identifying trends associated with fentanyl production, purity, synthetic impurities, and synthetic routes. Elemental impurities are now being used for source attribution since they do not break down compared to the organic components, and pharmaceutical products have tightly controlled metal contents.[3] Metals are unwanted elements that occur in drug formulations, and they can arise from active pharmaceutical ingredients, raw materials, synthetic additives, excipients, catalysts, and product processes and equipment used during manufacture. This research aimed to solve two major issues associated with fentanyl: (1) to use metals analysis data of seized fentanyl to differentiate between licit (pharmaceutical) and illicit sources via the United States pharmacopoeia-approved elemental impurities levels, and (2) profiling the metal content of seized fentanyl for same batch analysis (i.e., similar sources) using multivariate analysis and isotope ratio method.

The research was conducted in two major steps: (1) batch differentiation (i.e., inter-batch comparisons) and (2) pharmaceutical-diverted fentanyl against illicitly manufactured comparisons. The experiments analyzed 22 metals and metalloids (including some isotopes) namely C, Mo, N, Nb, Cr, Mn, Fe, Ni, T, Cu, Co, V, Si, Sr, Se, Al, Hg, Pb, Mg, Zn, Cd and As including 111Cd/ 114Cd, 111Cd/ 112Cd, 208Pb/206Pb and 207Pb/206Pb via Inductively Coupled Plasma/Optical Emission Spectrometry (ICP/OES) and Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) of seized fentanyl samples.[4-5] The use of specific instruments depended on the levels of metals being analyzed. The information obtained was used to develop a metals database, including seizure metadata.

This research will help Customs and Border Protection (CBP)/ Drug Enforcement Administration (DEA)/ Department of Homeland Security (DHS)/ Law Enforcement (LE) personnel clamp down on criminal drug networks, both within our nation and internationally, including dynamic patterns of criminal activities in pharmaceutical companies.

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Fentanyl; Metals; Attribution

B11 Determination of Plant DNA Recovery After Extraction of Ephedrine From Ephedra: A Pilot Study

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WITHDRAWN

B12 A Simple DNA Extraction Method and a Rapid PCR-Based Assay for Detecting *Ricinus Communis* L. DNA Co-Extracted With Ricin

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Learning Objectives: Ricin is highly toxic and can be easily extracted, making it a potential substance for biocrime or bioterrorism. This presentation aims to discuss the problem and propose a detection approach.

Impact Statement: This presentation will introduce new analytical methods for identifying ricin and addressing the threat of biocrime and bioterrorism. The potential options for supporting traceability investigations of this substance will also be discussed.

Abstract Text: Ricin is an extremely toxic poison derived from plants, making it one of the deadliest substances known.[1] Due to its easy extraction from the seeds of the *Ricinus communis* L. plant, this species has been studied as a potential threat for bioterrorist attacks. Its abundance in Brazil has led the country's authorities to consider ricin a major concern. Advanced chemical techniques are utilized to detect ricin, but an alternative method involves analyzing the DNA extracted simultaneously with the protein fraction using the DNA barcoding technique. This technique is effective due to the abundance of nucleic acids present in the seeds of castor beans, from which ricin protein is extracted.

We will describe a rapid and efficient adaptation of an automated DNA extraction method (2) and a Polymerase Chain Reaction (PCR) -based assay to detect *Ricinus communis* L. and its plant products, such as ricin, using a fast plant DNA barcoding approach.[3] The DNA extraction is based on optimizing the buffer for incubation before running the samples in an automated DNA extraction platform. This strategy has previously been used, even for wood. In terms of the analytical approach, we analyzed DNA fragments generated by PCR from specific regions of plant DNA. These fragments vary between plant species and can be used for forensic investigations and genetic identification of plant species. Although the strategy has been theorized but not universally applied to plants, it may also be valuable for other forensic investigations and genetic identification of plant species. The International Criminal Police Organization (INTERPOL) recently hosted its inaugural biosecurity conference and unveiled the Biotracker system. The presented analytical approach also shows promise for enhancing efforts to trace ricin. The proof of concept presented here, when applied to a real case, could form the basis for a more general and simplified analytical model in the field of genetic identification of plant species.

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Plant; Ricin; Genetics

B13 A Rapid Method for Identifying Source Species of Body Fluids: Implications for Wildlife Investigations

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Learning Objectives: After this presentation, attendees will understand how blood cell populations from different wildlife species can be differentiated from each other and from cell populations derived from human blood. Additionally, participants will learn how methods for analyzing features of individual cells such as autofluorescence and morphology can be integrated into the workflow of a forensic laboratory.

Impact Statement: This work can impact the forensic community by providing a fast and accurate method for identifying the source species for unknown bloodstains recovered from a crime scene. This can enable laboratories to quickly screen biological evidence samples and determine which are relevant to the investigation (i.e., human vs. non-human deposits) or provide probative information for cases involving animal trafficking and/or abuse.

Abstract Text: Differentiation between human bodily fluids and body fluids derived from wildlife species is an ongoing need for caseworking laboratories. Illegal trafficking of wildlife, such as for medicinal or culinary purposes, would require use of species identification technologies to properly identify bear tissues or fluids among other animals. Additionally, killing and/or abuse of protected or domestic species would possess the same need for differentiation to ensure accurate and timely species identification. Determining the source species for evidentiary samples, particularly those containing blood deposits, can be challenging due to small amounts of sample, degradation, and the presence of cell populations from multiple tissue sources. Therefore, the goal of this research is to develop a new signature system that can be used to identify the source species for unknown blood stains and test it on a series of mock evidence samples approximating those encountered in forensic casework. This research aims to provide an efficient and effective technique that can be easily integrated into the procedures and workflows used by forensic agencies involved with wildlife investigations.

To accomplish this, blood samples from a series of wildlife species (deer, bear, rabbit, dog) were collected along with human blood samples from 20 different contributors. Blood from each source was aliquoted onto glass microscope slides in triplicate (~20ul for each spot) and allowed to dry for varying periods of time ranging between one day and two months. Samples were collected from each surface using a wet swab method and the blood cell populations were eluted from the swab using PBS buffer. Cells were then analyzed using flow cytometer equipped with an excitation laser at 488nm and detector channels for measuring emissions between 500nm and 800nm.

Results from human and canine samples showed significant differences in the intensity of autofluorescence at wavelengths between 500-600nm as well as between 600-700nm (median autofluorescence ~100 RFU and ~500 RFU for human and canine, respectively, $p < 0.001$). Additionally, differences were observed in the forward scatter and side scatter profiles that capture the overall size of the cell and its structural complexity (median FSC and SSC values).These results were consistent in samples that had been aged for up to 90 days and could be detected even in mock casework samples. Blood cell populations from other species (bear, rabbit) showed some variation in the distribution of autofluorescence values between ~200RFU and ~1500RFU compared to both human and canine samples. However, these changes showed greater intra-animal variability across replicates. Overall, these results suggest that information about the source species could be determined for samples approximating those encountered in casework, especially if the provenance is not completely unknown. Further, autofluorescence profiles can complement the information obtained from standard DNA profiling techniques, particularly in agencies that are resource limited.

Species Identification; Autofluorescence; Flow Cytometry

B14 The Mysterious Case of a Fatal Accident During Skydiving: A Fracture of the Pelvis From Parachute Opening Shock (POS)?

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Learning Objectives: This presentation will depict a case of a fatal accident during skydiving to demonstrate the importance of correctly interpreting the data collected on the scene, the subsequent postmortem examination, and the engineering report on the parachute and the flight. After attending this poster, attendees will better understand the importance of continued research and improvement in parachute design and skydiving safety protocols to prevent such incidents in the future.

Impact Statement: This presentation will significantly impact the forensic community by presenting a unique case of an open-book pelvic fracture resulting from parachute opening shock, contributing to the relevant literature.

Abstract Text: Skydiving, encompassing competitive, recreational, and military activities, is a high-risk endeavor where accidents can lead to severe injuries or death, despite technological advancements[1]

In this case, a 49-year-old military skydiver was found lifeless in a private garden, with significant blood loss evident beneath his body that had soaked into the soil. Prior to the autopsy, an X-ray revealed a fracture of the pelvis with diastasis of the pubic symphysis, a dislocated appearance and diastasis of the right sacroiliac joint, and a suspected fracture of the right iliac wing and the left sacroiliac branch. The autopsy confirmed these fractures and also revealed a laceration of the right internal iliac artery. The cause of death was determined to be acute hemorrhagic shock. A review revealed that this case is unique in the relevant literature.[2-4]

The case was investigated by a multidisciplinary team comprising a forensic pathologist, a radiologist, and engineers. The team determined that the event was accidental, and that the parachutist's death resulted from hemorrhage caused by the laceration of the internal iliac artery due to the open-book fracture of the pelvis. This fracture likely occurred during the parachute's opening phase, known as opening shock.[5]

Supporting this hypothesis, video footage showed the parachutist was alert until the parachute deployed, with a subsequent soft landing. The high-velocity blood spatter pattern on the parachute bag indicated blood leakage during free fall, confirming the pelvic injury occurred during the opening phase.

By corroborating the forensic data with the engineering report on the parachute and the flight, the dynamics of the accident were clarified, identifying the parachute opening as the critical moment for the fatal injury.

Thus, this work underscores the importance of a thorough analysis, combining precise interpretation of circumstantial and autopsy data with meticulous forensic investigation to determine the manner of death. Continued research and improvements in parachute design and skydiving safety protocols are recommended to prevent similar incidents in the future. This case highlights the need for proper training and positioning during parachute deployment. Skydivers should be educated about the risks associated with improper body positioning during the opening phase. Additionally, manufacturers may consider enhancing harness designs to better distribute forces during high-G events, potentially mitigating such injuries.

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Parachuting Accidents; Skydiver; Pelvis Fractures

B15 A 3D-Printed Object Topography Analysis and the Viability for Forensic Examination

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Learning Objectives: Attendees of this presentation will learn how modern technologies like the Cadre Forensics TopMatch-GS system are being used to identify microscopic topographical signatures on 3D printed objects. Attendees will understand how these features can link objects to specific printers or determine if multiple items originated from the same printer. The presentation will cover the use of Virtual Comparison Microscopy (VCM) as well as Cadre's comparison algorithms to objectively compare and interpret these topographies for forensic analysis.

Impact Statement: This presentation will demonstrate the potential for forensic analysis of 3D printed objects using VCM, showcasing its ability to capture microscopic topographies present within 3D printed objects. This presentation aims to advance the understanding of forensic identification methodologies, offering new techniques for the identification of 3D printed objects and individuals involved in criminal activities aided by 3D printing technologies.

Abstract Text: The increasing accessibility and sophistication of 3D printing technology has led to a rise in the creation of untraceable firearms and firearm components, commonly referred to as "ghost guns." These 3D printed objects present a significant challenge to forensic investigations due to the difficulty in linking them to their original source. This study aims to address this issue by exploring the potential for same-source identification of 3D printed objects through the analysis of their microscopic surface topographies. Similar to the manufacturing process of traditional firearms, it is expected that the manufacturing processes utilized during the 3D printing process impart characteristics on the object that can be used to link the object to its original source. This study hypothesizes that the markings left from the hot end nozzle and the print surface of the 3D printer will impart characteristics on the objects that will serve as reliable identifiers of their origin. Preliminary data gathered from the study supports this hypothesis.

To further test this hypothesis with a more robust dataset, 10 hot end nozzles and 10 print bed surfaces will be used with 15 objects printed per nozzle/surface pair. These 150 objects will be inter-compared with objects from known same-nozzle/print surface origin and intra-compared with objects with known different-nozzle/print surface origin to assess the variability in markings found on the objects. To visualize these markings, the study employs the Cadre Forensics TopMatch-GS 3D scanner to capture detailed 3D scans of the 3D printed objects. The scans will be viewed using TopMatch's VCM application, which includes proprietary pattern matching algorithms to generate similarity scores, providing an objective measure of the topographical similarities between different prints. This process involves scanning multiple objects printed using the same and different hot end nozzles and print beds under controlled conditions to ensure a comprehensive analysis.

Along with visual comparison of the 3D printed objects, the system's powerful algorithms will provide similarity scores based on the topographical features that will allow for statistical analysis of the results. The study will include a thorough examination of known samples, generating box plots to visualize similarity score variability and employing Receiver Operating Characteristic (ROC) curves to evaluate the system's accuracy. In addition, confusion matrices will be used to assess the performance in distinguishing between same and different source.

In conclusion, this research advances the field of forensic science by introducing a novel method for the identification of 3D printed objects. The ability to link these objects to specific sources has important implications for criminal investigations involving 3D printed firearms. As 3D printing technology continues to evolve, the forensic techniques developed in this study will be crucial for addressing new challenges in forensic identification and maintaining public safety.

3D Printing; Toolmarks; Ghost Guns

B16 Exploring the Persistence of Human Scent for Forensic Purposes

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WITHDRAWN

B17 A Characterization of Decomposing Human and Animal Tissue in a Micro-Scale Biotope Through Simulated Rainwater Extraction

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Learning Objectives: After attending this poster presentation, attendees will understand human and animal decomposition chemistry in soil and some principles behind the characterization of Volatile Organic Compounds (VOCs). Attendees will be shown a microbiotope used for the analysis of human and animal decomposition chemistry.

Impact Statement: This poster presentation will impact the forensic science community by contributing to the understanding of human decomposition and how the chemical "fingerprint" of human VOCs can be differentiated from animal VOCs through qualitative and quantitative analysis. This presentation will demonstrate the difference in the chemical profiles when the extraction of the VOCs is facilitated by environmental processes, such as the percolation of rainwater through soil, providing a novel methodology for the measurement of decomposition samples in suspected clandestine burial sites.

Abstract Text: The decomposition of human and animal remains produces a complex matrix of VOCs, which are released into the surrounding environment. Past research regarding this field has focused on finding human-specific makers, but due to the emission of similar VOCs in various animal tissues, there is currently a gap in the literature when it comes to a clear differentiation of these VOCs.[1-4] Further research regarding human and animal decomposition VOCs could assist forensic investigators by providing them with the ability to locate victims for suspected clandestine burial sites through soil vapor sampling and standard chemical analysis methods.[5-8] Collating more research around human-specific markers can lead to the development of more effective screening methods for clandestine burial sites, while also improving the effectiveness of Victim Recovery (VR) canine training by honing on specific chemical "fingerprints" that indicate for human decomposition.

A novel approach for studying human and animal decomposition processes will be demonstrated in this research. For this study, a micro-scale biotope has been constructed by using multiple modified soil "chromatography" columns. The research will evaluate muscle, adipose, and bone tissue samples for both human and animal remains. The tissue types will be embedded in the soil of the columns at a set depth to simulate a shallow burial. One aspect that will be evaluated alongside the soil vapors will be the effect that the movement of percolated rainwater will have on the tissue types and whether these extracted rainwater samples can be used for the sampling of human decomposition VOCs at a suspected clandestine burial site. An adjustable flow rate controller was used to maintain a consistent drip-rate in the soil columns.

For the vapor samples, headspace extraction will be performed through Solid Phase Microextraction (SPME), followed by Gas Chromatography/Mass Spectrometry (GC/MS). For the rainwater samples, chemical extraction will be performed using Solid Phase Extraction (SPE), followed by GC/MS. Upon determining the presence of VOCs and/or other organic compounds, the gathered data will be quantitatively analyzed to measure the levels of each evaluated compound in the generated VOC profiles.

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Decomposition; Volatiles; Biotope

B18 Analyzing the Case of a Fatal Shooting on a Movie Set

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Learning Objectives: Attendees will learn about how a single-action revolver functions and how it will not discharge without pulling the trigger.

Impact Statement: This work will affect the forensic science community by showing how methodical testing can reveal whether a firearm is functioning safely and according to the manufacturer's design.

Abstract Text: This research presents the findings of an experimental study conducted to analyze the tragic incident of a fatal shooting on the set of the new film "Rust" and the type of projectile that had been discharged from the firearm on the movie set. The experiment involved firing bullets at a 20-pound block of ballistic gelatine with a piece of 2"x6" wood covering the exit part of the gelatine. The ballistic gelatine acts like human tissue because it slows down the bullet as it enters.

Observations were conducted through multiple trials, photographing the entryway of the bullet trajectories and the exit after encountering the gelatine. The first four trials consisted of pulling the hammer all the way back and pulling the trigger while pointing at the target to confirm that the Pietta .45 Long Colt did shoot as designed. All four times were successful as the handgun did shoot as intended. As the shots were fired, the bullets entered the 20-pound block of gelatine and slowed down considerably as they exited, merely making an indentation in the 2"x6" backstop. Following the successful shots, another test was conducted to determine whether the gun would fire if the trigger was not pulled. Ten trials were conducted by pulling the hammer back a quarter of the way and releasing the hammer, allowing it to free fall toward a loaded chamber at the 12 o'clock position. An additional ten trials of the hammer being cocked back halfway, again with a cartridge placed into the loading gate as normal, and oriented into the 12 o'clock position. When the hammer was released, allowing it to fall toward the live cartridge without pulling the trigger, the revolver still did not result in the discharge of the cartridge. The data was collected using an original 1873 Colt Single Action Army revolver and the Italian Pietta .45 Long Colt, which is an economical replica used on the movie set. Neither the original revolver nor the modern replica revolver resulted in a discharge when the trigger was not engaged. Only when the trigger was pulled did the cartridge fire.

Movie Set; Prop Gun; .45 Caliber Revolver

B19 Developing a Method for the Analysis of Products Claiming to Treat COVID-19

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WITHDRAWN

B20 Forensic Intelligence Derived From Crime Scene Evidence Using Text Embeddings

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Learning Objectives: After attending this presentation, participants will learn how artificial intelligence can be leveraged to identify patterns in textual data, particularly with respect to crime data. Specifically, they will explore key concepts of Natural Language Processing (NLP) and its application in clustering crimes with similar characteristics. This presentation integrates research from Forensic Intelligence and Crime Linkage to uncover relationships between cases. Additionally, attendees will understand how crime scene evidence directly influences criminal modus operandi in real-world scenarios.

Impact Statement: This presentation will impact the forensic science community by showcasing the significance of Forensic Intelligence applications and their potential to enhance the efficiency of investigations. Furthermore, this work aims to demonstrate, with the support of artificial intelligence, how comprehensive intelligence can be derived by analyzing a diverse collection of vestiges, contrasting with traditional literature that typically focuses on a single type of evidence.

Abstract Text: Uncovering patterns has been a key topic across various research domains. The behavioral patterns observed in criminal activities often reflect the modus operandi of individuals or groups.[1] Crime Linkage, a field dedicated to studying crime information, aims to establish connections between potential serial crimes.[2,3] Initially explored by psychologists, sociologists, and statisticians, this area has recently garnered attention from computer scientists, leveraging artificial intelligence techniques, although this latter discipline is in its initial stages.[4-6] Also, most of the related work has been focused on proprietary crimes, leaving questions on whether crime link conclusions can be drawn with other types of crimes, especially when analyzing big data.[7]

Another related field, Forensic Intelligence, represents a shift in forensic science from individual evidence analysis to a macro-level perspective capable of deriving useful intelligent information from a collection of crime evidence data.[8] Although it is considered a general framework, most of the studies have been treating Forensic Intelligence on one particular type of seized evidence, with a scarcity of work on overall crime scene data.[9]

Both topics (Crime Linkage and Forensic Intelligence) share great similarities, but because they come from different fields, they are often unrelated. This study lies at the nexus of Crime Linkage and Forensic Intelligence, with the support of artificial intelligence, to unveil relationships among crimes utilizing real crime scene information. This research introduces a novel approach to analyzing violent crime scene data by employing advanced natural language processing techniques. In summary, the project consists of three steps: a summarization or extraction of main forensic features from crime scene reports with the support of Large Language Models (LLMs); an embedding conversion (vectorization) with the support of transformers-based models; and data clustering, visualization and analysis.[10] The objective is not merely to identify common offenders across different crime datasets (as typically observed in Crime Linkage) but rather to discern patterns or relationships abstracted from the concealed embeddings. Results demonstrated that crimes share certain commonalities that allow them to be clustered. The vectors generated from crime scene information, combined with spatiotemporal data, can thus encapsulate criminal modus operandi, providing valuable insights for investigations. Moving forward, this model will facilitate the development of a tool designed to generate intelligence for law enforcement and the forensic community.

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Crime Scene Investigation; Artificial Intelligence; Criminalistics

B21 Deep Ultraviolet Raman Spectroscopy for the Forensic Analysis of Oral Fluids

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Learning Objectives: This work demonstrates the application of deep Ultraviolet (UV) Raman spectroscopy and chemometrics to analyze antihistamine drugs and assess the impact of thermal conditions on Oral Fluid (OF) samples on various fabrics. The study is relevant to both analytical chemists and forensic scientists, showcasing how deep UV Raman spectroscopy can efficiently analyze OF and other forensic samples with minimal sample consumption and pretreatment. Attendees will appreciate the potential of this technique for crime scene analysis. Additionally, the use of chemometrics, including multivariate Partial Least Squares Discriminant Analysis (PLSDA), to distinguish between pure OF stains and those containing cetirizine highlights the significance of these methods for interpreting forensic evidence. The presentation will also explore the biochemical composition of OF, underscoring its importance as a source of crucial forensic evidence.

Impact Statement: This research highlights the transformative potential of deep UV Raman spectroscopy combined with chemometrics for forensic analysis, particularly in the detection of antihistamine drugs and the evaluation of OF samples exposed to extreme thermal conditions. By demonstrating the method's ability to analyze various forensic samples with minimal pretreatment and sample consumption, this work advances the field of forensic science. The integration of multivariate PLSDA further enhances the capability to differentiate between pure and drug-contaminated oral fluid stains, offering significant improvements in forensic evidence interpretation. The study's insights into the biochemical composition of OF underscore its critical role in forensic investigations, potentially influencing practices in crime scene analysis and evidence assessment.

Abstract Text: This study explores the application of Deep Ultraviolet Raman Spectroscopy (DUVRS) for analyzing OFs in forensic contexts. The application of deep UV Raman spectroscopy and chemometrics to analyze antihistamine drugs and assess the impact of thermal conditions on OF samples on various fabrics was explored. Initially, DUVRS was utilized to detect cetirizine, a common antihistamine drug, in oral fluid samples without requiring sample pretreatment. Antihistamines are a class of compounds that are used to mitigate the symptoms associated with allergic reaction by inhibiting the action of histamine. However, due to their sedative effects, this class of drugs can hinder some vital psychological functions, thereby decreasing the safety in certain common crucial tasks (i.e., driving). From a forensic viewpoint, antihistamine drugs can cause intoxication and can sometimes be fatal when co-administrated with alcohol or other sedative drugs. The method demonstrated practical sensitivity for forensic applications, detecting cetirizine at concentrations as low as 50µg/mL. Additionally, the sensitivity was assessed for analyzing dried oral fluid stains spiked with cetirizine to simulate forensic scenarios involving bodily fluid stains. Combining DUVRS with multivariate statistical analysis enabled effective differentiation between pure OF stains and those containing cetirizine, highlighting DUVRS's high sensitivity for drug detection.

In the second phase of the study, the method was adapted to analyze OFs on various fabrics subjected to extreme thermal conditions for up to 48 hours, to estimate the time since deposition. Despite some spectral changes over time, the approach successfully detected OF bands even after prolonged exposure to harsh thermal environments. This research highlights the transformative potential of deep UV Raman spectroscopy combined with chemometrics for forensic analysis, particularly in the detection of antihistamine drugs and the evaluation of OF samples exposed to extreme thermal conditions. By demonstrating the method's ability to analyze various forensic samples with minimal pretreatment and sample consumption, this work advances the field of forensic science.

Raman Spectroscopy; Drug Analysis; Age Estimation

B22 The Distribution of Retroreflective Glass Beads in Soil Samples

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Learning Objectives: After attending this presentation, attendees will understand the value and challenges of analyzing retroreflective glass beads in soil samples.

Impact Statement: This presentation will impact the forensic community by adding an analytical strategy that could support or exclude scenarios where soil evidence is relevant.

Abstract Text: Currently, forensic laboratories do not routinely analyze anthropogenic materials found in soil samples. The primary reason is that the significance of most anthropogenic materials as evidence is not understood.[1-3] Analyses of a few specific anthropogenic materials, such as fibers and glass, have been developed as robust and useful disciplines in forensic science, and are routinely used in United States courts. However, the presence, prevalence, and persistence of anthropogenic materials are not understood in a forensic context. For example, these materials may distribute through the environment predictably such that, if they have a known point source, their concentration in soil samples could be used to infer distance from that point source.

One type of anthropogenic material found in soil is retroreflective glass beads. These beads are incorporated into pavement markings to improve nighttime driving visibility as a countermeasure for lane departures. Therefore, retroreflective glass beads found in soil adjacent to roads potentially are a new source of trace evidence for forensic investigations.

In this study, the 32-500µm fraction from 17 soil samples collected near roads from several locations in central Arizona were analyzed and compared to explore variations in spatial distribution and chemical compositions that could offer source discrimination. Bead concentrations in the soil samples ranged from 0 bead/g of soil to as much as 587 beads/g of soil. Analysis of spatial distribution of glass beads demonstrated that the bead concentration of retroreflective glass beads has a strong dependency with distance from a paved road. Soil samples taken near marked roads showed significantly higher concentrations of glass beads, averaging from 0.18 bead/g of soil sample to 587 beads/g of soil, while soil samples taken near unmarked roads had an average range of concentration of 0 bead/g of soil to 0.21 bead/g of soil. This is expected as glass beads come from pavement markings, thus soil samples near marked roads are expected to have higher concentrations of glass beads. Elemental concentrations by Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) for each sample showed discriminating differences among samples, for most of the elements. An analysis of variance for elemental concentrations was conducted, and results showed statistically significant differences, beyond random chance alone for half of the elements analyzed. For forensic comparisons, these exclusionary differences would support the scenario of the samples coming from different sources. The elemental concentrations of glass beads collected from the same location, but of varying distances, was also analyzed. The results show significant differences for only one or two elements across those samples. The combined results are consistent with the potential use of retroreflective glass beads as trace evidence.

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Trace Analysis; Glass; Soil Analysis

B23 Forensic Profiling of Cigarette Paper Using ATR/FTIR Spectroscopy and Machine Learning/Algorithms

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Learning Objectives: This research provides a detailed understanding of the differences between cigarette paper and other similar materials. Forensic experts and law enforcement personnel can use these insights to better assess the relevance of paper-based evidence found at crime scenes. Further, the application of Machine Learning (ML) algorithms demonstrates the growing importance of data science in forensic analysis by classifying and identifying papers based on their spectral properties. This study also emphasizes the effectiveness of the CatBoost classifier, which outperformed other algorithms. This insight is valuable for future forensic investigations that may require similar classification tasks.

Impact Statement: By demonstrating a method to accurately differentiate cigarette paper from other similar materials and identify specific brands, the study offers a novel approach to forensic evidence analysis. The ability to identify the brand of cigarette paper can offer crucial insights into the habits and social characteristics of individuals at a crime scene, potentially narrowing down suspect lists and establishing links between different pieces of evidence. The adoption of Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) spectroscopy and ML in forensic investigations represents a significant advancement in the field, allowing for rapid and accurate analysis of evidence and reducing the time and resources required for traditional forensic methods.

Abstract Text: Cigarette paper, often found at crime scenes, is potentially overlooked forensic evidence in investigations ranging from burglary and theft to kidnapping and homicide. This evidence can reveal significant details about the individuals present at the crime scene, such as their brand preferences, social and economic status, and behavioral patterns. Despite its potential value, cigarette paper is frequently overlooked, primarily due to the challenge of distinguishing it from other similar-looking materials, such as tissue, newspaper, and notebook paper. This study aims to address this challenge through two primary objectives: first, to differentiate cigarette paper from other types of paper, and second, to identify the specific brand of cigarette paper from a set of nine different brands. The research employs a combination of ATR/FTIR spectroscopy and advanced Machine Learning (ML) algorithms to achieve these goals.

The spectral data underwent preprocessing, including baseline correction and normalization, to ensure consistency and reliability. Principle Component Analysis (PCA) was then applied to reduce the dimensionality of the data, facilitating more efficient and accurate analysis. The reduced data set was used as input for various machine learning algorithms to classify the paper types and identify the specific brands of cigarette paper. In this work, 15 different ML algorithms were tested to determine the most effective model for this classification task. Among these, the CatBoost classifier stood out for its exceptional performance in both objectives. CatBoost, a gradient boosting algorithm, effectively handled the complex relationships within the spectral data, achieving high accuracy in distinguishing cigarette paper from other types of paper and identifying the specific brands.

This study provides a comprehensive and reliable method for the forensic analysis of cigarette paper, emphasizing the importance of advanced technologies in modern forensic investigations. The integration of ATR/FTIR spectroscopy and ML offers a powerful tool for law enforcement agencies, enabling more precise and efficient crime scene investigations. As forensic science continues to evolve, the incorporation of such innovative methods will be crucial in enhancing the accuracy and efficiency of criminal justice processes.

Cigarette Paper; Machine Learning; Forensic Analysis

B24 Low-Field and Benchtop NMR in Forensic Science: Challenges and Opportunities

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WITHDRAWN

B25 Cracking the Case: Toward the Development of Mass Spectral Analysis of Blow Fly Eggs for Postmortem Interval Determination

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Learning Objectives: Attendees can expect to learn about the use of Direct Analysis in Real-Time High Resolution Mass Spectrometry (DART-HRMS) and its applications in forensic entomology and how statistical analysis can be applied to the metabolome profiles revealed by the aforementioned technique to enable insect species identification that can be useful for Postmortem Interval (PMI) determination, as well as geographic provenance determination.

Impact Statement: Facile generation of metabolome profiles combined with the application of statistical analysis of these profiles reveals unique chemical fingerprints that enable species differentiation and accurate identification of necrophagous blow fly eggs. Furthermore, the mass spectral data acquired from blow fly eggs within a species collected from different regions may reveal provenance with high accuracy.

Abstract Text: Medicolegal forensic entomology utilizes the patterns associated with insect colonization of remains to estimate time since death, or PMI, a critical element in criminal investigations. Blow flies (Family: Calliphoridae) are capable of detecting remains within minutes of death, often serving as the initial colonizers. A "back-calculation" method exists in which the time since death has occurred (i.e., PMI) can be estimated by correlating the insect species identity, environmental conditions, the stage of decomposition of the remains, and the species-specific insect life cycle timelines. The calculation determines the time since the laying of the eggs, based on the assumption that the eggs were laid within a time period shortly after the death occurred. Precise species identification is essential for accurate PMI estimation, as developmental rates vary significantly among blow fly species as a function of temperature and weather conditions. Retrieved entomological evidence can also reveal important information about where the death occurred through determination of whether the insects colonizing the remains are endemic to the region where the remains were found. Therefore, knowing the identity of the species is essential.

Traditional identification methods based on morphological characterization are time-consuming and susceptible to error, particularly for the immature life stages (i.e., eggs, larvae, and pupae) because of their visual similarity across multiple species. Thus, if the specimens are viable, an experienced entomologist may rear them to adulthood to accomplish species identification based on the visually apparent gross morphological features of the emergent adults.

Reported here is an alternative novel approach employing mass spectral analyses and chemometrics for rapid blow fly egg species identification, which can be used to facilitate estimation of PMI. By analyzing 70% aqueous ethanol suspensions of eggs from multiple species representing the genera *Calliphora*, *Chrysomya*, and *Lucilia* using DART-HRMS, distinct mass spectral profiles were obtained for each species. Kernel Discriminant Analyses (KDA) for each genus effectively clustered these profiles, demonstrating the potential for accurate genus identification with accuracies of 88.75%, 95%, and 92.50%, respectively. In addition, application of KDA to the mass spectral data acquired from egg specimens of *Cynomya cadaverina* from three regions (Louisiana; Manhattan, NY; and Sauble Falls, Canada) showed the potential to determine geographic provenance for samples of the same species from different regions with an accuracy of 95.56%. Future studies aim to create a database of the species-specific chemical signatures for species identification of entomological evidence that will increase the evidentiary value of juvenile life stages in the determination of PMI and assessment of whether the remains were moved from one location to another.

Entomology; Species Determination; Species-Specific Chemical Signatures

B26 The Contamination of Training Aid Substrates

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Learning Objectives: The objectives of this poster project focus on the crucial aspects of contamination in canine training aids. First, the project aims to understand the impact of contamination on these training aids by recognizing how contamination can degrade their quality and affect detection proficiency. Second, it will evaluate non-target contamination during training by assessing the extent of contamination in different substrates under training conditions and understanding how these conditions influence contamination levels. Finally, the project will compare substrate suitability for training aids by examining contamination levels across various substrates to determine their appropriateness for use in training aids.

Impact Statement: This study significantly enhances our understanding of contamination issues in canine training aids, a critical factor in the proficiency of detection canines. By systematically comparing contamination levels across various substrate materials in training scenarios, the research identifies the key strengths and weaknesses of these materials. The findings provide crucial insights that will inform the development of improved manufacturing processes, storage protocols, and contamination mitigation strategies. This work will ultimately lead to more reliable and effective detection canines, with broad implications for security, law enforcement, and other sectors relying on these animals.

Abstract Text: The effectiveness of canine training aids is crucial for the proficiency of detection canines, but contamination can rapidly degrade their quality. This study addresses the problem of contamination in canine training aids by comparing the extent of contamination across various common substrate materials in real-world training scenarios.

The project focused on assessing non-target contamination during training. Substrate materials, including GetXent polymer tubes, cellulose-based materials, Polydimethylsiloxane (PDMS), and novel Fabric Phase Sorptive Extraction (FPSE) materials, were exposed to highly odorous environments to simulate training conditions. These odorous locations, such as open air, cars, and dog kennels, were chosen because they represent typical training environments where contamination could originate and later affect the detection canine's proficiency.

To test the substrates, triplicates were first equilibrated and sampled before being exposed to odorous locations for 4 hours. After this exposure, the substrates were sampled again to collect their headspace. The headspace above these substrates was sampled using Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS). The collected data was then analyzed to compare the contamination profiles of the various substrates.

The results indicated significant differences in non-targeted contamination levels among the substrates, suggesting variability in the resistance of different substrates for training aids. The study also demonstrated how various methods of handling training kits affect contamination levels. This finding underscores the importance of best practices in handling and deployment to minimize contamination risks. Proper handling procedures, such as exposing the training aids using an open container and maintaining clean storage conditions, can significantly reduce the contamination of training aids, thereby enhancing the accuracy and reliability of detection canines.

Interestingly, the study showed no correlation between cost and effectiveness. This suggests that more expensive materials do not necessarily offer better resistance to contamination. Therefore, selecting cost-effective substrates that meet the required standards of contamination resistance is feasible, ensuring the sustainability of canine training programs.

Finally, while GetXent tubes and PDMS training aids are gaining popularity in the operational community, there has been limited research on their strengths, limitations, and contamination potential. This study contributes to filling that gap by providing empirical evidence on their performance under real-world training conditions. The insights gained from this research can guide trainers in making informed decisions about the selection and use of training aids, ultimately enhancing the proficiency of detection canines.

In conclusion, the study underscores the importance of selecting appropriate substrate materials and adhering to best handling practices to minimize contamination in canine training aids. By doing so, trainers can ensure the effectiveness and reliability of detection canines, which are essential for various operational applications. The findings from this study pave the way for future research and development in the field, aiming to enhance the quality and efficacy of canine training aids.

Absorption; Solid Phase Microextraction; Forensic Analysis

B27 A Rapid Hand-Held Detector for Drugs and Explosives and Their Signature VOCs Using Novel Nano-Porous Molecularly Imprinted Polymer Sensors

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Learning Objectives: We are presenting a new chemical detection sensor technology. We will describe how the new technology is unique and its advantages for low cost chemical detection in the field.

Impact Statement: The Nanoporous Molecularly Imprinted Polymer Sensors (NPMIPS) chemical detection sensor technology has the potential to enable new, highly selective, and sensitive, field chemical detection instruments. These new field portable instruments will be capable of detecting multiple specific compounds of interest simultaneously, based on the MIP sensor design array. A new hand-held NPMIPS portable detector will bring the next generation of rapid, safe, and reliable sensing technology to users in the field for drug, explosives, toxic chemical and materials, and chemical warfare agent applications.

Abstract Text: Novel NPMIPS exploit nanotechnology to enable new micro sensor devices for chemical sensing through nanoengineered nanoporous materials. NPMIPS sensors achieve high selectivity and sensitivity through a nanoporous polymer material that has been molecularly engineered for analyte of interest specific collection and sensing. Sol-gel molecular imprinting technology creates molecularly imprinted polymers characterized with high imprinting factor (ratio between specific and non-specific extraction), robust nanocavities complimentary to the shape, size, and functional composition of the template molecule(s), remarkably high thermal (~500°C), solvent (any solvent or solvent mixture), and chemical stability (pH 1-13). The NPMIPS sensors are designed for analyte-specific sensing using an array of capacitive sensing elements to detect compounds of interest.[5-6]

The performance superiority of novel nanoporous molecularly imprinted polymer materials has been published in several recent publications by our research group.[1-4] For example, N.P. Kalogiouri et al. utilized Bisphenol A (BPA) -imprinted NPMIP in combination with High-Performance Liquid Chromatography-Ultraviolet (HPLC-UV) to monitor BPA in walnuts and have reported 10ng/g quantitation sensitivity.[1] Similarly, V. Samanidou et al. reported application of chloramphenicol imprinted NPMIP to monitor the presence of this antibiotic in milk samples. Analytical figures of merit included an imprinting factor 9.7, Limit Of Quantitation (LOQ) in HPLC-UV 50ng/g and 0.3ng/g in Liquid Chromatography/Mass Spectrometry (LC/MS)[4] The new NPMIPS could enable rapid detection of both the analyte and its Volatile Organic Compound (VOC) (e.g., cocaine and its signature VOC methyl benzoate), giving a potential confirmatory measurement capability for a compound of interest on the same sensing device, a new and powerful method of field chemical detection without sample preparation. The new NPMIPS sensors are amenable to thick film casting on substrates, integrate well into micro sensor device and batch fabrication processes, and may be molecularly engineered and synthesized for any desired analyte. The chemical specific collection material is very stable and resistant to high temperature (500°C), allowing for temperature cycling of the sensor by resistive heating to clear the previous sample to enable new measurements. Further, it is possible for the NPMIPS to have 10 to 20 different specific sensing elements, covering a broad range of drugs, explosives, or other analyte classes of interest.

NPMIPS nanotechnology will enable the development of new low-cost portable instruments for the simultaneous detection of drugs, explosives, and other compounds, and their signature VOCs in a hand-held device for use in the field. Chemical sensing in the field demands small, lightweight, low power, cost-effective, and precise instrumentation to provide the most reliable measurement result and optimal situational awareness for end-users. The new hand-held NPMIPS device will bring the next generation of rapid, safe, and reliable sensing technology to users in the field.

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Chemical Sensor; Molecular Imprinting; Portable Chemical Sensing Instrument

B28 Exploring Alternative Solvents for Dichloromethane as an Extraction Solvent of Sexual Lubricant Components Using Gas Chromatography/Mass Spectrometry

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Learning Objectives: Attendees will recognize the value of finding an alternative solvent for extracting sexual lubricant components that performs as efficiently as Dichloromethane (DCM).

Impact Statement: DCM-Methanol (MeOH) has been found to be proficient in extracting non-polar and polar components of sexual lubricants. However, a recent recommendation has been made by the Environmental Protection Agency (EPA) that may result in a ban of DCM usage in laboratories. Therefore, this study will provide the forensic community with an extraction solvent for sexual lubricants that results in a comparable recovery to DCM.

Abstract Text: After attending this presentation, attendees will understand the importance of finding an alternative extraction solvent in replacement to DCM that allows for an efficient recovery of sexual lubricant in comparison to DCM. The forensic community will find this information helpful due to a recent recommendation made by the EPA that suggests a ban of DCM laboratory use.

Recent research has proven that sexual lubricant may be useful as an additional form of evidence linking suspects to a sexual crime or victim.[1,2] Several extraction solvents have been evaluated for sexual lubricant extraction, particularly hexane and MeOH. However, hexane extracts the non-polar components and MeOH extracts the polar components. Thus, multiple extractions require multiple analysis, which increases turnaround. Therefore, studies have demonstrated that a DCM-MeOH (1:1) solvent system is appropriate to conduct one extraction to recover polar and non-polar components simultaneously.[3] However, recent recommendations made by the EPA to restrict the use of DCM affects the implementation of this solvent system for forensic lubricant analysis and therefore a comparable solvent must be evaluated for sexual lubricant extraction.

This study evaluates different solvents for the extraction of individual sexual lubricant components as well as manufactured lubricant samples. The extraction systems evaluated were Ethyl Acetate (EA), EA-MeOH (1:1), EA-MeOH (2:1), and 2-Methyltetrahydrofuran (2MTF). Preliminary data indicated that the EA-MeOH (1:1) solvent system was most efficient at extracting several sexual lubricant components in comparison to 2MTF and EA and thus the study was expanded to more lubricant components/samples and an additional solvent system. The EA-MeOH (2:1) solvent system was added to increase the extraction of more nonpolar components in relation to MeOH extraction of polar components. All extracts were analyzed in triplicate using gas chromatography/mass spectrometry. The resulting chromatograms from each solvent extraction were compared against the DCM-MeOH extraction chromatograms using Pearson correlations coefficients.

It is hypothesized that the EA-MeOH (2:1) solvent system will provide the most comparable results to the DCM-MeOH solvent system. Considering that the EA-MeOH (1:1) solvent system did extract some non-polar components but not in the same abundance as the DCM-MeOH (1:1) system.

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Forensic Lubricants; Extraction Solvents; Dichloromethane

B29 Time-Since-Deposition Signatures for the Analysis of Canine Body Fluids

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Learning Objectives: After this presentation, attendees will have a new understanding of how flow cytometry can be used to determine the Time Since Deposition (TSD) of canine bodily fluid samples. Attendees will also learn about biological signatures that can differentiate between human and canine bodily fluids.

Impact Statement: This presentation will impact the forensic science community by providing alternative scientific methods for animal cruelty cases and giving investigators a new method for analyzing animal bodily fluids and determining TSD. It could also assist in generating reference data and casework protocols for analysis of canine bodily fluids.

Abstract Text: Although dogfighting is a felony in all 50 states, increasing criminalization has done little to deter the estimated tens of thousands of people involved with the organization and commodification of dogfighting and related breeding in the United States. Dogfighting has grown into a substantial enterprise, which is often tied to other organized crime activities, including gambling, narcotics, and weapons trafficking.[1]

When investigating a suspected dog fighting event, canine bodily fluids such as blood or saliva are some of the primary types of physical evidence collected from the crime scene. However, one of the challenges with processing and interpreting DNA profiles generated from canine blood deposits is that DNA profiles provide identity of canines involved (through comparison with a reference sample) but cannot confirm whether the bodily fluids were deposited during the activity or at a different time under unrelated circumstances. Determining when these bodily fluids were deposited at the crime scene could add probative value to the DNA evidence, since it can be linked to both the date and location of the fight.

One proposed method of analysis uses flow cytometry to characterize changes in the autofluorescence profiles of canine blood or saliva across different TSD intervals. By comparing the changes of properties such as fluorescence intensity, forward scatter (which indicates the relative size of the cells), and side scatter (which indicates the size and density of the cell) over time, it may be possible to estimate the TSD of a canine bodily fluid after sample deposition. Therefore, the goal of this study was to develop a new set of biological signatures based on flow cytometry measurements that can be used to determine the time-since-deposition of forensically relevant canine bodily fluids, including blood and saliva.

To accomplish this, blood was taken from ten canines of different breeds and ages, then deposited onto a non-porous substrate in 15µL spots. Samples were then aged for up to 90 days and analyzed at fixed TSD timepoints (1, 2, 3, 7, 14, 30, and 90 Days). At each timepoint, blood deposits were collected from the surface using a wet swabbing method and allowed to dry prior to flow cytometry analysis. Autofluorescence profiles of individual cells were characterized using a 488nm excitation laser and detector wavelengths between 535nm-700nm. Fluorescence histograms showing the number of cells at varying fluorescence intensity were used to compare cell populations at different TSDs. Results showed that ~50% of the canine contributor samples showed high fluorescence at 523nm, 583nm, and 695nm within a week of deposition (e.g., 104 RFU at 3 days vs. 103 RFU at 7 days). After one week, fluorescence intensity continued to increase up to 30 days. However, after 90 days, all contributor cell populations showed a two-fold decrease in autofluorescence intensity (~400 RFU).

As part of this study, differences between blood cell populations of humans and canines were also investigated. Autofluorescence intensity from human samples were lower (approximately 25 RFU) compared to canine samples (approximately 100 RFU) across each TSD at 523nm for up to 30 days. Human samples also had higher median side-scatter area (SSC-ALog) at all timepoints than canine samples, though there was considerable overlap in size range between the two.

These results suggest that flow cytometry can be used to analyze an unknown blood sample and determine whether it originated from human or canine tissue as well as the sample's TSD at specific intervals up to 90 days. This indicates that it may be possible to link the relative age of canine blood samples to the known time period of a dog fighting event, leading to increased prosecution of dog fighting crimes in the United States.

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Canines; Animal Abuse; Bodily Fluid

B30 Implementing Y-Screen Protocols as a Tool for Sexual Assault Forensic Examination Kit Backlog Reduction

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Learning Objectives: From this presentation, attendees will learn how to implement Y-screening protocols to manage the backlog of Sexual Assault Forensic Examination (SAFE) kits more effectively. This presentation will explain the methodology for using Y-screening to identify and prioritize the most probative samples and will demonstrate the concept of autosomal-to-male DNA quantitation thresholds. Attendees will understand how these approaches can enhance forensic DNA analysis, resulting in shorter turnaround times and leading to more efficient processing of sexual assault cases.

Impact Statement: This presentation will provide the forensic science community with valuable insights into optimizing Y-screening protocols and integrating autosomal-to-male DNA quantitation thresholds. This presentation will demonstrate how the Baltimore Police Department's Forensic Biology Unit (FBU) has applied these techniques to improve efficiency, accelerate case processing, and make significant strides toward eliminating its backlog with a goal to reach real-time analysis.

Abstract Text: A prominent challenge facing many forensic DNA laboratories is a backlog of SAFE kits. Due to federal mandates, labs must now process all previously untested SAFE kits, some spanning back decades, placing a significant workload on labs that are already facing staffing shortages and budget constraints. All of this can make it difficult for labs to manage both backlogged and current cases. To help combat these challenges, many laboratories are adopting Y-screening protocols that streamline the analysis process and reduce turnaround times.

Y-screening is performed post-quantitation and is used to assess the presence of male DNA in SAFE kit samples. Using quantitation results and case circumstances, this method allows labs to prioritize the most probative samples—those most likely to produce male profiles—thereby decreasing the number of samples that require downstream analysis. By using this approach, labs can enhance efficiency and accelerate case resolution.

The Baltimore Police Department FBU first implemented its Y-screening protocol in the summer of 2021 and has since continued to refine and improve the process. In 2023, the FBU began evaluating an autosomal-to-male DNA quantitation threshold as a new criterion. In samples where the amount of female DNA is disproportionately higher than the amount of male DNA, the probability of obtaining a male profile is unlikely. This allows for even further refinement in sample selection, conserving time and reagents while optimizing the analysis workflow.

This presentation will demonstrate the successful application of Y-screening protocols in SAFE kit backlog reduction, as evidenced by the BPD's FBU. It will highlight ways to refine and improve processes, such as the use of autosomal-to-male DNA quantitation thresholds. Attendees will learn practical strategies for implementing these techniques, ultimately contributing to greater efficiency and faster turnaround times, while working toward the goal of real-time SAFE kit processing.

Backlogs; Y-Screening; Forensic DNA

B31 The Use of Quantitation Values to Infer the Possible Presence of Spermatozoa

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Learning Objectives: This presentation will introduce attendees to a novel approach to using differential extraction quantitation data as a gauge for the presence of sperm cells in a sample. Attendees will also learn how our laboratory system has implemented multiple quantitation data analysis methods as presumptive tests for the presence of spermatozoa.

Impact Statement: The presented work will impact the forensic biology community by introducing a new approach to inferring the presence of spermatozoa using quantitation results. We also provide guidance on implementing these methods into a multi-laboratory system in a way that increases the probative information obtained while following the recommended "Direct to DNA" approach to sexual assault casework.

Abstract Text: The National Institute of Justice (NIJ) recommends a "Direct to DNA" approach for the processing of sexual assault kits.[1] While effective to decrease overall processing time, the lack of traditional serology testing prevents the confirmation of spermatozoa in a sample. Research by Alderson et al. and Ridgley et al. indicates that when utilizing a differential extraction method, the average portion of male DNA in sperm fractions is high when sperm cells are present.[2,3] Both papers utilize a calculation termed the Fractionation Ratio (FR) but consider different thresholds for what percentage of male DNA is expected in a sperm fraction and the quantity of DNA in the fraction below which a sample will not be tested.

We developed an additional method using the Female-To-Male Ratio (FTMR) of both the sperm and non-sperm fractions to create a ratio we have termed the Differential Ratio (DR). It was determined that using the FR method in conjunction with the DR method captured the highest number of samples that contained spermatozoa. In particular, the DR captured samples made of dilute semen mixed with undilute body fluids from both male and female donors. There were a number of false negatives for both methods, and a false positive result was observed with the FR method using the thresholds described in Alderson et al.[2]

These results support the leading thought that these calculations should be utilized as a presumptive test for spermatozoa. Our laboratory system has implemented both the FR and DR into our processing of sexual assault kits—and any other samples subjected to a differential extraction—such that no additional sample is consumed, nor time spent by the analyst, but with a potential to retrieve additional probative case information. Using our in-house DNA analysis workbook, the FR and DR are automatically calculated and a positive result from either method is then reported to the submitting agency along with the results of DNA analysis.

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Spermatozoa; DNA Quantitation; Direct to DNA

B32 Seminal Fluid Identification in Different Semen Sample Types

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Learning Objectives: After attending this presentation, attendees will be informed of the differential efficiency in detecting seminal fluid in four forensic scenarios using these biomarkers: Prostate-Specific Antigen (PSA), Semenogelin (Sg), Y-chromosome profiling and microscopy.

Impact Statement: This presentation will impact the forensic science community by presenting, for the first time, an extensive study of the assessment of these four semen detection approaches, taking into consideration the characteristics of the donor.

Abstract Text: Semen detection at crime scenes is essential for sexual assault investigations. Presumptive and confirmatory tests have been developed to identify seminal fluid toward this purpose. While visual observation of sperm cells proves the presence of semen, this is not always possible. The detection of male DNA in a sexual assault sample may suggest semen is present but is not body fluid specific.

Currently, one of the main approaches to detecting seminal fluid is through the use of Lateral Flow Immunochromatographic (LFI) tests, based on antigen-antibody reactions. There are two types of LFI for the detection of semen: those based on PSA, produced in the prostatic epithelium; and those based on Sg, produced in the seminal vesicles, the vas deferens, the prostate, and the epididymis. Both proteins are secreted in semen, and to a lesser extent in some other body fluids. The question is: when to use one or another or both?

The aims of this study were: (1) to evaluate the effectiveness of PSA and Sg LFI tests to detect seminal fluid; (2) to correlate the positiveness of the tests with the presence/absence of spermatozoa; (3) to estimate the possibility of obtaining a full autosomal and Y-chromosomal Short Tandem Repeat (Y-STR) DNA profile; and (4) to assess the obtention of this profile using semen dilutions mixed with menstrual blood. To do this, PSA and Sg LFI tests were analyzed in six types of semen samples: single donor, pooled donor, vasectomized, 30-year-old frozen pooled donor (to simulate an unresolved case), low sperm count, and a reverted vasectomized donor sample. Serial dilutions of the samples were carried out from 1:2 to 1:32,000. Our results indicated that both tests were positive in all samples, ,except that Sg began to be negative from 1:2,000 in the 30-year-old pooled sample and from 1:8000 in the rest of the samples. Conversely, PSA began to be negative from 1:16,000 in the fresh frozen pool donor and from 1:32,000 in the 30-year-old group pooled sample, indicating better PSA performance than Sg, even in vasectomized samples. Cytology was done both with Christmas Tree stain and without stain, demonstrating that these results were independent of the presence of sperm cells.

LFI results for all samples were positive at the dilution 1:1,000. Previously published studies indicated that at this dilution, DNA profiling could be compromised. In regard to DNA analysis, from the dilution 1:2,000 onward, it was not possible to obtain quantifiable DNA to perform the STR analysis. In samples mixed with menstrual blood, PSA Semiquant showed positive results when tested up to 1 to 1,000. When using the sample pad of PSA immunochromatographic tests to extract DNA, it was possible to obtain both autosomal STR and Y-STR DNA profiles from positive tests, being able to produce full profiles from 1 part of semen diluted in 10 parts of menstrual blood, though profiles were not obtained from higher dilutions.

This research has demonstrated the importance of utilizing both detection of proteins (indicating possible presence of a body fluid) and DNA (identification of an individual) together as complementary techniques to detect semen when spermatozoa are not visible by microscopy or when microscopy equipment or expertise is not available. The variability of results could be related to different factors like medical conditions, age, and population affinity, among others. Future research aims to correlate the positivity of these tests at higher dilutions with DNA yield and to develop STR profiles and identify the Y chromosome for the presence/absence of male DNA to arrive at an unambiguous and powerful identification.

Bodily Fluid; PSA; Semenogelin

B33 Increased DNA Quantification Sensitivity: An Evaluation of Investigator Quantiplex Pro FLX

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Learning Objectives: Upon attending this presentation, attendees will gain an understanding of DNA quantification with increased sample input through use of lyophilized reaction reagents.

Impact Statement: This presentation will impact the forensic science community by introducing support for increased sample input to increase sensitivity in DNA quantification chemistries for screening purposes.

Abstract Text: DNA quantification is crucial for successful DNA profiling and provides key information about the nature of the evidence. With advancements in DNA quantification chemistries, detection of degradation, mixtures, and inhibition has become routine, requiring only 2µL of sample input for processing. However, as human identification testing has advanced to handle challenging samples such as low-template samples and complex mixtures, the need for increased sensitivity in quantification has emerged.

The research aimed to examine the potential for increased sensitivity using the Investigator Quantiplex Pro FLX kit. This kit features a novel lyophilized chemistry, allowing for an expanded sample target input range from 2µL to 18µL. The kit was evaluated with DNA concentrations as low as 0.0000625ng/µL. Additionally, DNA mixtures with high female-to-male ratios up to 1,000,000:1 and vaginal swabs spiked with semen were tested to determine sensitivity of male DNA detection. The spiked vaginal swabs were processed with the Investigator Casework GO! kit to test the efficacy of Y-screening and compatibility between the direct lysis chemistry of Investigator Casework GO! with the Investigator Quantiplex Pro FLX kit.

The results demonstrated the successful detection of DNA at the lowest concentration of 0.000625ng/µL. In high female-to-male ratio DNA mixed samples, male DNA was detected in all of the mixtures. For the vaginal swabs, male DNA was detected from swab cuttings as small as one-eighth of a swab spiked with semen diluted to an expected 0.0064ng of total DNA. In these spiked swabs, male DNA was detected even when female DNA was present at ratios higher than 19,000:1. These findings indicate that Investigator Quantiplex Pro FLX is a highly sensitive DNA quantification kit, suitable for a variety of forensic samples and workflows, including Y-screening commonly applied to sexual assault evidence. The increased sensitivity and expanded sample input range offer greater flexibility when working with low template DNA or extreme mixtures.

DNA Quantification; qPCR; DNA Mixture

B34 A Novel Method for the Identification of Source Tissue in Biological Mixtures

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Learning Objectives: After this presentation, attendees will be introduced to a new serological test that can detect saliva in single-source samples as well as in mixtures of bodily fluids. Attendees will also learn how variations in the fluorescence and morphological profiles of cells derived from separate tissues can indicate the source of bodily fluids for biological evidence.

Impact Statement: This work can impact the forensic community by allowing analysts to rapidly screen evidentiary samples for the presence of saliva and to determine if there is enough DNA present to obtain a profile based on the population of epithelial cells present. This will help laboratories predict which samples will have probative value before processing.

Abstract Text: Current serological tests often provide little information for trace samples and are usually destructive. Recently, flow cytometry has been proposed as an alternative approach for determining the source tissue of biological deposits. This uses the excitation of fluorescent molecules within cells to characterize their biochemical composition. Imaging Flow Cytometry (IFC) has an additional advantage by including a brightfield channel displaying cellular morphology, simultaneously allowing for the distinguishment of cell populations based on size, shape, and/or structural features. Therefore, this study aimed to develop and test a new flow cytometry-based screening approach for detecting the presence of saliva epithelial cells in samples approximating those encountered in DNA casework. This approach has several advantages for forensic laboratories. The process is non-destructive and can rapidly distinguish between cell populations derived from saliva, blood, vaginal, and epidermal tissue. Thus, IFC as a serological test could allow laboratories to decrease evidence backlogs and increase the probative value of single and mixture evidentiary biological samples.

For this study, dried swabs representing both single-source and mixture samples of blood and saliva were analyzed using imaging flow cytometry with a 20x objective for magnification. A gate was applied during the acquisition of cellular events to allow single cells to be collected and remove debris that would decrease the number of true events in the raw image files. Autofluorescence and morphological features were used to identify cell signatures that either presumptively detected or confirmed the presence and amount of saliva epithelial cells within each mock evidence sample. To ensure that this technique would be compatible with a typical DNA profiling workflow, portions of each dried swab underwent DNA extraction, quantification, and Short Tandem Repeat (STR) profiling so the saliva epithelial cell signatures could be correlated to the quantity and quality of DNA within the blood and saliva mixture.

Results showed that autofluorescence and morphological signatures from saliva epithelial cells could be easily distinguished from blood cell populations. Specifically, the contrast values for the saliva epithelial cell populations in the brightfield channel showed a two-fold increase over the contrast values from the blood cell populations. This signature was consistent for single-source samples as well as the blood and saliva mixtures. Additionally, differences were observed in the intensity of the autofluorescence at wavelengths between 550–650nm when comparing saliva and blood cell populations. Within this wavelength range, there was an approximately 15% increase in the intensity of saliva cells compared to blood cells. These signatures were then used to presumptively detect the presence of saliva epithelial cells in more than 25 different mock evidence samples containing mixtures of saliva and blood in ratios ranging between 1:1 and 1:20. Last, autofluorescent signatures were used to perform a front-end separation of contributor cell populations in saliva-blood mixture samples aged between one day to two weeks. Results indicated that front-end separation successfully enriched saliva and blood cell populations from mixture samples aged less than two days with contributor ratios of 1:2, 1:1, and 2:1.

Overall, these results suggest that imaging flow cytometry can quickly screen cell populations in evidentiary samples for the presence of saliva epithelial cells in mixtures of bodily fluids. Similarly, rapid detection of epithelial cell concentrations could allow forensic scientists to determine whether a sample contains enough DNA before initiating extraction. As a result, this method could enable laboratories to increase the probative value of biological samples representing either a single source or mixtures.

Forensic Science; Signature Identification; Fluorescence

B35 QIAxcel vs. qPCR: Evaluating Versatility for Forensic DNA Screening

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Learning Objectives: This presentation will compare the quantity and quality of DNA extracts predicted by the QIAxcel DNA High Sensitivity Kit and real-time Polymerase Chain Reaction (PCR) using Quantiplex Pro chemistry. The goal is to demonstrate the metrics provided by each chemistry for DNA extracts and their usefulness for downstream predictiveness.

Impact Statement: This study's findings on the QIAxcel's effectiveness in screening degraded DNA extracts prior to Capillary Electrophoresis (CE) or Next-Generation Sequencing (NGS) will help laboratories understand the added value of incorporating the QIAxcel. The data obtained from the QIAxcel could aid laboratories in their decision-making process when choosing between CE and NGS platforms. By providing valuable insights into the strengths and limitations of each technique, this research advances the ability of laboratories to streamline downstream processing of challenging casework.

Abstract Text: Real-time PCR (qPCR) has been the gold standard in forensic DNA for quantification since the 1990s, effectively predicting Short Tandem Repeat (STR) success and normalizing for STR-based methods. However, with forensic DNA capabilities evolving to include more NGS methods, such as Single Nucleotide Polymorphism (SNP) typing and mitochondrial DNA typing, qPCR may no longer consistently predict success, especially with degraded samples.

In this study, challenging samples were simulated by thermally degrading femur samples from two cadavers to four different levels of degradation. The Extra-Large Volume Bone Protocol on the EZ2 Connect Fx was used to extract DNA from the skeletal samples (n=5 replicates per degradation level). All DNA extracts were screened using standard qPCR with the Quantiplex Pro kit and fragment analysis on the QIAxcel using the QIAxcel DNA High Sensitivity kit to evaluate DNA quantity, degradation indices, and overall smear qualities. All extracts were then genotyped using the ForenSeq MainstAY Kit and Investigator 24plex QS Kit, with a subset of extracts genotyped using the ForenSeq mtDNA Whole Genome Kit and the ForenSeq Kintelligence Kit. All samples were normalized based on qPCR human target values. DNA quantity and degradation indices from both quantification chemistries were then compared with downstream success for all genotyping methods.

The data revealed that the total DNA concentration predicted by the QIAxcel correlated with the short target in qPCR. Both the QIAxcel and Quantiplex Pro were generally predictive of success with STR chemistries, MainstAY, and Investigator 24plex. However, the QIAxcel demonstrated greater predictive power for genotyping success with the mtDNA Whole Genome Kit compared to qPCR. Preliminary results with the ForenSeq Kintelligence Kit suggest that the QIAxcel may be a better screening tool for SNP genotyping success compared to qPCR.

Future studies will evaluate the sensitivity of the QIAxcel and explore additional sample types. Additionally, the QIAxcel DNA High Resolution Kit will be used to determine if it is a better predictor of downstream success than the QIAxcel DNA High Sensitivity Kit.

Forensic DNA; QIAxcel; DNA Quantification

B36 The Simultaneous Recovery of DNA, RNA, and Protein From Biological Samples for Forensic Applications Using a Novel Method

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Learning Objectives: Attendees will learn the value of analyzing multiple analytes from biological samples for solving crimes.

Impact Statement: There is growing interest in recovering other analytes such as RNA from trace biological samples. We have developed a novel method for the recovery of nucleic acids from biological samples while also keeping the proteins intact. Our method uses a simple, unified workflow, is amenable to automation, and is designed to be implemented in forensic testing laboratories after full validation.

Abstract Text: Due to increased sensitivity of commercial DNA profiling kits, analysis of trace amounts of DNA from biological samples is common in forensic testing laboratories. In addition to maximizing DNA recovery from trace biological samples, there is growing interest in recovering other analytes such as RNA from trace biological samples. Using mRNA markers for identification of body fluids is superior to traditional methods which lack specificity and consume limited biological material. mRNA can also help link a body fluid to a contributor's DNA profile through coding Single Nucleotide Polymorphisms (cSNPs). This is useful in establishing the link between the DNA profile obtained, the body fluid identified, and the events that occurred. When DNA is severely compromised, proteins can be successfully analyzed for body fluid identification as well as human individualization through Genetically Variant Peptides (GVPs).

This study aims to develop a highly efficient method for extracting nucleic acids from biological samples while also keeping the proteins intact. Using mild lysis non-destructive to proteins, we bind nucleic acids to a binding vector embedded on paramagnetic beads. With the nucleic acids bound, the protein-containing supernatant is collected and used for protein identification. The bound nucleic acids are then eluted from the beads and purified. Using trace amounts of blood, semen, and saliva, our results show that using our method, DNA and RNA recovery is comparable to a commercial bead-based kit. DNA was used to generate Short Tandem Repeat (STR) profiles and RNA was used to amplify body-fluid specific mRNA markers through cDNA synthesis, PCR amplification, and Capillary Electrophoresis (CE). Work is ongoing with supernatant fractions to identify proteins using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Our method represents the first multi-analyte recovery method for forensic application. It uses a simple, unified workflow, is amenable to automation, and is designed to be implemented in forensic testing laboratories after full validation.

Nucleic Acid Extraction; Bodily Fluid; Low DNA

B37 An In-Depth Look at DNA Casework on Fired Cartridge Casings Extracted Using a Modified Swabbing and Extraction Technique

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Learning Objectives: Attendees will be provided a summary of data regarding the analysis of Fired Cartridge Casings (FCCs) at the Illinois State Police (ISP). A brief description of our methods will be followed with graphs depicting submission data as well as result data from various angles that are applicable to day-to-day casework. Most importantly, we will highlight the success of our method and educate others on methods they can provide in their own labs.

Impact Statement: At many crime scenes, FCCs are the only evidence with a possible link to the offender(s). This poster presentation will show the success the ISP has achieved in obtaining DNA profiles from FCCs, a once thought near-impossible feat. We want to help educate more forensic scientists so their laboratory can also on-board this type of analysis.

Abstract Text: In 2022, the ISP validated and implemented a method for collecting cellular material from FCCs that was derived from a procedure developed by the Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATFE) Laboratory.[1] Generally, FCCs have not yielded enough cellular material of quality to generate DNA profiles that can be used for comparisons using traditional collection techniques. Often, there is no DNA detected at all. In the research conducted by the ATFE, it has been learned that the detrimental effects on the DNA may have more to do with the copper that comprises many FCCs than the heat from the fired gun. Therefore, minimizing the degradation effects of the copper is imperative.

ISP validated the rinse-and-swab method, derived from that of the ATFE, while utilizing the DNA IQ chemistry run on a Promega Maxwell instrument. ISP has modified the master mix to include Bovine Albumin Serum (BSA) and Gly-Gly-His (GGH) to counteract the effects of copper in hopes of providing a higher quantity and quality of recovered DNA. The FCCs are swabbed directly with this master mix and extracted immediately. The rinse-and-swab method was also part of a comparison study conducted by the Federal Bureau of Investigation Laboratory Division.[2]

The ISP has been utilizing this method on casework for over two years. Currently, an ongoing pilot program is in place evaluating FCC collection, laboratory case acceptance, workflow, and casework results. At this time, 441 cases have been completed statewide. Of these cases, 49% have provided results suitable for comparisons. In addition, 176 cases have profiles suitable for the Combined DNA Index System (CODIS) entry, which have resulted in 101 CODIS hits. Prior to onboarding this rinse-and-swab method, ISP rarely swabbed FCCs for DNA, opting to send them for latent print analysis. Because of this, we conclude this method of cellular collection on FCCs is a success.

Please note, statistics will be updated with more information for the poster presentation.

References:

1. Bille et al. An improved process for the collection and DNA analysis of fired cartridge cases. *Forensic Sci Int Genet*. 2020 May; 46:102238.
2. Elwick et al. Recovery of DNA from fired and unfired cartridge casings: Comparison of two DNA collection methods. *Forensic Sci Int Genet.* 2022 July; 59:102726.

Cartridge Casings; Forensic DNA; Touch Sample

B38 Streamlining Forensic Workflows: Field Test Results of Forensic Samples Using the HID NIMBUS Presto QNA System

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Learning Objectives: After attending this presentation, attendees will understand how the HID NIMBUS Presto QNA (HIDNP QNA) system increases throughput by automating purification, quantification, normalization, and amplification setup while maintaining results comparable to manual preparation methods.

Impact Statement: This presentation will impact the forensic science community by demonstrating a streamlined method for processing forensic samples from purification to amplification using the HIDNP QNA system while achieving reliable and consistent results.

Abstract Text: Incorporating automation into forensic laboratories is essential for increasing throughput and minimizing human error. While the HIDNP QNA has been previously validated for purification, its new capabilities for quantification, normalization, and amplification setup must be assessed and compared to manual methods. The software now supports the Quantifiler Trio kit for quantification and VeriFiler Plus, YFiler Plus, GlobalFiler, GlobalFiler IQC, and NGM Detect kits for amplification setup.

In this study, 29 forensic casework samples, including burned, buried, surface-decomposed bones, gum, hair, nail, blood on a cotton swab, and a dilution series were processed in duplicate (N = 58) through the HIDNP QNA system pipeline from purification to amplification setup. All samples were purified using the PrepFiler BTA Automated Protocol. Extracts processed with automation were quantified, normalized, and amplified in duplicate both on the instrument and manually to assess the reliability of the instrument. Extracts were quantified using the Quantifiler Trio kit on the ABI 7500 system and amplified with the GlobalFiler IQC kit. Amplified products were separated with a 3500 Genetic Analyzer, and data analysis was completed using Genemapper IDX v 1.6.

This study found similar DNA yields for quantification across between replicates. Additionally, all standard curve quality metrics from the instrument-prepared standards were within expected ranges, with r^2 values greater than 0.99, and slopes were within the acceptable range. The average autosomal allele recovery between automation and manual methods was consistent for most samples. Gum, hair, the dilution series, and all but one nail sample produced complete profiles for both methods. The average autosomal allele recovery from bones varied widely, from 4% to 100%, but results were comparable between automation and manual methods. Burned sampled yielded the highest recovery rates among the insult types. Blood on a cotton swab had an average autosomal allele recovery of 99% with the HIDNP QNA system versus 100% manually.

The time-saving capabilities of the HIDNP QNA system was evident: quantification took ~50 minutes manually versus ~30 minutes with automation, making the automated method 1.67 times faster. Similarly, normalization and amplification setup manually took ~1.5 hours, compared to ~35 minutes with the HIDNP QNA instrument, resulting in a 2.56-fold faster process. Overall, this study shows that the HIDNP QNA system can provide a streamlined, automated approach for purification, quantification, normalization, and amplification setup without compromising results for forensic samples.

Automation; STR Profiles; Forensic DNA

B39 The Simultaneous Determination of Time-Since-Deposition and Source Tissue for Forensic Biological Samples

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Learning Objectives: Attendees will gain an understanding of the use of morphological and autofluorescence characteristics measured by flow cytometry to provide simultaneous information for Time-Since-Deposition (TSD) and tissue source of forensically relevant biological samples.

Impact Statement: This research will impact the forensic science community by utilizing an analytical technique such as flow cytometry to provide important contextual information for DNA profiles developed from mock evidentiary samples. The ability to identify tissue source, associate that source with a DNA profile, and determine how long ago it was deposited could drastically increase the probative value of DNA profiles.

Abstract Text: Determination of tissue source and the time at which a biological material is transferred from its source to another surface, known as the time-since-deposition, would assist forensic analysts by providing crucial context for DNA profiles generated from biological evidence. Many methods for cell identification have been explored with mostly a focus on biomolecule markers such as mRNA and miRNA. Forensic laboratories have also developed methods for TSD estimation that focus on degradation of biomolecules. However, these methods have not been validated for use in a forensic science laboratory due to high false positive rates, limited application to different biological fluids, and the destructive nature of many existing methods. Flow cytometry is an analytical technique that is used to measure autofluorescence and structural properties of cells in a non-destructive and high-throughput manner. Past studies have utilized flow cytometry to identify tissue source and determine TSD; however, these signatures were always tested in separate experimental efforts and sets of samples, which may not be feasible to implement in the operational workflow of a forensic laboratory.

Therefore, the goal of this research was to develop a single laboratory technique for determining TSD and source tissue for an unknown biological sample. Initial experiments focused on blood samples that ranged in concentration from neat to 1:1,000 and varied in TSDs between 1 day and 6 months. Blood samples from three donors were deposited in quadruplicates onto microscope slides (two separate stains per slide) and air dried at ambient conditions. One set of stains was used as a reference sample. The other set of stains was used to perform Red Blood Cell (RBC) lysis to analyze White Blood Cells (WBCs). To separate the WBCs from the whole blood sample, RBCs were lysed using Ammonium-Chloride-Potassium (ACK) Lysing Buffer prior to analysis on the flow cytometer. A second method that did not utilize a WBC separation technique was also used by creating a gate based on events that were measured after running whole blood samples and data found in literature to separate WBCs from RBCs and platelets present in the whole blood sample. To test whether this technique could also be used in mixed tissue samples, blood/saliva mixtures from six donors were deposited in duplicates onto microscope slides and air dried at ambient conditions. After the designated time periods ranging from T = 0 days to T = 120 days, samples were collected using a sterile cotton swab moistened with deionized water and air-dried. Sampled were filtered through a 100µm mesh filter and analyzed using a flow cytometer equipped with 488nm excitation laser.

Results showed that autofluorescence profiles of blood cell populations showed incremental changes within the first two weeks (e.g., median autofluorescence in wavelengths between 535nm and 600nm increased from ~2,200 RFU at one day to ~2,500 RFU at 14 days). Samples that had been aged for more than two months showed a 40% decrease in median autofluorescence (~400RFU), which was consistent across each contributor tested. Results also showed that Forward Scatter (FSC) and Side Scatter (SSC) profiles collected from the same flow cytometry dataset were able to differentiate blood cells from saliva cells. Autofluorescence in wavelengths between 650nm and 700nm was capable of further discriminating between the two cell sources. These measurements were successful in detecting the presence of blood cells in a sample even when saliva was present in ratios between 1:5 and 5:1. The observed trends show promise for the development and implementation of flow cytometry as a method for TSD determination and cell identification in forensic laboratories.

Flow Cytometry; Cell Identification; Time-Since-Deposition

B40 Modified Extraction Protocols for Nuclear DNA Extraction From Human Hair Shafts

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Learning Objectives: This presentation will inform the audience about the progress made by our group in extracting nuclear DNA from hair shafts and generating a Short Tandem Repeat (STR) profile suitable for human identification.

Impact Statement: Results from this project will impact the forensic community in cases where the only evidence available are hair shafts.

Abstract Text: Hair strands are commonly collected as forensic evidence at a crime scene. Although hair shafts contain both nuclear and mitochondrial DNA, nuclear DNA markers are rarely used to genetically identify individuals. This is largely because the DNA in hair shafts is typically of low quantity and degraded, which makes generating an STR profile difficult and often unsuccessful. Therefore, developing and optimizing a method of DNA extraction that is more effective at recovering the small quantities of DNA and significantly degraded DNA present in hair shafts is critical to be able to successfully analyze hair forensic evidence. The goal of this research project was to optimize a DNA extraction method that would enable generating STR profiles from human hair shafts. To extract DNA from hair shafts, two DNA extraction methods were compared: the first one based on a modified protocol that uses the Qiagen QIAamp DNA Investigator Kit and the second one based on a modified protocol that uses the Biochain cfPure cfDNA Extraction Kit. Additional modifications were tested, such as type of lysis buffer used, altering the ethanol washes and centrifugation steps, and attempting a combined Qiagen-Biochain method. Most of the samples yielded very low amounts of DNA, less than 100pg per 5cm of hair, with high degradation indices. DNA recovered from both extraction methods did not inhibit subsequent Polymerase Chain Reaction (PCR) reactions.

The methods were tested on a variety of hair shafts from different donors and the resulting DNA quantities and STR profiles were compared. To date, hair shafts from 14 individuals have been tested, all of which were samples collected after The George Washington University-Institutional Review Board (GW-IRB) approval. Following extraction, samples were quantified with the Quantifiler HP DNA Quantification Kit (Thermo Fisher Scientific) and amplified with the GlobalFiler STR Amplification Kit (Thermo Fisher Scientific) to generate an STR profile on a 3500 Genetic Analyzer (Thermo Fisher Scientific). The DNA quantities and completeness of STR profiles varied from donor to donor, ranging from no STR profile to a profile two loci short of being a complete STR profile. However, results from the same donor were similar with regard to quality and quantity>

In our hands, both modified extraction methods are an improvement over methods that were previously tested in our lab with regard to DNA yield and quality. Thus far, the results from attempts at further optimizing these modified protocols are comparable and the modifications do not seem to greatly affect DNA quantity or STR profile quality from the same donor. There is great variation in the DNA quantity and completeness of a STR profile from donor to donor, yet 50% of the donors tested yielded STR alleles, showing the promise of generating partial profiles from hair shaft DNA. The cause of this subject-to-subject variation is yet to be determined and may be a result of differential levels of degradation in hair shaft DNA between individuals. Further efforts to optimize these protocols are ongoing with the goal of correlating morphological features of the hair to the likelihood of obtaining an STR profile of sufficient quality for identification.

Hair Shaft; STRs; Degraded DNA

B41 Direct to DNA: Revolutionizing Forensic Analysis by Streamlining the Process

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Learning Objectives: In this presentation, attendees will learn about the advantages of adopting a direct-to-DNA approach in the forensic laboratory. They will also assess the implementation process and review the impact of its use in a forensic laboratory over the past few years.

Impact Statement: Exploring new advancements in the forensic field is essential to improving crime-solving techniques and transforming forensic laboratory workflows. The direct-to-DNA approach increases efficiency by accelerating the screening process. In consequence, the adoption of this approach can assist in reducing forensic laboratory backlogs.

Abstract Text: Forensic evidence analysis conventionally involves multiple steps, evidently resulting in an overall time-consuming process. The first stage in a forensic DNA laboratory is evidence screening and sample preparation, where items of evidence are visually examined, and serological testing is performed on any suspected biological material. Alternate Light Sources (ALS) can also be utilized to assist in identifying the most suitable samples for DNA testing.

Identifying specific biological materials is typically done to name the potential sources of DNA before the extraction, quantification, and amplification steps of the genetic material. Examples of some of these sources are blood, semen, and saliva. Serological testing demands a substantial amount of hands-on time from an analyst.[1] This can be attributed to the need for both presumptive and confirmatory tests for evidence staining being labeled as suspected biological fluids. Serological testing also involves consuming more of an evidentiary sample when compared to the alternative—cutting only one portion of a sample and forwarding it directly to DNA testing, also known as the direct-to-DNA approach.

In the direct-to-DNA workflow, DNA analysis is performed without testing the evidence for possible biological material during the initial screening process, thus optimizing the chances of obtaining timely Combined DNA Index System (CODIS) -eligible DNA profiles.[2] In the direct-to-DNA approach, a cutting from a substrate, whether it be a swab or a piece of material, is directly forwarded for DNA analysis without identifying the biological material or the source of the DNA. This approach streamlines the forensic evidence processing workflow while consequently offering numerous benefits, including increased efficiency and improved reliability. The direct-to-DNA approach bypasses these traditional serological testing procedures and allows evidence to be moved directly into DNA analysis.[2]

This presentation will examine how the direct-to-DNA process significantly streamlines the forensic evidence screening workflow by comparing it to the traditional methods that involve serological testing. Though the direct-to-DNA approach is most often implemented during the screening of Sexual Assault Kits (SAKs), this presentation will also explore how the workflow can be expanded to be used on evidence of various cases, increasing automation in the laboratory and ultimately reducing backlogs. The benefits of the process along with the drawbacks were recognized through implementation and will also be further discussed.

References:

1. Sexual Assault Kit Initiative. (n.d.). *Improving laboratory efficiency: A direct-to-DNA approach*. https://www.sakitta.org/toolkit/docs/Improving-Laboratory-Efficiency-A-Direct-to-DNA-Approach-for-Testing-Sexual-Assault-Kits.pdf.
2. National Institute of Justice. (2017). *National Best Practices for Sexual Assault Kits*. https://www.ojp.gov/pdffiles1/nij/250384.pdf.

Direct-to-DNA; Backlogs; Streamlining

B42 Rapid DNA—Friend or Foe? Comparing Rapid DNA Analysis With Conventional Methodologies for Sexual Assault and Identification Cases

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Learning Objectives: This presentation will inform attendees on (1) Rapid DNA vs. Conventional Analysis; (2) Rapid DNA for Identification Cases: Improved methods for the pre-treatment for fresh, moderately degraded and highly degraded bones and teeth, including a comparison of Rapid DNA pre-treatment vs. conventional extraction method, (timeframe for fresh, moderately degraded and highly degraded bones or teeth), improved methods during Rapid DNA pre-treatment designed for a more efficient DNA recovery; (3) Rapid DNA for sexual assault cases: a validated method for evidence samples from sexual assault safe kits, a comparison of Rapid DNA pre-treatment vs. the conventional extraction method (criteria for Rapid DNA in sexual assault cases and timeframe), and a discussion of pre-treatment for separation of spermatic fraction and epithelial fraction from the evidence samples; and (4) advantages and disadvantages of Rapid DNA in sexual assault cases and identification cases

Impact Statement: Today, crime laboratories, especially DNA laboratories, have been confronting multiple challenges, including backlog of sexual assault kits, the encounter of many low-quality samples, and too many identification cases. This presentation will show some of the advancements we have made using Rapid DNA and how we can use this knowledge in the future to obtain great results in a shorter amount of time.

Abstract Text: The lack of techniques that could improve the recovery of genetic material has prevented analysts from obtaining results in a short amount of time. However, it is important to investigate alternative extraction methods and technologies that can be evaluated prior to receiving evidence in a crime laboratory, allocating time and resources to improve the analysis of evidentiary samples.

The DNA laboratory from the Institute of Forensic Sciences of Puerto Rico conducted a validation study to develop interpretation guidelines with Rapid DNA to evaluate evidence samples from sexual assault and identification cases. In this study, we compared the workflow of conventional analysis with Rapid DNA analysis for both sexual assault and identification cases. Also, we discussed the improvements we have made to the Rapid DNA pre-treatment of fresh, moderately degraded, and highly degraded bones and teeth for identification cases, and the Rapid DNA pre-treatment made to evidence samples for sexual assault cases. Finally, we reviewed the advantages and disadvantages of Rapid DNA for both identification and sexual assault cases.

For identification cases, we found that for fresh and moderately degraded bones, we obtained a full interpretable genetic profile while for degraded bones, we obtained a partial profile but for a fraction of the time compared to conventional analysis. In the case for sexual assault samples, we obtained a highly efficient separation of the spermatic fraction and epithelial fraction from the samples, but Rapid DNA has many limitations that could be considered currently a disadvantage to use for sexual assault cases. The use of Rapid DNA for the analysis of evidence samples from sexual assault cases is not yet federally approved since it lacks a quantitation step in accordance with the Quality Assurance Standards for Forensic Testing Laboratories (QAS). But once approved, it can be considered as an advantage for sexual assault cases.

Rapid DNA; Victim Identification; Sexual Crimes

B43 The Impact of Various Substrates and Deposition Time on Bacteria Associated With Saliva Samples and Its Implication on the Accuracy of a Bacterial Signature-Based Body Fluid Identification Method

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Learning Objectives: This research will inform the audience about how different substrates, including fabric and non-fabric materials with smooth, rough, and porous surfaces, affect the interaction and retention of biological fluids like saliva. It will also explain how these factors influence bacterial signatures and the determination of Time since Deposition (ToD).

Impact Statement: Advancing methodologies: The findings will provide valuable information on how various materials influence bacterial signatures, leading to the development of more effective forensic methodologies and tools. Improving forensic accuracy: by examining how different substrates affect the microbial composition of saliva and tracking bacterial changes over time, this research enhances the accuracy of bacterial-signature-based body fluid identification methods. Enhancing ToD determination: insights gained from this study will refine techniques for determining the time since deposition, improving the precision of forensic analysis, and contributing to more reliable data

Abstract Text: The presence of a person's DNA at a crime scene does not necessarily indicate that the sample donor is the perpetrator; thus, establishing the time frame during which the biological stain was deposited is critical for solving a case. The type of substrate on which biological evidence is found significantly impacts the microbiome of body fluids, as substrates differ in absorbency, porosity, and chemical composition. These factors influence how biological fluids like saliva interact with and are retained by the substrate. For example, smooth, non-porous surfaces may cause fluid pooling or dispersion, while rough, porous surfaces can either trap fluids or accelerate absorption. By understanding how substrates affect the microbial composition of human saliva, we can improve bacterial signature-based body fluid identification methods. Additionally, tracking bacterial changes over time on various substrates helps in determining the ToD, which is valuable for forensic analysis.

This study aims to assess the impact of various clothing and non-clothing substrates on the microbiomes of human saliva and their implications for bacterial signature-based methods. It also evaluates how storage time and substrate type affect these microbiomes and their accuracy in determining the ToD. Saliva samples were collected at four time points (week 0 [control], week 1, week 4, and week 8) from both male (n=5) and female (n=5) donors. Fabric cuttings (denim-cotton, silk, linen, wool) and sterile cotton swabs from non-clothing substrates (wooden flooring, bathroom tiles) were used. Bacterial DNA was extracted with the Qiagen DNA Investigator kit, quantified with the Qubit 2.0 Fluorometric Quantification method, and the variable region four (V4) of the 16S rDNA was amplified via Polymerase Chain Reaction (PCR). Results were visualized on a 1.6% agarose gel, then each sample was cleaned using Agincourt AMPure XP.

Preliminary results showed consistently higher average DNA yield in control samples (Week 0) compared to Week 1 treatment samples. As expected, the substrate control and reagent blanks from control samples didn't result in any detectable DNA. Conversely, the positive controls demonstrated the highest DNA concentrations, averaging 25.95ng/uL. Following the positive controls, denim cotton, wooden tiles, bathroom tiles, and wool exhibited average DNA concentrations of 21.57ng/uL, 13.02ng/uL, 10.59ng/uL, and 9.77ng/uL, respectively. In contrast, silk and linen had lower average DNA yields, measuring 0.00525ng/uL and 4.46ng/uL, respectively. The 16S rDNA high throughput sequencing (currently in progress) will provide crucial insights into the effects of different substrates on the human saliva microbiome structure and its impact on accuracy of bacterial signature-based body fluid identification method.

In conclusion, results from this research will help forensic scientists to have a better understanding on how various substrate materials influence bacterial signature-based body fluid identification method and in the determination of ToD. Ultimately, these findings will refine forensic methodologies, leading to more accurate biological evidence interpretations and enhancing the fairness of criminal investigations.

Forensic Analysis; Bodily Fluid; DNA

B44 Enhancing Success Rates in Sexual Assault Investigations With the SpermX Differential Extraction System

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Learning Objectives: Attendees of this presentation will learn about new technology that significantly improves the success rates of obtaining probative DNA profiles from perpetrators when testing Sexual Assault Kits (SAKs).

Impact Statement: The presentation will make forensic DNA laboratories aware of a superior differential lysis technology that results in obtaining male profiles from a higher number of sexual assault samples.

Abstract Text: Currently, most forensic laboratories obtain a Combined DNA Index System (CODIS) -eligible DNA profile from the perpetrator in approximately 40% of SAKs tested, despite up to 70% screening positive for foreign DNA.[1] One primary cause for this gap is the inefficiency of current differential extraction methods, which recover only 5–20% of the total sperm available in SAK samples.

Differential extraction is a specialized process used in forensic science to separate sperm cells from non-sperm cells in mixed biological samples, such as those collected in SAKs. This technique is critical for isolating male DNA from female DNA in cases of sexual assault, allowing for the identification of the perpetrator. However, traditional differential extraction methods often fall short in recovering sufficient sperm cells, leading to lower success rates in obtaining usable DNA profiles.

A novel differential extraction method known as SpermX utilizes a nanofiber membrane to separate sperm from non-sperm cells by capturing the sperm cells in the nanofiber membrane while the epithelial cells are lysed and flow through the membrane.[2] This technology has demonstrated the ability to recover six times more sperm than standard differential extraction methods. This presentation will evaluate the impact of SpermX on success rates in real-world SAK cases. By increasing the success rate of male DNA profile recovery, this technology can significantly enhance the return on investment in SAK testing.

The presentation will feature compelling data comparing the performance of SpermX to laboratory's current differential extraction method. These data show a significant improvement in DNA profile recovery rates, with SpermX achieving higher yields of male DNA. This improvement is particularly noteworthy in samples with low initial quantities of sperm where traditional methods often fail to produce a usable male profile. Attendees will gain insights into how implementing SpermX could impact their laboratory operations, enhance case resolution rate, and potentially lead to more successful prosecutions and justice for victims.

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Differential Extraction; Male DNA; SpermX

B45 Expanding On-Site Crime Scene Reconstruction Capabilities by Uncovering Microscopic Clues Using Mobile Laser-Induced Breakdown Spectroscopy (LIBS)

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Learning Objectives: By attending this presentation, attendees will gain an understanding of the advanced capabilities of innovative mobile LIBS technology, which uses enhanced imaging and sensitive elemental analysis for real-time results. This tool can be utilized onsite, enabling a rapid and thorough approach to trace evidence examination. It demonstrates high utility in crime scene reconstruction, case/evidence prioritization, and the improvement in investigative efficiency.

Impact Statement: This study will impact the forensic science community by demonstrating the proficiency of mobile crime scene methods and their ease of operation. With high accuracy and reliability, the full elemental characterization of a microscopic residue can be achieved onsite within two minutes. The advantages of adopting such methods promote a triage of investigative information and better prioritization of evidence collection and submission to the laboratory.

Abstract Text: Firearm-related violence encompasses a broad range of complex scenarios that each demand special investigative efforts. Trace evidence plays a crucial role as microscopic residues often transfer between surfaces during a shooting incident and other physical interactions. Identifying residue transfer helps investigators link suspects to the crime scene or to other individuals, supporting crime scene reconstruction. To fully utilize this information, the analysis and interpretation of evidence must be conducted efficiently to support subsequent investigative decision-making. Current protocols involve more time-consuming and costly methods at the laboratory, leading to longer turnaround times for evidence and decreased productivity in investigations. This study examines the effectiveness of mobile instrumentation that can be utilized at crime scenes for quick, thorough assessments of trace evidence.

Mobile LIBS technology provides novel capabilities for enhanced imaging, rapid single-particle targeted ablation, sensitive elemental analysis, and mobility to the crime scene. The instrument allows for a more comprehensive approach in a fraction of the time, making it an advantageous tool for crime scene reconstruction. Previous studies have demonstrated the utility of the mobile LIBS in identifying Gunshot Residue (GSR) with high accuracy (>98%) from hand and substrate samples through elemental and morphological characterization.[1,2] In this study, the versatility of the mobile LIBS is expanded beyond GSR to include various target materials, bullets, and cartridge case residues. Seven substrates (wood, drywall, glass, paint, concrete, and several automotive parts) were fired at using three types of bullets (Full Metal Jacket [FMJ], Jacketed Hollow Point [JHP], and Lead Round Nose [LRN]), resulting in either perforation or ricochet. Over 400 samples were collected from the shooter's hands, substrates, and recovered bullets using an adhesive collection stub, allowing for a comprehensive assessment of transfer. Composition controls from each substrate were also collected to determine the true microscopic features and elemental profiles of each substrate.

Preliminary microscopic examination revealed the transfer of residues from the substrate to the shooter's hands in many experiments. Additionally, residues from the substrates were discovered on recovered bullets and extracted for direct mobile LIBS analysis. Distinct results based on bullet type were evident as FMJ and LRN bullets held on to less trace material but exhibited a greater abundance of transfer onto the shooter's hands. In contrast, JHP bullet experiments showed less transfer to the shooter's hands but more retention in the bullet itself. Mobile LIBS analysis provided further morphological (color, size, shape) and elemental characterization of the GSR and non-GSR traces. By comparing spectral data from known composition controls to the questioned residues, the transfer of trace evidence was further confirmed. Thus, analytical confidence has been proven on hands and bullet samples for other traces that are inadvertently collected along with the GSR. This finding allows for stronger links to be made between people of interest and evidence from the crime scene.

This study demonstrates how the transfer of trace residues differs in collection surfaces, bullet types, and bullet-substrate interactions in shooting events and highlights the utility of mobile LIBS technology for fast identification of evidence. Its enhanced capabilities offer on-site convenience for assessing a wide range of microscopic traces that would otherwise go unnoticed or require lengthy laboratory analyses. The mobile LIBS enables the rapid and reliable acquisition of information, which can then be obtained and utilized to support efficient evidence prioritization and investigative decision-making processes.

References:

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2. C. Vander Pyl, K. Morris, L. Arroyo, T. Trejos, Assessing the utility of LIBS in the reconstruction of firearm related incidents, *Forensic Chem.* 19 (2020) 100251. https://doi.org/10.1016/j.forc.2020.100251.

Mobile Device; Trace Analysis; Gun Shot Residue

B46 Analysis of Inorganic Oxidizing Salts in Intact and Chemically Reacted Consumer Fireworks Using Microscopical and Spectroscopic Approaches

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Learning Objectives: After attending this presentation, attendees will gain a compositional knowledge of intact and chemically reacted consumer fireworks that can be used as explosive charge in Improvised Explosive Devices (IEDs), with a focus on identifying the inorganic oxidizing salts they contain. The audience will learn about the strengths and limitations of the microscopic and spectroscopic techniques used.

Impact Statement: This presentation will impact the forensic science community with emphasis to the trace evidence community by demonstrating the efficiency of an analytical scheme that integrates light microscopy, micro-Raman spectroscopy, Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) spectroscopy, and canning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) to identify the inorganic oxidizing salts present in both intact fireworks and the particles and residues resulting from the chemically reacted fireworks.

Abstract Text: Cases such as the Boston marathon bombing (2013) and the increased use of inorganic explosive mixtures, including consumer fireworks, in IEDs have led to the need to better understand their composition to assist practitioners in their rapid analysis and identification. This study aims to demonstrate an analytical scheme integrating light microscopy, micro-Raman spectroscopy, ATR/FTIR spectroscopy, and SEM/EDS that forensic investigators can utilize to identify and discriminate the inorganic oxidizing salts in the intact and chemically reacted consumer fireworks.

Twelve consumer firework samples and six inorganic oxidizers reference samples were analyzed in this study. The firework samples included artillery, firecrackers (ladyfingers), Roman candles, and fountains across three different brands for each type. For the chemically reacted samples, triplicates of each firework were ignited, and the remaining particles and residues were collected. A decantation method was used to separate the inorganic oxidizers in the intact samples before their recrystallization and a wet swabbing technique was used to collect and subsequently recrystallize inorganic oxidizers on glass slides in the chemically reacted samples. First, the intact and chemically reacted samples were investigated by microscopical means to preliminarily identify the inorganic oxidizers present, followed by micro-Raman analysis to confirm their identity. Individual crystals were located using the Raman microscope to facilitate targeted spectral acquisitions. For ATR/FTIR and SEM/EDS analyses, the intact fireworks were cut open and crushed into a fine powder. For the chemically reacted samples, residues were directly sampled or scraped from the firework remnants.

Following microscopical examinations, potassium perchlorate (KClO4) was identified as the most common inorganic oxidizer in both the recrystallized intact and chemically reacted firework material. Microscopy also allowed the identification of four types of inorganic oxidizers in the recrystallized chemically reacted samples, potassium perchlorate (KClO4), potassium nitrate (KNO3), barium nitrate (BaNO3)2, and potassium chlorate (KClO3). A lower amount of oxidizers present in the recrystallized chemically reacted samples was observed as well as unhindered growths of crystals, which rendered the identification of inorganic oxidizers difficult. Euhedral crystals with well-defined, symmetrical shapes and sharp, flat faces were first formed and identified using microscopy before being absorbed by larger anhedral crystals with no well-formed faces, preventing the localization of individual crystals to collect the Raman measurements. Using ATR/FTIR, it was determined that artillery fireworks contained nitrate salts while fountain fireworks contained mixtures with both nitrate and perchlorate salts. Using SEM/EDS, it was determined that while intact and chemically reacted fireworks shared many common elements such as magnesium, aluminum, silicon, chlorine and potassium, other elements were only found with intact fireworks. Those included fluorine and barium whereas titanium was only found in one chemically reacted fountain sample.

Microscopy; Spectroscopy; Trace Analysis

B47 An Artificial Intelligence-Based Detection of Ignitable Liquid Residues in Fire Debris Using a Deep Convolutional Neural Network

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Learning Objectives: The attendees will understand how to develop an Artificial Intelligence (AI) workflow through a transfer learning technique to classify Gas Chromatography/Mass Spectrometry (GC/MS) data that can be applied in forensic analyses. The attendees will also learn the operability of deep Convolutional Neural Networks (CNN) to classify untrained class data, which is the practical problem of forensic evidence.

Impact Statement: AI is transforming forensic science, providing an asset to analysts. The impact of this study is to present the potential of scalogram representations to avoid retention time alignment issues that are commonly encountered in chromatographic data. The combination of scalograms and a CNN offers a promising inter-laboratory applicability to accurately detect Ignitable Liquids (ILs) residues in fire debris samples.

Abstract Text: Due to the complex chemical composition of ILs and the noteworthy matrix interferences, the interpretation of GC/MS data to detect the presence of IL residues in fire debris samples is challenging.[1] Traditionally, chemometric tools are used to process and extract analytical signals based on mathematical algorithms to assist in interpreting sample attributes.[2] The application of AI into the fields of science has been emerging and revolutionizing, and it is expected to show promise in forensic fire debris analysis. CNNs have been one of the remarkable AI algorithms for solving image classification tasks for the past few years.[3] Transfer learning is to re-train an existing CNN and leverage knowledge to another task, with noticeable performance in an efficient training fashion.[4] This work aimed to propose an AI-based workflow via transfer learning with a CNN to classify GC/MS data and detect IL residues from synthetic sample matrices. It was also hypothesized that the proposed AI workflow could be applied to analyze inter-laboratory data without retention time alignment, an unavoidable issue in chemometric analysis.

In this work, four brands of regular gasoline collected from local gas stations in Huntsville, TX, and nylon 6,6 carpet were chosen as the investigated IL and matrix, respectively. Nine levels of concentrations (0.4-100µg/20-mL headspace vial) were made from two-fold serial dilutions of stock solutions to prepare neat IL and simulated fire debris samples. All samples were analyzed by Headspace/Solid-Phase Microextraction coupling with GC/MS (HS/SPME-GC/MS). Summed Ion Mass spectra (SIM) were produced by extracting *m/z* 55–156 range and retention times between 3 and 13 minutes in each GC/MS datum, followed by a conversion procedure to generate scalograms. Finally, a pre-trained CNN, ResNet-50, was fine-tuned on 390 data for a new task (i.e., classifying the scalograms into "positive of gasoline" or "negative of gasoline")[5]

Results showed that the ResNet-50 was re-trained to detect gasoline residues with 100% validation accuracy. By being tested with new sets of intra-laboratory data, the model obtained 100% accuracy in detecting neat gasoline residues across all concentrations. The limit of detection of the model to detect simulated fire debris samples was 25µg/20-mL headspace vial with 91 ± 1 % accuracy. Regarding inter-laboratory data (collected from the National Center for Forensic Science), the model acquired 88 ± 1 % and 84 ± 1 % accuracy in correctly detecting neat gasoline and simulated fire debris samples, individually.[6-8]

In conclusion, an AI workflow was developed to yield an intelligent model for detecting gasoline residues from fire debris samples. The development of the model utilized a small training data set, enabling its application into limited data that are available in real fire case scenarios. Additionally, the model could predict samples outside of the training distribution or tested by different laboratories without aligning the retention time shift. The implication of this work was to demonstrate an AI-based, cost-effective, and reliable system to facilitate IL analysis. The proposed AI workflow can also be adopted by analyses of other types of forensic evidence.

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Artificial Intelligence; GC/MS; Fire Debris Analysis

B48 The Detection of Organic Explosive Residues From Outdoor Explosions Using Confocal Raman Microscopy

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Learning Objectives: After attending this presentation, attendees will acquire an understanding of the strengths and limitations of confocal Raman microscopy to identify organic explosive residues from controlled explosions on a variety of substrates.

Impact Statement: This presentation will impact the forensic science community by demonstrating a workflow involving a simple liquid extraction for the rapid and accurate identification of smokeless powder constituents, TNT, and RDX post-blast explosive residues using confocal Raman microscopy.

Abstract Text: Following an explosion involving an Improvised Explosive Device (IED), accurately identifying its explosive charge in post-blast debris is a crucial step that can significantly benefit subsequent investigations. In particular, IEDs utilizing smokeless powders have become more prevalent due to their ease of acquisition. Additionally, military grade explosives such as TNT or RDX bombs are often recovered from war zones by criminal elements. A majority of studies on explosive detection involve the analysis of intact explosives or post-blast debris containing partially burned grains of explosive compounds. This study aims to employ confocal Raman microscopy as a means to identify organic explosive residues recovered from post-blast debris of simulated IEDs without the presence of grains or particles.

With the help of the Montgomery County Fire Marshal's Office (MCFMO), four IEDs were constructed (two utilizing a smokeless powder and two utilizing a TNT/RDX mixture). The IEDs were placed into polypropylene toolboxes along with electrical tape, a cell phone, wooden paint sticks, copper wire, nuts and bolts, and batteries to serve as a variety of substrates suitable for sampling as well as mimic components commonly found in IEDs. The post-blast debris of the substrates were sampled with pre-wetted acetone swabs, taking care to avoid collection of any unburned particles. The swabs were then soaked in acetone for three minutes to extract the analytes. A 10µL drop of the extract was then placed onto a steel microscope slide and allowed to dry before analysis with the confocal Raman. Additionally, a standard reference mixture containing 50ppm each of ethyl centralite, dibutyl phthalate, diphenylamine, and nitroglycerin in acetone was prepared to identify characteristic bands associated with each constituent as well as assess any peak shifts in these bands when compared to the spectra obtained from the swab extracts. Raman spectra from the post-blast substrates were compared to spectra obtained from the 50ppm standard mixture. From the smokeless powder IEDs, constituents such as ethyl centralite (deterrent), dibutyl phthalate (plasticizer), diphenylamine (stabilizer), and nitroglycerin (explosive) were identified on all of the post-blast substrates sampled except for the wooden paint sticks. While detection of TNT and RDX was unsuccessful in one detonation, residues for TNT were successfully identified in the post-blast debris of the second IED. Additionally, crystals were observed in the dried extracts from the TNT/RDX swabs, which displayed characteristic peaks for RDX when analyzed with the confocal Raman microscope.

This study demonstrates the benefits and limitations of a developed methodology using a confocal Raman microscope for the analysis of organic explosive residues, as well as provides a methodological framework for practitioners for post-blast debris sampling and subsequent analysis using confocal Raman microscopy when no unburned or partially burned particles are present.

Raman Microscopy; Explosion; Trace Analysis

B49 The Application of a Hand-Held Raman Spectrometer for the Classification of Synthetic- and Petroleum-Based Hydrocarbon Fluids

Javier E. Hodges, BS, Sam Houston State University, Huntsville, TX; Kailee R. Marchand, BS, Sam Houston State University , Fairfield, TX; Jorn Yu, PhD, Sam Houston State University, Huntsville, TX*

Learning Objectives: The attendees will learn a rapid and effective analytical method to distinguish synthetic-based and petroleum-based hydrocarbon fluid products using a hand-held Raman spectrometer. The technique demonstrates the potential use of Raman spectroscopy for source tracing of physical evidence.

Impact Statement: Raman spectroscopy provides great discrimination power for chemical analysis of physical evidence. In some cases, Raman spectroscopy can be applied for sample classification and quality control. The forensic science community will benefit from this presentation by becoming aware of this recent development toward utilizing Raman spectroscopy data for the field analysis of evidence.

Abstract Text: Hand-held Raman spectrometers offer a rapid and field-deployable platform for chemical tests of physical evidence. In the case of testing pure chemicals, identification can be achieved by comparing the unknown spectrum to that collected in the Raman spectrum database. In the case of mixture analysis, the chemical signatures of major components may be detected. In this work, we investigated the capability of Raman spectroscopy for hydrocarbon fluid sample classification.

Hydrocarbon fluids are very common in our daily lives. They are used for heating, cooking, drying, transportation, and as engine fuel. These materials can be made using petroleum-based or synthetic-based manufacturing processes. While petroleum-based hydrocarbon fluids are refined, synthetic-based hydrocarbon fluids are manufactured by organic synthesis, enabling these products to achieve higher performance. Overall, synthetic-based hydrocarbon fluids are chemically engineered for a specific molecular composition with a more tailored and uniform chemical structure. To differentiate hydrocarbon fluids with different manufacturing processes, expensive and time-consuming chemical separation and identification analyses are required. This work analyzed hydrocarbon fluids with different manufacturing processes with a hand-held Raman spectrometer.

A total of six different sources of hydrocarbon fluids were tested in this study. Two of the fluids were petroleum-based, and four of them were synthetic-based. These hydrocarbon fluids are formulated and manufactured as coolants and hydraulic fluids. Each fluid was sampled into seven aliquots in 2-mL vials. Each aliquot was tested ten times using a hand-held Raman spectrometer (HandyRam). The HandyRam was equipped with a 785nm laser. Raman spectra were all collected with autointegration. No data post-treatment was performed.

After side-by-side visual comparisons of Raman spectra between two types of hydrocarbon fluids, it was found that both petroleum-based and synthetic-based hydrocarbon fluids showed a typical predominate Raman scattering peak at 1,450cm-1, likely from a CH2 bending vibration. However, the synthetic-based hydrocarbon fluids exhibit four more observable peaks at 1,310, 1,145, 1,069, and 895cm-1. The petroleum-based hydrocarbon fluids exhibit a weak Raman scattering peak at 1,610cm-1, which is not observable in the synthetic-based samples. The pattern of Raman spectra between synthetic-based and petroleum-based hydrocarbon fluids is visually distinguishable. The results demonstrate that Raman spectroscopy can detect the chemical features of hydrocarbon fluids with different manufacturing processes.

Because most field investigators are not chemists, the next step in our research is to train an AI system to interpret the results. The research team envisions an AI-powered Raman spectrometer to assist field investigators in the critical decision-making of testing unknown samples. This work is significant because the results demonstrated the capability of Raman spectroscopy in distinguishing hydrocarbon fluid samples with different manufacturing processes. We believe Raman spectroscopy is a promising technique in the forensic investigation of physical evidence, including source tracing and classification tasks.

Raman Spectroscopy; Source Tracing; Classification

B50 The Impact of Glass Vial Type in Measuring Raman Spectrum of Liquid Hydrocarbon Samples

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Learning Objectives: The attendees will learn how the same type of glass vials purchased from the same source can result in obscure Raman scattering and the importance of vial composition for obtaining adequate quality of Raman spectra for liquid hydrocarbon samples. Attendees will become aware of how sample containers produce interference and understand a protocol to overcome this issue.

Impact Statement: Raman spectroscopy is a widely used and well-regarded test for chemical analysis. The forensic science community will benefit from this study's presentation of a vial testing method for Raman spectroscopy. The impact of glass vial selection to guarantee an interpretable spectrum will be presented. The new procedure prevents time and money from being wasted on inadequate results and, therefore, enhances the reliability of outcomes.

Abstract Text: Raman spectroscopy has been increasingly used in forensic science over the recent decades due to its myriad benefits. This method can be developed to become relatively inexpensive, rapid, non-destructive, and accepted by the scientific community, making it valuable in forensic chemical identification and classification. It also has confirmatory potential when paired with multivariate statistical methods, even when it is a portable instrument.[1] However, further use of Raman spectroscopy has been found to have difficulties with vial types. Vials labeled as the same composition of glass can lead to varying results for the same sample with this spectroscopic method. The varying results obtained from the same sample may be due to the composition of glass vials producing interfering fluorescence in the spectra.

Thus far, research on the relationship between fluorescence and Raman spectroscopy with reference to sample vials is limited. Several methods are available to overcome the obstacle of fluorescence in Raman spectroscopy. These methods include Shifted Excitation Raman Difference Spectroscopy (SERDS), adjusting laser excitation wavelength, and computational methods in postprocessing.[2] When these methods are not implemented, the detection of Raman scattering signals may be adversely impacted by the composition of the vials used to conduct experiments. By applying a protocol that detects the interference of vials, laboratories can limit the room for error and consequently save time and money.[3]

In this work, a quartz cuvette was used as the ideal sample holder that produces minimal interference for detecting Raman scattering signals. Several Type 1 borosilicate glass vials, including Amber Type 1 Borosilicate vial (B75562), Clear Vial Black Foam Lined Cap (V1526C-FM), and Clear Vial PTFE Lined Cap (V1526C-TFE), were tested using a hand-held Raman spectrometer (HandyRam). The HandyRam point-and-shoot method was used to obtain each container's spectra in two different instances. In one instance, each container held a standard neat jet fuel sample, while the other spectra were obtained with no sample in the vial. Raman scattering signals were collected with autointegration, and no post-measurement data treatment was completed on the spectra. All Raman spectra were obtained with a 785nm laser.

Although the V1526C-FM and V1526C-TFE vials are made of borosilicate, these vials produce high background signals that peak at about 1,384cm-1 in the spectra, leading to inconclusive results for liquid hydrocarbon samples. The background signal of the B75562 vial appears similar to that of the quartz cuvette. The background signals produced from the V1526C-FM and V1526C-TFE vials could be detected and noted as problematic by taking the background spectra of the vials. As a result, the spectra of the B75562 vial and quartz cuvette containing the standard neat jet fuel sample displayed a better quality of Raman spectra. In contrast, V1526C-FM and V1526C-TFE vials containing standard neat jet fuel samples produced inconclusive results. These results illustrate the importance of taking the spectra of sample containers before sample testing to guarantee adequate quality of the Raman spectra.

In summary, since glass vials are not specifically made or marked for Raman spectroscopic techniques, it is crucial to collect a background spectrum of the sample container prior to sample testing. This study demonstrates the critical role of the vial glass type in collecting acceptable Raman spectra for liquid hydrocarbon samples. This work proposes a testing protocol to ensure high-quality spectra are collected. The results of this work include an awareness of the importance of vial composition when using Raman spectroscopy.

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Raman Spectroscopy; Type 1 Borosilicate Vial; Fluorescence

B51 The Detection of Bio-Based Lighter Fluid and Biodiesel Residues on Wood and Fabric Debris

Mengliang Zhang, PhD, Ohio University, Athens, OH*

Learning Objectives: After attending this presentation, attendees will understand how their plant oil source and the type of substrates affect the chromatographic and mass spectral profiles of fatty acid-based Ignitable Liquids (IL).

Impact Statement: This presentation would benefit the scientific community by providing key information about the detection of commonly encountered in forensic labs but less studied IL, such asbio-based lighter fluid and biodiesels, by both Gas Chromatograph/Mass Spectrometry (GC/MS) and Direct Analysis in Real Time-Mass Spectrometry (DART-MS).

Abstract Text: The American Society for Testing and Materials (ASTM) E30.01 Criminalistics committee is currently working on a newly proposed standard, "Classification of Ignitable Liquids in Fire Debris Analysis," and the fatty acid-based products are proposed to be a new category of IL classification. Examples of fatty acid-based products include bio-based lighter fluid, torch fuel, charcoal starter fluid, and biodiesel. Biodiesel and bio-based lighter fluid, as IL are the trans-esterified products containing Fatty Acid Methyl Esters (FAME), which can be detected in fire debris by GC/MS analysis without derivatization. Compared to other petroleum-based IL, studies on biodiesels and bio-based lighter fluid in forensic settings are relatively rare, and the exploration of techniques other than GC/MS for their detection is very limited. In this study, we investigated the detection of fatty acid-based IL products on wood and fabric substrates and debris using GC/MS and DART-MS methods. In addition to the commercial bio-based lighter fluid and biofuels, we prepared biodiesels with different plant-based oils in the lab to study how the plant oil sources would impact their chemical profiles in the final products.

The IL residues on substrates and debris were extracted following the ASTM E1412 activated charcoal method, and the extracts were analyzed using both GC/MS and DART-MS.[1] The FAME standard solution, biodiesel standard solution, and biodiesel samples prepared in our laboratory with ten different plant-based oils were studied and compared. The results showed that the variety and relative distribution of FAME compound in the biodiesel products were dependent on the types of plant oils. Specifically, the main FAME compounds, such as C16:0, C18:0, C18:1, C18:2, and C18:3, were identified in the samples, and their relative quantity varied significantly based on their plant oil source. The biodiesels prepared from cooked/waste oil and straight oil gave similar FAME profiles from the GC/MS and DART-MS analysis. The results also suggested that both GC/MS and DART-MS could effectively detect FAME compounds present in the substrate and debris samples. The variation of compound ratios affected by the substrates and burning conditions was also studied in the project.

Reference:

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Ignitable Liquids; GC/MS; DART-MS

B52 Real Bullets, Plastic Guns: Evaluating the Strength of 3D-Printed Gun Parts

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Learning Objectives: Attendees can learn more about the 3D design/printing process. Attendees can learn more about ghost guns, which are becoming increasing popular around the world and how they differ from privately made firearms; how they are made; what harm can be done with them; how feasible it is for someone to assemble/design a firearm; what damage can be reasonable expected from a privately made firearm; and what trace evidence is left behind after firing a plastic gun or a gun with plastic parts.

Impact Statement: 3D printing is a popular endeavor among many enthusiasts and hobbyists around the world. With easy access to 3D printers, materials, and, most importantly, the creative design of guns and gun parts, the rise of privately made firearms is an inevitability. Privately made firearms (including ghost guns) can be assembled from commercially available kits or readily available parts combined with 3D printed parts. The identification of firearms based on fired bullets is founded on the microscopic agreement of individual marks left on a fired bullet to the interior of the questioned barrel; 3D printing a functional gun barrel would successfully fire a projectile and leave no rifling on the bullet because the barrel would be printed out of plastic and lack the hardness to leave marks on a metal projectile. A 3D printed barrel that is unregulated would be difficult to trace and control, especially without advancements in forensic science and law enforcement. The language of the law needs to advance just as much as investigative techniques. Forensic investigation of privately made firearms is a complex new landscape that requires an interdisciplinary approach centered on technological advancement in order to better understand this evolving terrain.

Abstract Text: Privately made firearms, also popularly referred to as "ghost guns," are firearms created and assembled by an individual; these firearms are not manufactured or marketed by a corporation or government and, as such, many of them lack serial numbers and are unregulated. "Ghost guns" refers to guns without a serial number, which actually only comprises a small portion of the true umbrella term, privately made firearms. Privately made firearms can be entirely 3D printed, assembled from "buy build shoot" kits (can be purchased with a firearm already up to 80% assembled), or be composed of 3D printed parts retrofitted onto an existing gun. With the accessibility of 3D printing and the freely shared files of 3D printed guns and gun parts, the appearance of privately made firearms in any of the three forms has increased in the past years in several countries. 3D printing gun parts, except for the lower receiver, is legal according to the language of some state laws. Because firearm parts are designed to be interchangeable to allow for customization or repairs, it is possible to exchange an original manufactured gun part for a 3D printed part, this is a problem in a legal sense when used either for concealment or forensic evasion. The identification of firearms based on fired bullets is founded on the microscopic agreement of individual marks left on a fired bullet to the interior of the questioned barrel; 3D printing a functional gun barrel would successfully fire a projectile and leave no rifling on the bullet, being that the barrel would be printed out of plastic and lack the hardness to leave marks on a metal projectile.

The goal of the experiment was to determine if a 3D printed barrel is capable of producing a lethal projectile when retrofitted onto the original firearm. A commercially available 3D printer (Makerbot Replicator+) was used to print an exact replica of the SBS barrel (a side-by-side barrel) of a Derringer Cobray Model DD in .38 Special/.357 Magnum modified to be chambered for a .25 ACP cartridge using PLA Tough filament (the strongest filament compatible with the original extruder of the 3D printer). The modification to accommodate a smaller cartridge allows for more plastic material in an effort to maintain the integrity of the barrel when the gun is fired. The results showed that the 3D printed barrel did not have the strength to survive multiple test fires and that the bullet was not capable of penetrating ballistic gelatin deep enough to cause lethal damage. The lack of accuracy and penetrating power of the bullet can be attributed to the lack of rifling on the 3D printed barrel, which is essential for spin-stabilization of a bullet.

3D Technology; Forensic Science; Firearms

B53 The Collection Efficiency of the Activated Charcoal Adsorbent for the Detection and Identification of the DMNB by GC/MS From the Headspace of the Suspected Post-Blast Debris

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WITHDRAWN

B54 The Characterization and Discrimination of Soil by Major Elemental Analysis Using Laser-Induced Breakdown Spectroscopy: A Case Study of Nirasaki in Central Japan

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Learning Objectives: This presentation shows the effectiveness of analyzing soils by Laser-Induced Breakdown Spectroscopy (LIBS) based on the case study in the Nirasaki area in central Japan. The spatial distribution of the major elements in soil samples reflected the geological background of sampling sites. When three elements, potassium, magnesium, and calcium, were combined, the discrimination power of the 74 surface soils was 94%. Our result demonstrated that LIBS can discriminate soils derived from similar geological backgrounds and the possibility of provenance estimation.

Impact Statement: LIBS can characterize soils based on their geological backgrounds. Despite soils derived from similar geological backgrounds, 94% of the pairs of each soil could be discriminated.

Abstract Text: In forensic soil examinations, elemental analysis is informative for discriminating and characterizing soils.[1] However, soils from similar geological backgrounds are often difficult to discriminate or estimate their provenances. This study aimed to utilize elemental analysis by LIBS to forensic soil discrimination using fine fractions in the Nirasaki area in central Japan. The study area is surrounded by different geology from place to place, such as granodiorite, accretionary prisms, volcanic debris, and lava in the neighborhood (within ca. 10km). Therefore, the soils derived from different parent materials are distributed. For this geological setting, this area was previously studied for forensic soil color examination.[2] Organic matter decomposition, iron oxide removal, and sieving in 53µm were treated on 74 surface soils and pelletized.[3] The samples were analyzed by LIBS equipped with a 266nm laser by three lines (25 laser shots per line) per sample. The emission lines of major elements were selected as follows: 288.1nm (Si), 334.9nm (Ti), 257.5nm (Mn), 518.4nm (Mg), 393.4nm (Ca), 588.9nm (Na), 766.5nm (K), 495.8nm (Fe), and 396.1nm (Al). The intensity of each LIBS emission line was normalized to the aluminum emission line, and discrimination power of the emission line ratio was evaluated. The discrimination power was calculated as the percentage of pairs that were discriminated from each other out of total number of pairs of samples. Discrimination of each sample was conducted using the three-sigma criteria.

As a result, the K/Al, Mg/Al, Ca/Al, Si/Al, and Fe/Al ratios were high in the western area, which is mainly derived from granodiorite or their fans, terrace, and river plain sediments.[4] The Mn/Al ratio was relatively high from the central to the eastern region, which originated from andesitic volcanic debris and fan deposits. These differences in geological background were considered to be reflected in the emission line ratios of each area. The discrimination powers of K/Al, Mg/Al, Ca/Al, and Fe/Al ratios were high at 76.9, 75.0, 73.8, and 67.0%, respectively. Combining K/Al, Mg/Al, and Ca/Al can discriminate 94.2% of the sample pairs. Our result demonstrated that the major elemental ratios from LIBS analysis could discriminate 94% of the samples and the possibility of estimating the provenance of unknown soil samples.

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Soil Analysis; Spectroscopy; Method Development

B55 An Investigation Into Chromatographic Methods for the Preservation, Stability, and Analysis of Organic Gunshot Residue

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Learning Objectives: After this presentation, attendees will understand how the storage conditions (temperature and length of storage) affect the detection of organic gunshot residues by various confirmatory chromatographic methods. Additionally, the attendee will gain insight into how Gas Chromatograph/Mass Spectrometer (GC/MS) micro Electron Capture Detector (μECD), Gas Chromatograph/Mass Spectrometer/Flame Ionization Detector (GC/MS/FID), and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) can be incorporated into the current workflow of forensic laboratories for Gunshot Residue (GSR) analysis.

Impact Statement: This presentation will impact the forensic science community by providing recommendations on the best way to preserve GSR evidence for minimal loss of information of organic gunshot residues and describes the advantages and disadvantages of different chromatographic methods. This study demonstrates the results of assessing three different storage conditions and five different times from collection to analysis to assess the stability of Organic Gunshot Residues (OGSR). These results impact the forensic community by adding to the current knowledge of OGSR stability, analysis, and workflow recommendations.

Abstract Text: Following the discharge of a firearm, OGSR and Inorganic Gunshot Residues (IGSR) are produced and deposited on nearby areas, including the hands of the shooter. Most forensic laboratories analyze IGSR by scanning electron microscopy-energy dispersive x-ray spectrometry to classify particles by morphology and elemental composition.[1] Recently, OGSRs have gained traction by providing complementary information that gives extra confidence in the examiner's results due to their relatively low prevalence in the environment.[2] Due to the increase in gun violence in the United States, forensic organizations are compiling research and knowledge for the future adoption of OGSR detection and increasing the evidentiary value of GSR.[3] This study targets some of the gaps in knowledge to better understand the capabilities and limitations of analytical techniques that can be used for OGSR while exploring recommendations for the best practices to store and preserve OGSR in these analytical workflows.

In this study, two separate validations were performed for the analysis of OGSR using GC/MS coupled to an FID or μECD using a microfluidic splitter. The effect of solvent used to extract and reconstitute the samples is also investigated. Figures of merit include repeatability, reproducibility, selectivity, linear dynamic range, Limits Of Detection (LODs), and Limits Of Quantitation (LOQs). A previously validated GC/MS/FID method from our group found MS LODs to range from 40 to 120ppb and the FID LODs to range from 35 to 180ppb. ECD LODs are also in the low parts per billion range. Testing using hexane has shown an increase in sensitivity, which leads to lower LODs. GC/MS has inferior LODs and LOQs when compared to LC/MS/MS, which makes it more difficult to detect trace levels of OGSR.[2] Equipping a secondary detector to a GC/MS can increase sensitivity for certain compounds that exhibit poor response in an MS and allow for better performance. The μECD has a high sensitivity for nitro-containing compounds; however, its limitation is the lack of detection of DPA, EC, and AKII. Conversely, the FID has the ability to detect almost any compound but has similar detection windows to the MS, which does not add any increased sensitivity, just extra confidence in the results. On the other hand, LC/MS/MS showed the highest sensitivity. However, it is a more expensive instrument compared to a GC/MS, even with configurations that include extra detectors (FID or μECD).

Three analytical techniques were used in this study to assess various storage conditions and time of analysis after collection. These techniques include GC/MS/FID, GC/MS/μECD, and LC/MS/MS. The time of analysis and storage condition for an OGSR sample may vary between laboratories. To simulate this, authentic residues were recovered from hands and stored in three different conditions: room temperature, a freezer at -30°C, and an extreme heat environment to simulate if a sample were to be left in a vehicle (~80–100°F). Samples were then tested the same day of firing, 2-day, 1-week, 2-week, and 1-month time periods to determine the effect of time in each of the three conditions.

This presentation discusses recommendations on the collection, storage, and analysis of OGSR comparing the use of chromatographic methods to benefit laboratory decision-making in the future adoption of OGSR analysis.

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Gun Shot Residue; Stability; Gas Chromatography

B56 A Study of Casing Markings of Semi-Automatic Firearms Modified With a Glock Switch

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Learning Objectives: After attending this presentation, attendees will understand the difference between casing markings fired in the modified and unmodified fashion of semi-automatic firearms with the addition of a Glock switch. A Glock switch is an illegal modification that allows the firearm to alternate between semi-automatic and fully automatic fire.

Impact Statement: This presentation will impact the forensic science community by bringing awareness to the increased use of Glock switches on semi-automatic firearms and its potential effect on firearms examinations.

Abstract Text: This report will present research completed involving three modified firearms. The firearms used included two Glock 17's and one Glock 19. Each firearm fired three magazines (17 cartridges per magazine): one in the semi-automatic mode and two in the fully automatic mode. Standard manual examination of the 153 casings in the study was performed using a LEEDS comparison microscope at 11x magnification. Side-by-side comparisons were made of semi-automatic casings and fully automatic casings fired from the same firearm to compare striation patterns on the breech face. No discernible differences were found in the striation patterns fired from the same firearm with and without the Glock switch. Once manual comparison on casings was completed, the LEEDS comparison microscope was used in conjunction with the SPOT Imaging Solutions application to evaluate firing pin drag mark length, ejector marks, and drag mark width on each casing at 22x magnification. The increased magnification was needed to ensure sufficient detail. Statistical analysis was completed on measurements using a two-tail t-test for two dependent means at 95% confidence to compare markings made in the semi-automatic mode with markings made in fully automatic mode on casings from the same firearm.

Unlike the comparison of striations, results of the two-tail t-tests from this portion of the study showed that in two firearms, the drag mark length was significantly different between the semi-automatic and fully automatic casings fired from the same firearm. Furthermore, in one firearm involved in this portion of the study, the ejector mark location was significantly different between the two modes.

Although the chance of an incorrect conclusion reached by a trained firearms examiner is likely rare, the results of this study indicate that firearm examiners must nonetheless be aware that markings may change on casings due to the use of a Glock switch.

Firearm Examination; Modified Firearms; Glock Switch

B57 One-Step Non-Contact Sampling and Analysis of Trace Chemical Residues Using an Electrostatic Wand and Paper Spray Mass Spectrometry

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Learning Objectives: After attending this presentation, attendees will understand how electrostatic sampling can rapidly and inexpensively analyze chemical residues on surfaces while preserving underlying fingerprint and touch DNA evidence, with potential on-site use.

Impact Statement: We present a simple, portable, combined sampling and mass spectrometry ionization device for non-destructive analysis of surface evidentiary residues.

Abstract Text: When forensic chemists encounter trace powder residues, such as a baggie with cocaine residue or a fragment of an exploded pipe bomb with residual traces of explosive compounds, the residues are often analyzed using mass spectrometry. However, analysis of residues on evidentiary surfaces with mass spectrometry typically involves solvent rinses or swabbing, which destroys potential underlying fingerprint and touch DNA evidence. In previous work, we demonstrated that a simple electrostatic sampling device could be used effectively for non-contact sampling of chemical residues on surfaces.[1] The device is a hand-held Van de Graaff Generator (VDG) sold as an inexpensive children's toy. After sampling, the captured residue was analyzed offline using mass spectrometry with an electrospray ionization source. In the current work, we have changed the methodology and the sampling substrate so that the electrostatic wand can be used both for non-contact sampling of a surface and also as an ambient ionization source. In this way, the method has become a combined, online process using the same device for sampling and ionization.

In the combined method, a triangular piece of filter paper is connected to the output of a commercially available hand-held VDG. Initial experiments verified that the handheld VDG was an effective voltage source to drive ambient Paper Spray Ionization (PSI) from the paper triangle, supplying ~27 kV.[2] Drug residues were deposited directly onto the paper and allowed to dry. A spray solvent was then pipetted onto the paper, and the voltage was applied from the VDG, with the tip of the paper triangle positioned in front of the inlet to the mass spectrometer. Signals for 100pg of methamphetamine, hydrocodone, and cocaine were each obtained in Tandem Mass Spectrometry (MS/MS) mode on an ion trap mass spectrometer using the VDG to drive the PSI.

After demonstrating that the hand-held VDG could effectively drive PSI, the feasibility of non-contact sampling was tested on various surfaces. Cocaine was used as a model compound, with 1µg of cocaine deposited on rubber, stainless steel, and pine wood surfaces. For sampling, the hand-held VDG with the paper attached was held such that the tip of the paper triangle was held a few millimeters above the surface. The voltage was applied to the handheld VDG to facilitate surface charging and electrostatic evidentiary particle capture at the triangle tip. Then, the tip was moved in front of the mass spectrometer inlet, spray solvent was added, and the VDG voltage was applied to drive PSI of any captured sample. Cocaine signal was observed in each MS/MS spectra, with the signal-to-noise ratio being the lowest for the cocaine sampled from the stainless-steel surface. These preliminary experiments demonstrate non-contact capture followed by direct PSI using this device.

In follow-up experiments, the sampling and analysis of transferred residues were tested. A gloved finger was loaded with 100µg of a drug and rubbed against a rubber substrate. Drug residues transferred from the glove to the substrate were electrostatically sampled and ionized using PSI driven by hand-held VDG. Signal was obtained for both of the test compounds, atropine and procaine. Additionally, transferred residues were detected using this methodology with a fieldable ion trap mass spectrometer instrument. In this experiment, a pseudoephedrine tablet was rubbed onto a gloved finger, and that finger was rubbed on a rubber luggage handle. The luggage handle was sampled and analyzed using the VDG and PSI ionization on a fieldable instrument. This experiment extends the technique beyond benchtop equipment and has positive implications for on-site crime scene, defense, and homeland security applications beyond the laboratory.

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Mass Spectrometry; Touch Sample; Seized Drugs

B58 A Quantitative Assessment of the Stability of New AR-Style Rifle Breech Face Characteristics

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Learning Objectives: Much of the research performed in forensic firearms is qualitative based on observations made using a comparison microscope. However, this study uses quantitative data from Three-Dimensional (3D) comparison algorithms to assess changes that occur to the breech face area of firearms when brand new. Attendees will learn when firearm examiners may need to be conservative in making elimination conclusions due to potential differences observed within cartridge case breech face marks over consecutive shots.

Impact Statement: The time between when a shooting incident occurs and when the suspect firearm is recovered is critical. This study will present quantitative data to inform firearm examiners about the extent to which the breech face surface of AR-style rifles changes when brand new, emphasizing the importance of newer 3D imaging systems for firearm forensics research.

Abstract Text: Firearm surfaces have been observed to change; however, previous research into this topic has been mostly qualitative in nature where a firearm examiner uses a microscope to examine a sequence of fired cartridge cases for any changes. New 3D imaging technologies have been developed to acquire high-resolution surface topographies of firearm evidence. These 3D scans can be compared using algorithms that take into account the heightmap of features present on the surface. These algorithm scores allow for the presentation of quantitative data to assess changes in firearm surfaces over consecutive shots.

Three AR-style rifles from each of two different manufacturers were acquired for this study. One hundred rounds of 5.56 x 45mm caliber Herters range ammunition were purchased and fired through each rifle for a total of 600 shots. The first ten, last ten, and every fifth cartridge case in between were collected from each rifle for a total of 36 cartridge cases per rifle. All fired cartridge cases will be assigned a unique identifier that will be engraved onto each cartridge case to track firing order and from which firearm each was fired. 3D topography scans will be acquired of all collected cartridge cases using a Cadre Forensics TopMatch-GS 3D instrument. The Cadre Forensics breech face comparison algorithm will then be used to compare the cartridge cases within and between firearms, providing an objective similarity metric. Time series analysis will be used to assess whether statistically significant decreasing trends are present. These quantitative data will be coupled with visual examination to support all conclusions.

It is hypothesized that there will be small changes, but that these changes will stabilize within the first 100 shots. Preliminary data from a 9mm Luger caliber pistol showed no statistically significant decrease in scores over the first 100 shots, indicating no significant changes were occurring to the breech face surface of the firearm. However, AR-style rifles generate double the chamber pressure when compared to 9mm Luger pistols, so some changes are expected to be observed due to this increase in pressure.

3D Technology; Firearms; Ballistics

B59 A Modified Procedure for Extracting Primer Gunshot Residue From Scanning Electron Microscopy Carbon Stubs and Trace Analysis Utilizing the Graphite Furnace-Atomic Absorption Spectrometry Technique

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Learning Objectives: After attending this presentation, attendees will: (1) understand the development and validation of a modified procedure for extracting primer Gunshot Residue (GSR) elements from adhesive carbon stubs collected for Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) analysis; (2) gain knowledge of the Graphite furnace/Atomic Absorption Spectroscopy (GTA/AAS) technique for trace analysis of primer GSR elements; and (3) recognize the advantages of the modified extraction method, including its simplicity, high sensitivity, and potential impact on expediting forensic investigations.

Impact Statement: The development and validation of a modified procedure for extracting primer GSR elements from adhesive carbon stubs and utilizing GTA/AAS for trace analysis have significant implications for forensic investigations related to gun crimes. This alternative technique offers a practical solution, particularly in situations where SEM/EDS is unavailable or impractical. By addressing the limitations of SEM/EDS, such as cost, expertise requirements, and parts availability, implementing this modified procedure can help alleviate case backlogs and expedite the identification of shooting incidents and potential shooters. The simplicity, high sensitivity, and reproducibility of results make this method a valuable tool in forensic analysis, ultimately contributing to the effective resolution of gun-related criminal cases.

Abstract Text: **Background:** Gun crimes have become a global concern in countries with lenient firearm acquisition regulations and weak prosecution systems. The rise in homicide and suicide cases involving firearms necessitates the use of contemporary forensic techniques to identify shooting incidents and potential shooters. One such technique involves the analysis of primer GSR using SEM/EDS. In combination with SEM-EDS, the application of graphite furnace atomic absorption spectroscopy (GFA-AAS) imparts an extensive application in conducting elemental analysis of GSR from crime scene.[1,2] However, the widespread implementation of SEM/EDS is hindered by its high cost, the need for advanced expertise, and challenges related to troubleshooting and importing parts, particularly in developing countries. Consequently, the backlog of cases awaiting primer GSR analysis continues to grow. In such situations, an alternative, reliable technique that utilizes the same adhesive carbon stubs collected for SEM/EDS analysis becomes essential.[3]

Aim and Objectives: This study aimed to develop and validate a modified procedure for the extraction of primer GSR-related elements from SEM/EDS adhesive carbon stubs employing simple extraction techniques and finally analyzing by GTA/AAS.

The modified procedure involved the following steps: (1) removal of the adhesive carbon layer from the stub: The carbon layer was carefully peeled off from the stub using acetone and a scalpel; (2) digestion of the trace material on the adhesive carbon: The removed adhesive carbon material was transferred into a 15-ml plastic centrifuge tube, and the adhered material was digested in an airtight condition with 2ml of 10% nitric acid (HNO_3) for a minimum of 12 hours at 80°C ; (3) vortexing and centrifugation: after digestion, the sample tube was vortexed for at least 5 minutes at a speed of 2,000–2,500. The vortexed contents were then centrifuged at 4,000rpm for 5 minutes; and (4) analysis by GTA/AAS: The supernatant extract from the centrifuge tube was transferred to sample cuvettes and analyzed using GTA/AAS for the identification of lead (Pb), barium (Ba), and antimony (Sb) in combination.

Notably, this extraction method does not require the use of chemical modifiers to address matrix interference. The sensitivity of the method is remarkably high, enabling the successful detection of GSR-related elements (Pb, Ba, and Sb) at levels as low as parts per billion (ppb).

Results: The method demonstrated a sensitivity of ≥1ppb for Sb and ≥10ppb for Pb and Ba, which are critical detection elements present in trace amounts in positive sample stubs. The Coefficient of Variation (%CV) for Sb, Pb, and Ba ranged from 7.8% to 17.4%, 13.7% to 19%, and 5.8% to 18%, respectively. The bias for Sb, Pb, and Ba ranged from 0.6% to 13%, 4.31% to 19.12%, and 0.6% to 7.2%, respectively.

Discussion: The developed extraction method offers a simple, robust, and sensitive approach with excellent repeatability and reproducibility of results. By utilizing adhesive carbon stubs and GTA/AAS, this alternative technique provides a practical solution for primer GSR analysis, particularly in situations where SEM/EDS is unavailable or impractical. Implementing this method can help alleviate the backlog of cases and expedite forensic investigations related to gun crimes.

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Forensic Analysis; Gun Shot Residue; Inorganic

B60 Outpacing the Law: NPS—A Global Threat Challenging Forensic Analysis

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Learning Objectives: The attendees will have a thorough knowledge of New Psychoactive Substances (NPS) emergence, their analytical strategies, and special emphasis on challenges faced by forensic scientist during their detection in seized and biological samples. The rapidly and constantly evolving nature of NPS poses a greater challenge to forensic analyst in identification. The attendee will overview the main practical challenges and their possible solutions during analysis of seized and biological samples in forensic laboratory.

Impact Statement: The presentation will give an insight and understanding of the analytical challenges and their possible solutions regarding qualitative detection and reporting of NPS using Gas Chromatograph/Mass Spectrometry (GC/MS). This will help the forensic science community in uplifting the standardization and standard operating procedures for proper identification and interpretation of NPS in forensic samples. Consequently, the content of this presentation will help in coping with the current NPS identification challenges.

Abstract Text: NPS have become a growing concern in recent years for communities and especially for forensic analytical laboratories due to their potential health risks and the difficulty in detecting them. Forensic experts face numerous challenges in the qualitative detection of NPS using GC/MS. These challenges include the rapid emergence of new substances, the lack of reference standards for identification, and the complexity of analyzing specimens with diverse compositions along with MS library matching limitations, and the chemical nature of NPS as being thermolabile.[1-3] Additionally, the interference from major constituent peaks during qualitative analysis can result in the missed detection of minor and trace constituents.[4]

This paper explores such challenges and their mitigation as well as the need for a sensitive and selective analytical approach. The qualitative identification of NPS involves presumptive and confirmatory tests. GC/MS is most common technique for confirmation of seized drugs. Various strategies like choice particular method parameters, MS libraries, derivatization techniques, extraction techniques, and data interpretation expertise are applied for correct identification of NPS in forensic samples. There is a need for thorough understanding of NPS emerging trends, their profiles, and collaborations for forensic lab with law enforcement agencies and research institutes to address these challenges so that the accuracy and reliability of qualitative detection methods can be improved as per need. The development of comprehensive databases and collaborative international efforts for data sharing are also critical, enabling the timely identification of new NPS. Alternative techniques like High-Resolution Mass Spectrometry (HRMS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) may be applied if capacitated by the lab for achieving enhanced sensitivity and specificity. This technical note provides an insight into some of the key parameters that should be discussed and monitored for exact and timely identification and profiling of NPS encountered routinely.

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New Psychoactive Substances; Forensic Analysis; Forensic Library

B61 The Enhancement of Latent Fingerprints Using Waste Biomass-Derived Biocompatible Fluorescent Graphene Nanosheets

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Learning Objectives: Attendees will learn about green synthesis and the characterization of nanoparticles, the development of latent fingerprints on different surfaces (porous and non-porous), and toxicological studies of the synthesized nanoparticles.

Impact Statement: Green synthesis and characterization of nanoparticles aims to significantly advance the forensic science community by introducing eco-friendly and sustainable methods for nanoparticle synthesis. Using these nanoparticles, developing latent fingerprints on both porous and non-porous surfaces promise enhanced sensitivity and clarity in crime scene investigations. Comprehensive toxicological studies also ensure that the synthesized nanoparticles are safe for forensic practitioners and the environment. This multi-faceted approach not only pushes the boundaries of forensic technology but also aligns with global sustainability goals, setting a new standard for innovation in forensic science.

Abstract Text: The application of biocompatible and non-toxic nanomaterials for latent fingerprint detection is gaining significant interest in the forensic science field.[1] In this study, waste chickpea seeds, a natural resource, were bioprocessed to synthesize non-toxic Graphene Nano-Sheets (GNS) with high fluorescence. High-temperature pyrolysis was used to create the GNS, and a variety of methods were used to characterize them, including Fourier Transform Infrared (FTIR) analysis, fluorescence spectroscopy, UV-Visible (UV-Vis) spectroscopy, X-ray Photoelectron Spectroscopy (XPS), and Transmission Electron Microscopy (TEM).[2,3] The synthesized GNS exhibited excitation-independent emission at approximately 620nm with a quantum yield exceeding 10%, showing distinct blue fluorescence under a UV lamp.[4,5] Biocompatibility was assessed, revealing cell viability rates of 88.28% and 74.19% even at high concentrations (50 and 100mg/mL, respectively). Additionally, the antimicrobial properties of the synthesized GNS-based coatings were evaluated using a pathogenic strain of *Bacillus cereus* through live/dead cell counts and plate counting methods, confirming their biocompatible and antimicrobial nature. This highlights their potential use in safe fingerprint detection. The developed chickpea-originated fluorescent GNS-based spray coatings were tested on various non-porous surfaces, including plastic, glass, silicon, steel, and soft plastic. Results confirmed that GNS could effectively detect latent fingerprints on multiple non-porous surfaces, which were easily visible under a UV lamp at 395nm. The color-tunable behavior of the developed GNS-based spray coating makes it ideal for the visual enhancement of latent fingerprints.

These findings underscore the high potential of the fluorescent GNS spray coating to increase the sensitivity and stability of crime trace detection on non-porous surfaces. The prints developed showed the Level I and Level II characteristics clearly. The prints also developed on the porous surface. By utilizing sustainable and natural resources, this study offers an innovative and eco-friendly approach to forensic latent fingerprint detection, aligning with global sustainability goals while enhancing forensic technology.

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Fingerprint; Accuracy; Fingerprint Identification

B62 An Assessment of the Permeability of VOCs Between Containers Housing Detection Canine Training Aids

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Learning Objectives: After attending this presentation, attendees will learn how problematic cross-contamination of volatile odorants is among co-located aids and the estimated longevity of optimal storage containment systems for canine training aids over time prior to odorant permeation and cross-contamination.

Impact Statement: This presentation will impact the forensic science community by depicting the issues of improper storage containment systems for canine training aids. Moreover, it will provide beneficial information on the selection and longevity of various storage containers in hopes of reducing cross-contamination and improving a canine's detection accuracy toward trained odorants.

Abstract Text: Canine training utilizes training aids comprised of real materials or odorants corresponding to illegal and legal materials for detection purposes. However, improper storage of aids could severely affect a canine's performance, hindering their reliability, and thus emphasizing the need for suitable containers. A recent study utilized Solid Phase Microextraction -Gas Chromatography/Mass Spectrometry (SPME-GC/MS) to assess the headspace of 11 new explosive training aids from three vendors for initial cross-contamination.[1] Though the explosives in this study were purchased new, the results depicted significant levels of cross-contamination from one of the three vendors. Evident cross-contamination could have resulted from how the samples were shipped or packaged, as it was noted that this vendor utilized plastic bags and packed aids together.[1] With possible issues of cross-contamination being caused by plastic bags and aids packaged together, ideal containment systems need to be further investigated. Therefore, this research utilized SPME-GC/MS to further explore levels of cross-contamination within old co-located drug training aids and to assess the permeation of odorants in various storage containment systems.

In this study, potential cross-contamination between previously used drug canine training aids kits containing cocaine, cocaine base, methamphetamine, 3,4-Methylenedioxymethamphetamine (MDMA), heroin, and black tar heroin from three locations was assessed using SPME-GC/MS. The main odorant evaluated for each drug was acetic acid for heroin and black tar heroin, benzaldehyde for methamphetamine, methyl benzoate for cocaine and cocaine base, and piperonal for MDMA. Small amounts of sample were collected and transferred into 20-mL vials. Following equilibration, the samples' headspace was extracted using SPME prior to thermal desorption in the GC inlet. Triplicates were performed for each drug in each location.

The co-located aids assessed from each location depicted significant amounts of cross-contamination, with acetic acid being predominantly detected in each sample. Additionally, there was no detectable limit of piperonal in MDMA or any sample; however, a high intensity of dimethyl sulfone was detected and measured.

With significant contamination being detected with these co-located aids, a further study explored the efficacy of different storage containers in preventing cross-contamination. Containment systems included glass Training Aid Delivery Devices (TADDs), canning jars, Mylar bags, Bitran bags, and Ziploc bags. Heroin and MDMA mimic training aids were formulated using dimethyl sulfone, the major odorant seen in the cross-contaminated samples by MDMA, or acetic acid, the main odorant of heroin, mixed into a cellulose substrate. The devised mimics were each stored in identical containers inside a case.

Cross-contamination in each container was evaluated by transferring small portions of the cellulose-containing no major odorant in each container into separate 20-mL vials without depleting the quantity and intensity of the heroin and MDMA training aid mimics. Samples were collected on the initial day, followed by monthly sampling to monitor cross-contamination.

The assessment of various storage containment systems depicted the odorants from heroin and MDMA mimic training aids tend to cross-contaminate earlier in some containers than others. This study provides information to individuals regarding the issues of utilizing improper training aid storage containers over time. Additionally, this research provides insight into the importance of implementing routine training aid checks for cross-contamination to ensure the odor profile remains consistent during storage.

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Cross-Contamination; Permeability; Training Aids

B63 The Prevalence of GSR on Ammunition Packaging

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Learning Objectives: This presentation will summarize the results of a Gunshot Residue (GSR) prevalence study performed on ammunition packaging in Houston, TX, area sporting goods stores. After attending this presentation, attendees will become familiar with the prevalence of GSR, the need for mitigating techniques during collection, and the need to apply the appropriate interpretation on a case-by-case basis.

Impact Statement: This presentation will impact the forensic science community by identifying the potential for elevated levels of GSR in specific environments and the importance of interpreting the results effectively in both analytical reports and subsequent testimony as well as identifying mitigating techniques to sample collection.

Abstract Text: The prevalence of GSR can be of concern in specific environments. Accordingly, specific environments with elevated levels of GSR may impact the interpretation and subsequent reporting GSR analytical results. To help build on this understanding of GSR in the surrounding area, a localized study of GSR from various sporting goods stores was conducted to determine the prevalence of GSR and identify the potential for transfer.

In this study, ammunition boxes were sampled throughout sporting goods stores in the greater Houston area. A random sampling of brands and calibers were sampled in each store. Each box of ammunition was on a public facing shelf easily accessible by customers. Sampling occurred in a similar manner from seven stores on six different dates. 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/EDS) with an automated software to detect particles characteristic of GSR. The same standard operating Procedures (SOPs) employed for evidence analysis were followed in this study.

A total of 90 GSR stubs were collected from seven sporting goods stores from the greater Houston area. A total of 16 GSR particles were confirmed. Confirmed particles were from a variety of brands and calibers. This prevalence has the potential to lead to transfer to an individual who may not have otherwise had an association with the discharge of a firearm. The results of this study will highlight the benefit of localized prevalence studies, collection-mitigating techniques, and appropriate use of reporting statements to accurately communicate the significance of GSR analysis results.

In conclusion, this presentation will demonstrate the prevalence of GSR in sporting goods stores around the Houston area, touching on the potential for transfer of GSR, collection- mitigating techniques, and the importance of interpretation and reporting of analytical results.

Gunshot Residue (GSR); GSR Prevalence; Evidence Handling

B64 Advancing Trace Evidence Identification Through ATR/FTIR Spectroscopy and Machine Learning for Textile Fibers Analysis

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Learning Objectives: The integration of Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) spectroscopy with machine learning offers a powerful tool for the non-destructive analysis of textile fibers in forensic investigations. The creation of an ATR/FTIR spectral database will enhance the ability of forensic experts to identify unknown or suspect fibers, thus providing critical evidence in criminal cases. The ability to quickly and accurately identify fiber types can narrow down the suspect pool in the investigations, where other forms of evidence, such as DNA or fingerprints, are unavailable or inconclusive. By providing a reliable method for linking suspects, victims, and crime scenes through fiber evidence, this study supports the purpose of ensuring justice through precise and scientifically sound forensic analysis.

Impact Statement: The development of a spectral database for textile fibers represents a significant advancement in forensic science, providing a standardized and systematic approach to fiber analysis. This resource could be continuously expanded as new fiber types are introduced into the market, ensuring that forensic capabilities keep pace with changes in textile manufacturing. Moreover, the integration of machine learning into forensic workflows reflects a broader trend toward the adoption of artificial intelligence and data-driven methods in forensic investigations. These technologies offer the potential to automate complex analytical processes, reduce human error, and improve the speed and accuracy of forensic investigations.

Abstract Text: Fibers are a ubiquitous form of trace evidence encountered in a variety of criminal investigations, particularly in cases of rape and physical assault. The analysis of these fibers can establish critical connections between crime scenes, victims, and perpetrators, often providing the only tangible link that places an individual at a scene or in contact with another person. The ability to study fiber components and assess their transferability is therefore a cornerstone of forensic science, offering insights that can corroborate testimonies or refute alibis. This study aims to explore the ability of FTIR spectroscopy in ATR sampling mode, in integration with machine learning to characterize and classify a diverse set of textile fibers.

The study focused on a comprehensive analysis of 104 fiber samples, which included natural fibers such as cotton and wool (43 samples) and synthetic fibers, including terry wool (61 samples). ATR/FTIR spectroscopy was selected for this analysis due to its non-destructive nature and ability to produce detailed spectral data that reflect the molecular composition of the fibers. The fingerprint region of the spectra (1,800–450 cm^{-1}) proved to be particularly valuable, as it contained prominent peaks that are indicative of specific chemical bonds and structures unique to different fiber types. This region of the spectrum is often the most informative for identifying materials because it captures the fundamental vibrations of molecular bonds, which are characteristic of specific substances.

Additionally, the study employed Principal Component Analysis (PCA) for data reduction to handle the complexity of the spectral data obtained from the ATR/FTIR analysis. The reduced data were then subjected to machine learning algorithms using PyCaret library of Python framework, allowing identification of the most effective approach for classifying the fiber samples. The study explored multiple algorithms, and each algorithm was evaluated based on its accuracy, Area Under the Curve (AUC) score, precision, recall, F1-value, kappa, MCC, and the ability to generalize to new data. The most effective algorithm was selected for further validation, ensuring that the chosen model was robust and reliable for practical forensic applications. The approach not only provided a rapid and non-destructive means of characterizing fibers but also showed promise for high accuracy in distinguishing between different fiber types. The creation of an ATR/FTIR spectral database, as proposed in this study, would allow for the quick identification of fibers found at crime scenes, thereby aiding in the investigation process. By comparing unknown samples to a comprehensive library of known fiber spectra, forensic experts could identify fibers with a high degree of confidence, supporting the identification of suspects or victims.

Forensic Analysis; Fibers; Machine Learning

B65 Addressing Challenges in Mineral Identification by Raman Spectroscopy

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Learning Objectives: After attending this presentation, attendees will understand the challenges with mineral identification by Raman spectroscopy and the analytical techniques for overcoming them when using advanced Raman imaging techniques like Particle-Correlated Raman Spectroscopy (PCRS)

Impact Statement: This presentation will impact the forensic science community by offering practical approaches for the reliable identification of soil minerals using Raman spectroscopy.

Abstract Text: Raman spectroscopy is a valuable non-destructive tool for investigating the chemical properties of diverse samples. However, challenges with regards to mineral identification set important limits for the practical use of Raman spectroscopy for forensic soil analysis. These limitations consist of fluorescence (including for minerals that may have characteristic spectra1), orientation effects, spectral masking by substrates, sample destruction (especially regarding dark samples), and natural variations within a mineral variety, which can cause meaningful spectral differences with regard to both manual and library identifications.[1] These have additional relevant repercussions with respect to applications of new advancements in Raman spectroscopic imaging, such as hyperspectral imaging and PCRS. With Raman imaging methods, a large number of Raman spectra (thousands to tens of thousands or more, potentially) are collected so spectral acquisitions must be very rapid for each data point (on the order of 1 second), thus materials that are weak Raman scatterers are not necessarily able to be identified and methods such as photobleaching become impractical.

This presentation will feature the results and conclusions from the method development of PCRS for the forensic discrimination of soil minerals, specifically with regard to overcoming limitations to mineral identification by Raman spectroscopy with short analysis times (1second collection, 1 acquisition per spectra). Samples analyzed in this study included the standard 60 common minerals from the Cargille Comminuted Mineral Set M-4 in addition to minerals from topsoil samples collected from diverse locations in the Northeast United States, which had been prepared for traditional forensic soil microscopical analysis (undergoing washing, sieving, and heavy mineral separation).[2] The individual mineral grains were then each probed by Raman microspectroscopy using two different lasers excitations (532nm and 785nm).

Several practical solutions were discovered during the course of this research to address challenges to the Raman identification of minerals. The first, and potentially most valuable, was the creation of a large and robust spectral library of diverse minerals capable of automated searching, which was made from Raman spectra in the RRUFF mineral database containing close to 10,000 different Raman mineral spectra.[3] This ensured that the greatest number of spectral variations within a mineral were included in the analysis. Next, although many mineral species do enable characteristic identification, some minerals have indistinguishable Raman spectra. For these minerals where distinct identification is not possible, classes of minerals were identified instead. Additionally, the use of two laser excitations for the interrogation of mineral grains afforded a greater number of minerals to be identified. Another critical feature is using an appropriate sample substrate for the desired method to avoid interference. In this research, four substrates were investigated (glass, aluminum, silicon, low-E glass), each having advantages and limitations with respect to research with Raman spectroscopy. For PCRS analysis, low-e glass slides proved to be best because they have no spectral interference nor any surface features to interfere with automated imaging. Despite the limits of Raman spectroscopy for the identification or classification of minerals, it is a valuable tool for forensic soil investigations, especially when paired with automated particle imaging techniques such as PCRS.

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Raman Spectroscopy; Forensic Soil Analysis; Mineral Identification

B66 The Characterization of Residues Left by the Impact of Different Types of Firearm-Propelled Projectiles

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Learning Objectives: This report addresses the characterization of residues produced by different types of projectiles when they impact various materials. The study aims to enhance the forensic analysis of crime scenes involving firearm use by identifying the elemental composition of residues found on impacted surfaces.

Impact Statement: This study not only characterizes the elemental composition of residues left by different types of projectiles on fabric targets but also provides support for the development of more robust forensic protocols. These protocols can assist in the accurate identification of firearms and calibers used in crimes, contributing to more effective investigations and the advancement of forensic sciences.

Abstract Text: The analysis of firearm discharge residues has become very frequent in forensic activities due to the frequent use of these instruments in criminal offenses.[1] When a shot is fired, the projectile is propelled at high speed and temperature, potentially impacting different types of targets at a crime scene and leaving residual particles along its path. Thus, to respond to technical questions formulated by the justice system, instrumental analysis methods have been developed to detect and characterize these residual elements from discharges that are deposited on clothing, parts of the victim's body, and other various targets that are part of our daily lives.[2] However, Criminalistics still lacks predictive elemental characterization studies of the passage of different types of projectiles through the most common everyday targets. In this context, this work aimed to identify the most frequent residues left by different types of projectiles on fabric targets.

Samples were collected by firing different types of projectiles (jacketed, semi-jacketed, and lead alloy) using three firearms (Glock 9mm pistol, Taurus .38 Special revolver, and Taurus .357 Magnum revolver) at cotton fabric targets from a distance of two meters. The residues around the entry holes were analyzed using Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS). Elemental compositions were quantified in terms of weight and atomic concentration percentages. Multivariate statistical analyses, including Principal Component Analysis (PCA) and Soft Independent Modeling of Class Analogy (SIMCA), were conducted to identify patterns and classify the residues. The PCA revealed two distinct clusters: one for .38 caliber residues and another grouping 9mm and .357 residues, indicating differences in elemental compositions. The SIMCA model showed an accuracy of 84.72% in classifying the residues. The findings support the development of robust forensic protocols to identify firearms and calibers used in crimes, contributing to more effective investigations and advancements in forensic sciences.

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Ballistics; Gun Shot Residue; Crime Scene Investigation

B67 Standards Development Activities in Human Forensic Biology

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Learning Objectives: 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: The 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. It will also increase awareness regarding training, tools, and resources that support implementation and compliance monitoring.

Abstract Text: The Organization of Scientific Area Committees for Forensic Science (OSAC) was created in 2014 to strengthen the nation's use of forensic science by promoting the use of discipline-specific standards. To this end, the OSAC drafts standards, and best practice recommendations are forwarded to Standards-Developing Organizations (SDOs), such as the Academy Standards Board (ASB). In turn, the SDO then further develops and publishes them as American National Standards Institute (ANSI) 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. ASB develops standards with the ANSI framework and provides training to support those standards. The OSAC also reviews standards and recognizes them on the OSAC Registry. The OSAC Registry serves as a central repository of high-quality, consensus-based, technically sound standards. The standards go through a technical and quality review process that encourages feedback from the forensic community. Standards and best practice recommendations are then placed on the Registry following a consensus vote of both the OSAC subcommittee and the Forensic Science Standards Board.

This presentation will provide updates related to standards development in human forensic biology testing. These include:

- What's new with the ASB DNA Consensus Body
- All published ASB standards and best practices and documents on the OSAC registry
- Documents that are currently being drafted by the Human Forensic Biology Subcommittee of OSAC and those under development by the ASB

To date, ASB has 17 standards and two best practice recommendations published. The OSAC Registry has 14 published standards and 1 best practice recommendation along with 5 proposed standards and best practice recommendations within its repository. An update to these numbers will be provided during the presentation. All published standards and best practice recommendations are available for immediate download at aafs.org/academy-standards-board and from the OSAC Registry. 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.

Serology; Forensic DNA; Standards

B68 An Evaluation of Semen Extraction Efficiency and Recovery After Washing From Reusable Period Underwear

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Learning Objectives: The attendees of this presentation will learn about the ability to extract sufficient sperm cells from reusable period underwear in order to develop complete and quality DNA profiles. The attendees will also learn about the persistence of semen in the underwear after washing and any degradation or inhibition that may be present in the developed profiles.

Impact Statement: This presentation will impact the forensic biology community in a positive way by leading to the development of a recommended protocol for the effective analysis of period underwear.

Abstract Text: Various items of evidence for sexual assault cases are submitted to the forensic biology section of a crime laboratory to develop a DNA profile that can identify the perpetrator of the crime, including clothing items like pants, shirts, and undergarments.[1] The type of undergarments as well as the materials they are made up of can make it difficult for a DNA analyst to obtain enough DNA to generate a usable profile. Period underwear are a new class of undergarments and feminine products that women can use during their menstrual cycles or for minor urine leakage. These non-disposable feminine products with absorbance and leak-proof capabilities can be washed and re-worn multiple times.

The research began with the process of cutting open the crotch area of the three period underwear brands (Hanes, Knix, and Thinx) in order to determine the crotch area composition and sperm recovery by layer. Common materials present in the crotch area of all the brands consisted of a moisture-wicking layer, which allowed the underwear to feel dry to the touch, absorbency layers, and a leak-proof layer, which allowed the body fluid to remain in the underwear and not end up on other clothing items.[2-4] The top layers of the crotch area, which are the moisture-wicking and absorbency layers, retained the majority of the cellular material, so all the layers of the crotch area were evaluated together for each brand. Thinx had significantly reduced sperm counts compared to Hanes and Knix (35.90% vs. 82.13% and 74.75%, respectively).

Simulated post-coital samples were placed on the period underwear and evaluated as having not been washed, washed once, and washed twice. All samples were analyzed through the entire forensic workflow, including sperm counts, extraction, quantification, amplification, and capillary electrophoresis. More DNA was recovered from Hanes and Knix than from Thinx, which was congruent to sperm counts. Sperm and DNA yields were analyzed by comparing the post-coital samples to both liquid and swab controls. The ability to obtain sufficient spermatozoa and DNA from the samples were evaluated using profile completeness and peak heights as well as the evaluation of any inhibition or degradation present in the samples. No inhibition or degradation was observed in the samples. Overall, the ability to extract sufficient sperm and DNA from reusable period underwear was present but more significant in the brands Hanes and Knix than from Thinx. Based on the data, we recommend cutting strains through to the leak-proof layer for maximal sperm and DNA yields.

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Extraction; Semen; Recovery

B69 Swab Technology Is Advancing: Are We Putting Research Evidence Into Action?

Jillian Conte, PhD, National Institute of Justice, Peckville, PA; Tracey L. Johnson, MSFS, Department of Justice/Office of Justice Programs/National Institute of Justice, Washington, DC*

Learning Objectives: After attending this presentation, attendees will understand which swab types are best to use for specific evidence types and sources of DNA. Attendees will also be aware of research gaps that exist for studying the use of different swab types for different evidence types.

Impact Statement: This presentation will impact the forensic science community by providing detailed information and graphics that are evidence-based on using different types of swabs to collect biological evidence.

Abstract Text: Forensic biology technology advances quickly: DNA typing technologies are increasing in sensitivity, resolving DNA mixtures is improving, and isolating and targeting of male-specific DNA continues to become more streamlined. However, one part of the forensic biology workflow that has remained relatively unchanged is the type of swab used to collect samples. Swab composition technology has advanced, yet cotton swabs continue to be the primary choice for biological evidence collection.

This research relates the results of a systematic review and analysis to determine which swab types are best for collecting biological evidence. We reviewed research literature and described the results below. An article was included in the review if it is an original research article, discusses more than one swab brand or material, has a forensic focus, and reports data from real-time quantitative Polymerase Chain Reaction (qPCR) or forensic DNA typing. Articles were excluded if they were not primary research (literature/systematic review) or not written in the English language or if the product was a thesis or dissertation. The literature was collected through searches in Web of Science, PubMed, and EBSCO in September 2023. Removal of duplicates and selection of articles was performed in Rayyan. Additional articles were identified through a review of the bibliographies of initially selected articles.

The analysis was organized by substrate (porous, n = 9; non-porous, n = 8) and source of DNA (n = 5). Forty-one substrate-DNA source combinations have been researched, and 13 substrate-DNA source combinations have an identified best-performing swab type. Nylon swabs were the best performing swab for glass-saliva, ammunition-saliva, skin-semen, textiles-touched surface, and leather-touched surface. Cotton swabs were the best-performing swabs for directly on swab-blood, explosive devices-blood, glass-saliva, and ammunition-touched surface. For collection of semen from the vagina and acellular DNA directly on a swab, rayon swabs have performed best. Recovering DNA from a touched drywall surface calls for using a bicomponent fiber swab and a foam swab is best for recovering saliva from wood. Polyester and microfiber swabs were studied throughout the literature and did not perform optimally.

Our study revealed multiple research gaps and trends in the current body of research. There are also several limitations in the study, including heterogeneity of data and selection bias during the literature search and study identification. The primary observations made are that DNA extraction chemistry needs to be considered with swab type, swabs made of the same material do not perform the same when compared to each other, and inter-operator swabbing is not different. This work highlights research gaps that should be addressed for substrate-DNA source combinations and can guide practitioners in making evidence-based decisions on implementing different swab types into their workflow.

Swab; Forensic DNA; Biological Evidence

B70 A Quantitative Assessment of Extraction Efficiencies Obtained From Various Methods and Sample Types

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Learning Objectives: This presentation will provide attendees with an understanding of the importance of quantitatively assessing the extraction efficiency of a method starting with a known amount of DNA and through the absolute quantitation digital Polymerase Chain Reaction (PCR) offers. Additionally, it will provide information about the statistical significance, if any, that different extraction methods and DNA sample types may have on the subsequent extraction efficiency.

Impact Statement: This presentation will impact the forensic community by providing quantitative data and statistical analyses regarding the significance of an extraction method on the resulting percent yield of extracted and purified DNA. This information can be used to aid forensic laboratories in choosing the most appropriate DNA extraction method for specific sample types to maximize efficiency.

Abstract Text: In order for a forensic Short Tandem Repeat (STR) DNA profile to be obtained from biological samples, a minimum amount of extracted and purified DNA is required. In most cases, this amount ranges from 0.5 to 1.0 nanograms. Certain sample types encountered in forensic laboratories may present challenges when it comes to obtaining the minimum required amount of extracted DNA. These challenges can be compounded when an extraction results in low efficiency, meaning that a large percentage of the genetic material necessary for the downstream forensic DNA typing is lost during the extraction process, which can result in a partial DNA profile.

A variety of studies evaluating the impact of extraction methods on the ability to obtain a full DNA profile are available. In these studies, extraction efficiency is quantified by identifying the number of samples that produced full profiles divided by the total number of extracted samples.[1,2] However, this study aims to focus on the amount of DNA present pre- versus post-extraction to quantify the amount of DNA lost to each method in the hopes of identifying ways in which this can be minimized, as well as to provide a benchmark of average efficiencies attained after extraction for comparison against alternative methods used by a forensic laboratory. Additionally, the data obtained from this study was used to determine if there is a statistically significant difference between the overall recovery of two specified extraction methodologies for three different sample types.

For this study, two methods of DNA extraction were assessed: manual extraction carried out via silica spin columns and automated extraction carried out via silica-covered magnetic beads. Three sources of DNA (human fibroblast cells, whole blood, and extracted DNA) were extracted at nominal concentrations of 50, 20, 10, 5, and 1ng by the two methods. To determine the starting amount of DNA in the whole blood and cells, the method of flow cytometry was used. White blood cell counts were measured by flow cytometry and reported by the blood bank. Cultured cells were counted through flow cytometry measurements at the National Institute of Standards and Technology (NIST). The extracted DNA used was Component A of Standard Reference Material 2372a: Human DNA Quantitation Standard. These values were used to approximate the amount of total cellular DNA tested at each concentration prior to extraction. Dilutions of the starting material were performed to generate each desired concentration. The resulting extracts were quantified with the use of digital PCR with nuclear DNA assays designed and optimized in-house.[3] Preliminary results indicate extraction efficiencies across all quantities of DNA, cell types, and extraction techniques ranging from 34.9% to 99.0%. Human fibroblast cells and whole blood samples were found to have higher efficiencies on average. Further work is being explored to determine any statistical significance regarding the differences observed between cell types, sample concentration, methodologies, and kit chemistries.

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Forensic DNA; Extraction; Quantitation

B71 Innovative Lab-on-a-Disc Technology for Rapid Differential DNA Extraction in Forensic Science

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Learning Objectives: This presentation will provide attendees with a comprehensive understanding of the lab-on-a-disc principle and its groundbreaking application in differentiating forensic evidence. Participants will explore the fundamental concepts of microfluidic technology in forensic science, delve into the design and operation of a lab-on-a-disc platform for automated differential extraction, and gain insights into the intricate process of separating female epithelial cell DNA and male sperm cell fractions. Moreover, attendees will examine a comparative analysis between the innovative lab-on-a-disc method and traditional manual techniques.

Impact Statement: This research promises to revolutionize the forensic science community by introducing an advanced methodology for differential DNA extraction. The automated lab-on-a-disc platform offers significant advantages, including dramatically reduced processing time, increased accuracy and reproducibility through minimized human error, potential cost savings due to reduced labor requirements, and improved efficiency in processing sexual assault evidence.

Abstract Text: Lab-on-a-disc is an innovative microfluidic technology that consolidates various laboratory functions onto a rotating disc, allowing for automated analysis of biological samples, including sexual assault evidence.[1-5] This sophisticated platform employs a series of miniaturized processes such as mixing, separation, and detection, significantly enhancing the efficiency of laboratory workflows.

In this study, a specialized lab-on-a-disc was developed for differential extraction from sexual assault samples. The disc integrates multiple processes seamlessly, including sample transfer, two-step separation, concentration, and washing. When a pre-lysed sexual assault sample, primarily containing female epithelial cells, is introduced onto the disc, it automatically separates the sample into distinct fractions: cell debris or fabric (contaminant fraction), epithelial cell DNA (female fraction), and sperm cells (male fraction). The sperm cell fraction is subjected to an additional washing step, leading to the retrieval of purified and concentrated sperm cells, which can then undergo lysis and Short Tandem Repeat (STR) analysis.

A comprehensive case study was conducted to compare the lab-on-a-disc method with traditional manual extraction techniques using both mock and actual sexual assault samples. The results indicated that the lab-on-a-disc method yielded comparable or superior quality DNA results while significantly reducing processing time to less than 10 minutes, compared to the 2–3 hours typically required for manual procedures.

This groundbreaking automated platform holds the potential to minimize human error, improve reproducibility, and reduce operational costs by decreasing labor requirements. Additionally, it opens avenues for expanding its application to encompass the entire differential DNA extraction process from raw sexual assault samples, ultimately providing both epithelial cell DNA and sperm cell DNA as final outputs. The adoption of this technology could represent a significant leap forward in forensic science, streamlining processes and enhancing the efficiency and accuracy of DNA analysis in sexual assault cases. By leveraging the capabilities of the lab-on-a-disc, forensic laboratories could improve their response times and the reliability of their analyses, ultimately contributing to more effective justice outcomes.

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Lab-on-a-Disc; Sexual Crimes; Differential DNA Extraction

B72 A New DNA Collection Tool Kit: Bringing Versatility and Flexibility to Analysts

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Learning Objectives: Attendees will learn about a new novel DNA collection tool that brings flexibility and versatility to DNA analysts. The presentation will demonstrate that the new tool can: (1) increase the amount of DNA recovered from potential evidence samples, and (2) allow multiple attempts of DNA genotyping through Direct Polymerase Chain Reaction (PCR) and traditional methods.

Impact Statement: Our patent-pending novel sample collection products provide a toolkit that can provide versatility and flexibility to DNA analysts. By utilizing storage cards in a manner that can collect a wide range of samples, the tool will be able to reduce the current burdens in DNA analysis.

Abstract Text: The demand for DNA testing continues to grow and has contributed to an ever-increasing backlog of samples that require analysis. Traditional DNA typing encompasses a multi-step process that can take a considerable period of time. A significant amount of investment has been made to shorten the time it takes to generate a DNA profile. Direct DNA amplification (direct PCR) was developed to genotype samples from the source, avoiding the time-consuming extraction and quantitation processes and alleviating backlog issues. However, Direct DNA amplification can be limited by sample type, collection methods used, and regional requirements.

Storage cards, such as FTA Elute cards and Nucleic-Cards, are traditionally used to preserve blood and saliva samples that are provided for diagnostic purposes (clinical and forensics). Research has shown that when storage cards are used in more unorthodox scenarios such as collecting trace evidence, they can improve DNA recovery and analysis. However, when moistened to collect dry samples, storage cards can tear, deform, and lose their integrity.

Our toolkit is designed to provide the end-user with the flexibility to choose the appropriate collection and analysis methods (choosing from direct PCR, traditional, or both protocols) that would increase the likelihood of success. Furthermore, the use of the tool would allow repeat testing as direct PCR methods would not destroy the entire sample. The tools can utilize commercially available storage cards (e.g., Qiagen FTA Elute cards or Copan Nucleic-Cards) to collect samples so they can be analyzed using Direct PCR or traditional methods when required. The toolkit comes with interchangeable platforms that allow for collection from various surfaces (angled/trace) and maintain the structure and integrity of the card, regardless of whether it has been moistened. In addition, an internal water source is available to moisten the card when required, eliminating the potential hazards of manual pipetting and exposure to contamination.

In this study, the collection device was used to collect dry saliva, blood, and mock sexual assault evidence samples that had been deposited on various apparel materials. Our preliminary results show that in comparison to cotton and nylon swabs, the new tool can significantly increase the amount of DNA collected from the biological fluids deposited. When 10µl blood was deposited on cotton, the device was able to recover 0.85ng/µl DNA; in comparison the swab recovered 0.36ng/µl. This trend was observed regardless of whether the fluids were deposited on cotton, polyester, or jean. In addition, our study has demonstrated that analysts can choose between direct PCR or traditional methods. When 10µl of single source biological fluid was deposited, a single storage card from the device (from either FTA Elute cards or Nucleic-Cards) could be used to generate four full STR profiles. Using 1.2mm punches from the cards and direct PCR protocols, full STR profiles were observed using GlobalFiler (Applied Biosystems™) and PowerPlex Fusion 6C (Promega) STR amplification kits; full STR profiles from the STR kits were also observed using the remaining card and traditional methods.

Our patent-pending novel sample collection products provide a toolkit that can provide versatility and flexibility to DNA analysts. By utilizing storage cards in a manner that can collect a wide range of samples, the tool will be able to reduce the current burdens in DNA analysis.

Direct PCR Amplification; Bodily Fluid; Backlogs

B73 Differential Extractions—Defining the Problem

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Learning Objectives: Attendees will gain a better understanding of why current differential extraction procedures often yield less than ideal results and how to evaluate the success of current procedures and/or apply changes to the current procedures.

Impact Statement: After attending this presentation, attendees will have an increased understanding of why current procedures used for differential extractions often provide less-than-ideal results and how to evaluate the success of current procedures and/or process changes to current procedures. This presentation will impact the forensic community by providing a detailed description of the range of outcomes observed when using differential extraction methods, increasing participants' understanding of the reasons for this variation and by providing examples of how to evaluate the procedures used in their laboratory.

Abstract Text: The original procedure for differential extraction was published in *Nature* in 1985 by Gill, Jefferys, and Werrett in one of a series of papers that introduced the possibilities of the uses of DNA identification.[1] The differential extraction procedure has undergone minor modifications over the years but, from a chemistry standpoint, remains similar to the originally published procedure. The process allows for the consecutive lysis of female epithelial cells (E-cells) and male sperm cells from a sexual assault sample. Thus, the removal of the E-cell lysate is followed by the lysis of sperm cells. Some version of this process is used by most laboratories for analysis of sexual samples. This could be hundreds to thousands of samples per year, depending on the size of the jurisdiction served by the laboratory.

In the process of exploring ways to improve current differential extraction procedures, we found that careful characterization of the samples, the process, and the outcomes was needed. All samples were dried onto cotton swabs, as this is the most common swab type used in Sexual Assault Examination Kits (SAEK). The samples used included swabs with known and unknown amounts of buccal cells, known amounts of sperm, unknown amounts of vaginal epithelial cells (from anonymous donors) ± known amounts of sperm and unknown amounts of cells on post-coital samples. Samples were extracted using the Qiagen EZ1 protocol for differential extraction, with and without modifications, and with an experimental process for differential extractions.[2] For each extraction done, the original swab was recovered from the spin basket and DNA from any cells remaining on the swab was recovered using Acrosolv from MicroGEM Bio.[3] This fraction is referred to as the Material Fraction (MF). All extracts, Epithelial Cell Fraction (EF), Sperm Fraction (SF), and Material Fraction (MF) were quantitated using Quantifiler Trio (Thermo Fisher). Total amounts of male and female DNA in each fraction were calculated based on quantitative Polymerase Chain Reaction (qPCR) results for the Y and small autosomal probes and the volume of each fraction.

The amount of total female DNA per half swab from donor swabs and mock evidence post-coital swabs showed almost a 10,000ng difference from the lowest to the highest amount. The amount of female DNA remaining on the swabs post-extraction and the amount of male DNA remaining on the swab post-extraction was substantial in some samples and maybe donor specific. Procedures to facilitate the release of sperm from the cotton were explored and chosen based on what is known about sperm surface chemistry. Additional time with a protease and higher temperatures facilitated sperm removal from the cotton while not affecting the quality of sperm profiles and reducing the amount of sperm remaining in the MF.

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Forensic DNA; Sperm Cell Recovery; Differential Extraction

B74 Criminalistics Gone Wild (in the Courtroom)—Human Factors, Activity Level, and Probability, Oh My!

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Learning Objectives: After attending this presentation, attendees will have gained insight into how forensic reporting and courtroom testimony have been impacted by innovative technologies and new machine learning tools. Specifically, attendees will hear how the different forensic disciplines have been changing and adapting to address current challenges and mitigate biases in reporting and testimony.

Impact Statement: Forensic sciences have long struggled with how to report and testify forensic results in a succinct and comprehensive manner. This panel of experts will explore the challenges in reporting and testifying in today's advanced technological environment in a manner that offers the transparency that the field and public demands.

Abstract Text: In more recent years and with the emergence of technological advances and increased media exposure of the forensic science community, the public has increased its interest and focus in the forensic sciences. As such, there has been an increasing need for practitioners to be more comprehensive in their explanation of tests and results in a court room. More specifically, forensic practitioners are expected to explain the potential for bias in their processes and be well versed in discussing other relevant topics such as how they addressed human factors concerns, the false positives and false negatives of the testing utilized, and the limitations of specific methodology and instrumental techniques.

During this interdisciplinary session, current reporting and court testimony challenges will be discussed, along with recommendations in overcoming those challenges. In addition, attendees will participate in discussions regarding technological advancements and how they are steering future approaches for presenting forensic results in court. The session will be comprised of an interactive panel of forensic experts representing different disciplines to ask and answer questions, provide feedback, and offer different perspectives in how the different disciplines are navigating through the increased public demands for clear, transparent and objective reporting and testimony. Experts from trace, DNA, and seized drugs will discuss how their disciplines have been shedding more light onto the potential limitations of applied methodology in their respective fields as well as the increased participation in collaborative research to explore better ways to objectively report forensic results. In addition, a representative from Jurisprudence will foster an environment of meaningful dialog on how the forensic science community as a whole can address current courtroom testimony challenges that are significantly impacting public perception of forensic sciences. Finally, the panel of experts will answer questions on the potential role that artificial intelligence tools play in helping practitioners meet the increasing needs of law enforcement, medical examiners, and public health agencies.

Testimony; Reporting; Criminalistics

B75 A Proteomic Analysis of Semen for Human Identification and Body Fluid Classification

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Learning Objectives: During this presentation, attendees will learn about semen proteomics and the impact of environmental degradation on both forensically relevant DNA and protein markers. Attendees will also learn about mass spectrometry and its use for inferring genotype and body fluid classification.

Impact Statement: This presentation will impact the forensic science community by shifting human identification into a dual DNA-proteomics perspective and will also shed light on circumstances that may require a more proteomics-informed strategy.

Abstract Text: This research explores the prospect of a proteomics approach to semen analysis. Forensic analysis of human semen has historically been conducted under the microscope for body fluid confirmation or using Short Tandem Repeat (STR) analysis for human identification. However, these methods can prove challenging if samples are heavily degraded or if the source of the sample is vasectomized or azoospermic. Unlike DNA, protein has peptide bonds that are more resistant to cleavage, making protein markers potentially good candidates for degraded samples. Furthermore, there are hundreds of proteins in seminal plasma that can be used for human identification and body fluid classification, thereby obviating the requirement of spermatozoa in the semen sample. In terms of human identification from proteomic information, DNA markers can be passed down genetically and can be inferred in the protein sequence. These Genetically Variant Peptides (GVPs)1 offer the prospect of more discriminating analysis, with random match probabilities near 1 in 50,000 from human semen samples. For body fluid classification, there have been a number of studies demonstrating the use of Body Fluid-Specific Peptides (BFPs)2 to body fluid classes such as blood, semen, saliva, and vaginal fluid. However, their utility under degrative conditions is not well understood.

This research focuses on the ability to detect DNA information in the form of STRs and proteomic information in the form of GVPs and BFPs for pristine and degraded human semen. For sample processing, semen was diluted with DPBS buffer and aliquoted into fractions for STR typing and proteomic analysis after manual degradation. For proteomic analysis, a dialysis-based method was used instead of a detergent-based method, as this yielded the largest number of unique peptides. For DNA analysis, samples were extracted using the Qiagen EZ1 DNA investigator kit, qPCR with Quantifiler Trio, and STR typing using Powerplex Fusion 6C. Results from STR analysis of DNA material displayed no apparent degradation after seven days of incubation at 70°C, both in terms of loss of STR alleles and in qPCR degradation index, thereby illustrating how well established and robust STR typing is. For the GVP analysis, 77 GVPs have been identified from pristine seminal fluid. However, after incubation at 70°C for seven days, the number of detectable GVPs reduced to 32. Proteins most resistant to environmental degradation include proteins such as SEMG1 and SEMG2, which function as gel-forming coagulation factors whereas proteins which exhibit the most degradation include MUC5B and PIGR, which function in sperm protection and immunity. Further, BFPs declined from 58 in pristine samples to 34 after seven days of degradation. This resulted in the identification of a robust set of 34 peptides, of which three are unique to humans, that can be used for reliable body fluid classification. Results from site-specific glycosylation analysis reveal that most glycosites from semen glycoproteins consist of the same glycan structure with varying degrees of fucosylation. Further study is being conducted to explore the relationship between these glycans and epigenetic information such as age. Further work is also being explored for ancestral classification using GVPs from semen and determining peptide-specific detectability using extracted ion chromatogram abundances.

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Proteomics; Semen; Degradation

B76 An Advanced Nucleic Acid Lateral Flow Assay for Rapid and Sensitive Identification of Human Vaginal Fluid Using Molecular Beacon Technology

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Learning Objectives: Attendees will learn following: (1) that an alternative method of rapid test exists that is completely protein free and hence highly reproducible, with more weather resistance and shelf life; (2) that a rapid test based on lateral flow assay can be confirmatory and have multiplexing possibilities of up to six different antigens/markers within a single strip that is 6–12mm wide; (3) that with proper focus on science and research, we may utilize micro-RNAs as forensic investigators: and (4) new methods of Gold-DNA conjugation.

Impact Statement: In my presentation, the forensic science community will gain insights about: (1) how criminalistics is very important to understand "'what'" crime took place and '"how"' it was performed; (2) how body fluid identification plays an important role in all forensic investigations and that it needs a special attention for further research and advancements; (3) how we can utilize micro RNAs for development of rapid tests, Nucleic Acid-based Lateral Flow Assays (NALFAs) and that these tests will not be presumptive but confirmatory; (4) how we do not need to rely on 1 or 2 protein markers for rapid body fluid identification. Instead, we can combine up to 6 markers in a single rapid test; (5) we are the first to try and develop a nucleic acid lateral flow assay for vaginal fluid detection(6) we are also the first to reduce the limit of detection to sub picomolar levels; and (7) We also demonstrate for the first time an alternative way of Gold-DNA conjugation in a lateral flow assay.

Abstract Text: The detection of vaginal fluid poses several challenges, particularly in forensic and clinical settings. Key challenges include the lack of specific biomarkers unique to vaginal fluid, rapid sample degradation, small sample volumes, interference from other substances, complex composition, the high cost and time of traditional methods, cross-reactivity, ethical and privacy concerns, and the need for standardization and validation. To address these challenges, we present a protein-free NALFA designed to identify body fluids by leveraging DNA-based rapid tests and molecular beacon technology.

Our approach targets micro-RNAs, enabling the simultaneous testing of multiple antigens within a single assay. The system employs a spot-based rather than a line-based format, utilizing a single gold conjugate for both test and control spots. Multiple reaction zones are situated in separate lanes to prevent cross-reactions, allowing for distinct flow dynamics and reaction times in each lane. This setup ensures optimized conditions and predictable, reproducible sample flow, contributing to the assay's high sensitivity, with detection limits below 1pmol of antigen. The semi-quantitative analysis capability, combined with miniaturization, reduces sample and production material usage, leading to cost-effective and faster overall assay times.[1]

By targeting specific micro-RNAs unique to vaginal fluid, our assay overcomes the challenge of identifying reliable biomarkers. This specificity reduces the likelihood of false positives and increases the accuracy of vaginal fluid detection. The use of nucleic acid-based detection mitigates the issue of sample degradation. DNA and RNA are more stable than proteins, over wider range of temperatures ensuring reliable results even when samples degrade over time. The high sensitivity of our assay allows for accurate detection with minimal sample volumes, addressing the issue of small sample sizes commonly encountered in forensic cases. Our method's specificity for micro-RNAs reduces interference from other body fluids and environmental substances, minimizing false positives and enhancing the reliability of results. By focusing on nucleic acids rather than proteins or other complex components, our assay simplifies the detection process, making it more robust against the complex composition of vaginal fluid. Traditional methods, such as DNA methylation and sequencing, are costly and time-consuming. Our NALFA approach eliminates the need for specialized equipment and skilled personnel, significantly reducing both costs and assay time. Our assay's design allows for standardized protocols that can be easily adopted in various forensic and clinical laboratories, ensuring reproducibility and reliability across different settings.[1]

Using our advanced NALFA approach, we have demonstrated confirmed detection of seminal fluid and vaginal fluid from human samples with no cross-reactivity with blood, saliva, or other frequently tested body fluids. DNA mimics of micro-RNAs were used as antigens for assay development, which was later validated with human samples. Three to four different micro-RNA markers for each body fluid were tested and verified, ensuring robust detection. Given the challenge of vaginal fluid detection in sexual assault cases and crime investigations, our focus includes a detailed discussion on this application.

Our researched and validated approach represents a significant advancement in molecular diagnostics, offering a highly sensitive, rapid, and cost-effective solution for the identification of body fluids. Thus, we provide a streamlined, reliable alternative to current state-of-the-art methods, making it a practical choice for forensic and clinical applications.

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Bodily Fluid; Criminalistics; Crime Scene Investigation

B77 Integrating Genetic and Epigenetic Sequencing for Forensic Application in a Single Workflow

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Learning Objectives: Attendees will learn about the advancements in simultaneous genetic and epigenetic sequencing for forensic purposes, the novel methodology developed for integrating genetic and epigenetic analysis into a single workflow, the application of this methodology in forensic profiling ,including age prediction and Body Fluid Identification (BFID), and the evaluation of the efficiency, sensitivity, and specificity of the developed approach through various fluids.

Impact Statement: This presentation will introduce a groundbreaking methodology that integrates genetic and epigenetic sequencing into a single workflow, significantly enhancing forensic profiling. By streamlining processes and combining analyses, this method promises to save time, resources, and sample consumption. This innovation has the potential to revolutionize forensic science, providing more comprehensive and efficient tools for human identification, age prediction, and body fluid identification.

Abstract Text: Epigenetic research has increasingly become crucial in forensic science, aiding in age estimation and BFID. Traditionally, molecular workflows have required separate processes for genetic and epigenetic analyses, leading to increased complexity and resource use. To overcome these inefficiencies, we have developed an innovative methodology that combines both genetic and epigenetic sequencing into a singular, cohesive workflow.

Our approach employs an enzymatic-based library preparation method integrated with hybridization capture techniques, simultaneously targeting core Short Tandem Repeat (STR) loci and CpG markers essential for BFID and age prediction. The library preparation involves multiple steps: hairpin addition, splicing, copy strand synthesis, adapter ligation, protection, deamination, and amplification. We designed 584 custom probes for effective target enrichment, followed by sequencing using the MiSeq FGx platform. A unique bioinformatics pipeline was created to resolve base pair readings (A, T, G, C, and methylated C) using a two-base coding mechanism by comparing R1 and R2 reads, with data interpretation performed via IGV and MixtureAce software.

For validation, blood, semen, buccal, and vaginal fluid samples were collected with informed consent, alongside a control sample. The DNA extracts were sheared to an average length of 250bp using the M220 focused-ultrasonicator (Covaris), enriched with methylation controls, and processed using the Duet multiomics solution +modC (Biomodal).[1] Following library preparation, samples were pooled, captured using Fast Hybridization Target Enrichment (Twist Bioscience), and sequenced on a microflow cell. Our results indicate a high conversion efficiency, with sensitivity exceeding 96% and specificity at 99.8%. We achieved average coverage of 30x for CpGs and up to 10x for most STR loci. The target enrichment process successfully demonstrated differential methylation patterns across the tested body fluids and enabled the acquisition of accurate partial STR profiles.

This groundbreaking research highlights a novel workflow that concurrently sequences STRs and CpGs, signifying a major leap forward in forensic science. By unifying genetic and epigenetic profiling into one streamlined procedure, our method provides a versatile and effective approach that aims to boost the forensic community's analytical proficiency and resource efficiency.

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Epigenetics; Genetics; Next Generation Sequencing

B78 A Microfluidic Device for the Rapid Serological Identification of Forensically Relevant Body Fluids

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WITHDRAWN

B79 The Identification and Optimization of MicroRNA Biomarkers for Accurate Body Weight Prediction in Forensic Samples

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Learning Objectives: Attendees will learn about a novel forensic DNA phenotyping approach for accurately predicting body weight class based on the Body Mass Index (BMI) using metabolism-related microRNA markers in DNA extracts from dried whole blood and saliva samples. Attendees will also gain an understanding of how the additional physical descriptive information obtained from this approach can assist in investigating cases with unknown individuals involved.

Impact Statement: This presentation will positively impact the forensic DNA community by introducing useful microRNA markers for accurately predicting the body weight class of unknown individuals, which can be easily integrated into the existing forensic DNA phenotyping panels. This research aims to acquire environmentally impacted phenotypical information from DNA evidence without increasing the sample consumption, time, and resources.

Abstract Text: Accurately profiling unknown individuals from biological samples is crucial in forensic DNA analysis. Conventional Short Tandem Repeat (STR) testing, while effective for direct identification, falls short in cases lacking suspects or database matches. Thus, an alternative method of using DNA evidence is needed to reveal more information about unknown individuals, such as their appearance traits or Externally Visible Characteristics (EVCs). Forensic DNA Phenotyping (FDP) utilizes genetic markers, such as Single Nucleotide Polymorphisms (SNPs), to predict EVCs of an unknown individual, including eye, hair, and skin colors.[1,2] However, predicting body composition, such as body height, weight, and muscularity, remains challenging due to the complex effects of environment and metabolism on genetic markers.

To enhance the capabilities of current FDP, it is critical to explore additional biomarkers like microRNAs (miRNAs), which are small, single-stranded, non-coding RNAs that mediate post-transcriptional gene regulation.[3] Their small size and protection by RNA-binding proteins give miRNAs exceptional stability, making them ideal for forensic samples subjected to long-term storage or environmental degradation.[4,5] Recent biomedical research has analyzed the mechanism of miRNA interference on mRNA and assessed their ability to control gene expression regarding the body metabolism.[6] Moreover, miRNAs responsible for regulating BMI-related genes affected the production of adipose tissue, leading to increased weight and fat percentage.[7] This responsiveness, unlike SNP markers that remain unaffected by metabolism, underscores the potential of miRNA markers for predicting EVCs influenced by environmental factors, including body weight.

This study introduces an innovative FDP approach by exploiting miRNA expression in DNA extracts from blood and saliva samples to predict an individual's body weight class. Our methodology encompasses Bioelectrical Impedance Analysis (BIA) for comprehensive body composition measurement, meticulous sample collection adhering to ethical guidelines, and DNA isolation followed by Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) for miRNA analysis. Our preliminary studies on dried whole blood DNA extracts showed that the expression of specific miRNA markers, including miR-486-5p, miR-885-5p, and let-7i-5p, demonstrated a positive or negative correlation with body weight. Using statistical models built upon the miRNA data, a 100% accuracy was achieved in predicting body weight categories (underweight, normal, overweight, obese), regardless of ethnicity or biological sex. Saliva, another commonly encountered body fluid type in forensic casework, was collected and analyzed in addition to blood to identify predictive miRNA markers for body weight. An expansion in the size and diversity of the donor population, including various ages, heights, weights, and ethnicities, was conducted to develop and maximize the confidence of our miRNA body weight prediction panel.

In conclusion, our study has shown the close association between body weight and miRNA expression in blood and saliva DNA extracts, highlighting the usefulness of miRNAs in predicting environmentally impacted EVCs beyond traditional FDP markers. This project not only aims to enhance current FDP by incorporating body weight prediction as a ready RT-qPCR miRNA analysis assay, but also seeks to set the stage for adding these validated markers into a commercial forensic high-throughput sequencing panel that is already employed in many forensic laboratories. The ability to detect miRNAs from DNA extracts can also eliminate the need for additional sample consumption and processing time, allowing for easy integration into the current forensic case workflow and more subject identity information for investigators in less time, especially in cold cases, missing-person searches, and victim identification in mass disasters.

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Body Weight Prediction; microRNA Analysis; Forensic DNA Phenotyping

B80 Dead or Alive? Biomarker Identification for Postmortem Blood Detection

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Learning Objectives: After attending this presentation, attendees will consider the possibility of using D-dimer as a biomarker to detect postmortem blood at crimes scenes.

Impact Statement: This presentation will impact the forensic science community by presenting a broad study of the applicability of D-dimer and immunochromatographic tests to identify postmortem blood.

Abstract Text: The detection of hemoglobin and glycophorin A through immunochromatographic tests is currently used to identify blood at crime scenes. However, depending on the forensic scenario, it may be important to determine whether a person was alive at the time of blood deposition. Based on the rapid onset of fibrinolysis after death, this pathway could be considered to identify potential biomarkers for postmortem blood. Fibrinolysis is the natural process that breaks down blood clots after healing a vascular injury. Among the fragments produced in fibrinolysis are soluble fibrin monomers crosslinked between two adjacent outer D domains detectable in plasma, known as D-dimers. Thus, D-dimer could be a potential biomarker for postmortem blood.

SERATEC has developed the PMB immunochromatographic assay to simultaneously detect human hemoglobin and D-dimer. The main goal of this study was to assess the possibility of using the PMB test to detect postmortem blood. Additionally, evaluation of D-dimer levels in peripheral, menstrual, and postmortem blood was carried out, and the ability to obtain Short Tandem Repeat (STR) profiles from postmortem blood was assessed. All postmortem blood samples reacted positively for the presence of hemoglobin using the SERATEC PMB tests, and all but one degraded sample showed a positive result for D-dimer. All peripheral blood samples from living individuals showed positive results for hemoglobin and negative results for D-dimer detection, except for one liquid sample with a weak positive, probably due to a previous blood condition. Menstrual blood samples gave positive results for hemoglobin but variable results for D-dimer. The DIMERTEST Latex assay was used for semi-quantitative measurement of D-dimer concentrations, with postmortem and menstrual blood yielding higher D-dimer concentrations compared to living peripheral blood. Full STR profiles were developed for all postmortem samples tested except for one degraded sample, pointing to the possibility of not only detecting postmortem blood at the crime scene but also the potential identification of the victim.

Postmortem Blood; D-Dimer; DNA

B81 The Design and Development of Novel Single Multiplex System Incorporating 26 Rapidly Mutating Y-STRs: 26 RM Yplex

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WITHDRAWN

B82 The Recovery of Probative aSTR and Y-STR Profiles From Single or a Few Micromanipulated Sperm Cells

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Learning Objectives: This presentation will provide attendees with an understanding of our sperm cell micromanipulation and genotyping methodology. This includes single sperm cell collection, direct sperm cell lysis, genetic analysis methods suitable for the low template DNA in sperm cells, and the application of probabilistic genotyping to both single source and mixture sperm samples.

Impact Statement: Potential impact on the forensic science community by sperm micromanipulation prior to DNA analysis would be to improve the recovery of probative genetic information from the male perpetrator(s) in challenging multiple semen donor sexual assaults. By isolating individual sperm cells for direct lysis and amplification, this approach avoids the loss of valuable sperm DNA while simultaneously avoiding mixtures of the multiple male semen donors and victim DNA. This approach for single sperm genetic analysis could also be applied to late-reported sexual assault, historic and remnant samples, as well as those from multiple sperm contributors.

Abstract Text: The ability to recover an autosomal STR (aSTR) profile of a semen contributor relies on the separation of sperm DNA from epithelial DNA. However, the sperm cells present in samples from delayed reported assaults can be weakened by prolonged exposure to the cervico-vaginal environment, which can result in premature sperm cell lysis to produce a sperm-epithelial DNA admixture. Because of this, it is typically not possible to recover aSTR profiles from samples collected 48–72 hours after a sexual assault using standard extraction and analysis methods, as the sperm DNA may be masked by the abundance of victim DNA.[1-3] For these samples, Y-chromosome STR (Y-STR) analysis is often used subsequent to aSTR analysis, as this approach preferentially amplifies the Y-chromosome. Y-STR profiles can provide useful investigatory leads, but unfortunately do not hold the same statistical weight as aSTR profiles.

We hypothesize that some of the limitations of standard sexual assault sample analysis can be overcome by using simplified micromanipulation to preferentially select rare individual or few sperm cells for direct lysis and amplification. We have previously demonstrated the successful application of our micromanipulation approach to epithelial cells.[4-12] In this study, we optimized and applied our micromanipulation methodology to sperm. This simplified approach utilities a water-soluble adhesive on the tip of a needle to collect individual sperm cells for direct transfer into a lysis solution. Following direct lysis, samples undergo a modified STR amplification, suitable for low template DNA samples.

Y-STR and aSTR analysis was performed on subsamples containing 1–5, 10, and 25 sperm cells collected from five semen donors. Each cell subsample was evaluated using five replicates, with a consensus profile generated from the replicates. For single cell aSTR analysis, the maximum allele recovery was 50% due to the haploid nature of sperm cells. For single cell replicates an average of ~20% recovery was achieved with a maximum recovery of 47%. Average allele recoveries for 2–5 cell replicates ranged from ~47–73% (with increasing percentages observed with increasing cell number). For 10 and 25 sperm cells, recoveries of 86.7%, and 99.0% were observed, respectively. Additionally, this method was applied to simulated sperm-epithelial cell (buccal and vaginal) admixtures with single source aSTR profiles obtained from collected sperm cells.

A similar trend was obtained for Y-STRs with average percent recoveries of ~13–65% for 1–5 cell subsamples, 78% for 10 cells, and 94% for 25 cells. A maximum of 92% recovery was achieved from a single sperm, with all other cell inputs having replicates resulting in full profiles. Compared to aSTR samples, greater variability in recovery was observed for Y-STR analysis, which can be attributed to only 50% of sperm cells containing a Y-chromosome. In addition to standard Y-STR analysis, Y-Targeted Pre-Amplification (Y-TPA), an in-house-developed nested Polymerase Chain Reaction (PCR) approach, was used for enhanced Y-STR analysis of single sperm cells. This resulted in an improved average allele recovery of ~25% and a maximum recovery of 100%. When applied to simulated sperm-epithelial cell admixtures, probative partial Y-STR profiles were recovered through use of Y-TPA.

Probabilistic genotyping was employed to further improve the recovery of aSTR DNA profiles from single sperm. The database matching and sample-to-sample matching features of FaSTR were used to successfully cluster single sperm aSTR profiles to specific donors. Once clustered, probabilistic genotyping replicate analysis (STRmix) was conducted, resulting in highly probative LRs and diploid STR profiles from each of the donors, potentially suitable for reference database searching. This PG approach was used to recover single source aSTR DNA profiles from complex 2–4-person semen mixtures.

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Forensic DNA; Single Cell; Probabilistic Genotyping

B83 The Humanitarian DNA Database (HDD): An Update

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Learning Objectives: Attendees of this presentation will learn about the HDD, a resource at the Center for Human Identification (CHI) at the University of North Texas Health Science Center at Fort Worth for associating missing and unidentified human remains in the United States (Texas) with families living outside of the United States.

Impact Statement: This presentation will discuss the potential to increase the identification of missing and unidentified remains using a collaborative effort to associate family references outside of the United States to DNA profiles discovered and genotyped in Texas by the Center for Human Identification.

Abstract Text: Due to the increase of global migration, Missing Person (MP) and Unidentified Human Remains (UHR) deceased person cases can have a cross-border impact. Often these cases are difficult to resolve in a national DNA database system. CHI at the University of North Texas Health Science Center at Fort Worth in collaboration with the Federal Bureau of Investigation (FBI) have developed the HDD to assist with the identification of deceased non-United States citizens recovered in the United States.

Conceptualized in 2018 in response to the challenges encountered by international agencies and non-governmental organizations in identifying deceased individuals recovered along migration routes in the Southwestern region of the United States, the building of the database was initially slowed due to the COVID-19 global pandemic.

Operated by CHI and supported by the FBI's Combined DNA Index System (CODIS) software, the HDD enables family references voluntarily contributed by foreign nationals to be compared to genetic information from unidentified, deceased individuals. The HDD database is air-gapped (an information technology term referring to isolation of a computer from any form of the internet) and contains DNA profiles from UHRs in Texas processed at CHI. With permission from the FBI, CHI can accept reference profiles that were generated by non-CODIS laboratories, including those from outside of the United States and search them in the HDD. The laboratories providing these profiles must meet certain quality criteria established by CHI and the DNA data must be reviewed by CHI prior to searching.

Allowing for the searching of DNA profiles generated by other laboratories in the HDD allows for potential associations to be made that would have not otherwise occurred and is being done in a humanitarian effort to provide closure to the families of missing persons. This is of particular interest with regard to unidentified human remains found along the Mexico-United States border.

In addition to the outreach and collaboration with international and non-governmental agencies for this effort, CHI is also the recipient of a grant from the United States State Department entitled *Reducing Human Trafficking Through Forensics in Central America* that involves the provision of technical and legislative support to countries in Central America. Countries in Central America have been significantly impacted by human trafficking and forensic DNA is an investigative tool that can assist the laboratories and governments within these countries to help combat it. While forensic DNA has not traditionally been viewed as a means of combatting human trafficking, the ability to properly develop, expand, and maintain DNA databases will add tremendous value in Central America, specifically Guatemala, El Salvador, Honduras, Panama, Costa Rica, and Belize. As a result of this grant, family reference profiles that were developed at the Instituto Nacional de Ciencias Forenses, which is the national DNA laboratory in Guatemala, are being shared with UNTCHI for searching in the HDD.

We will present an overview of the process to establish the HDD and present metrics on the successes of searches from the inception. We will also discuss future possibilities for the HDD.

Forensic DNA; Missing Persons; Unidentified Persons

B84 When You Come to a Fork in the Road, Recognize It: Mapping the FIGG Decision-Making Landscape

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Learning Objectives: Upon attending this presentation, attendees will come to recognize the decisional complexities involved with Forensic Investigative Genetic Genealogy (FIGG). Case studies will illustrate lessons learned, while others will prompt discussion by leaving open questions. Attendees will be guided through the FIGG process map of exactly where data-driven decision-making ends and data-absent gaps and risks begin.

Impact Statement: This presentation will impact the forensic science community by raising awareness of the need for responsible and ethical development of a standardized FIGG laboratory pipeline, while at the same time allowing for innovation and exception.

Abstract Text: FIGG has revolutionized the world of human identification but, with the revolution, has come the responsibility to recognize where data-driven decision-making ends and data-absent risks begin.

Standardization of FIGG is in its infancy. Policy is advancing. The majority of FIGG cases can and do follow a more or less defined path, but certain types of cases still challenge our decision-making efforts along that path toward a best outcome. Is a case a violent offence, an Unidentified Human Remains (UHR), or a threat to public safety? What if none of the above, but maybe? Can it still comply with the FIGG database terms of service? Also consider those tough cases that have precious little evidence remaining. Because blind spots exist in the FIGG pipeline, we must still rely on technologies designed for use with Short Tandem Repeat (STR) systems, so that we may find ourselves without the data required to take informed risks. A sample that is judged useless for STR analysis may actually be the best sample for FIGG, but how can we know? Suppose that sample is the last hope of solving a case? How do we weigh the risks of consuming the sample versus waiting for technology to improve? Once the decision is made to proceed, how do we determine which Single Nucleotide Polymorphism (SNP) processing method, if any, is best without data that characterizes the full range of outcomes? And how do we regulate the decision-making path without excluding innovation and exception? In this talk, we present case studies that illustrate the decisional complexities involved with FIGG. Some case studies represent lessons learned, while others leave us with open questions. The focus of this talk is to describe the current FIGG landscape to reveal where data-driven decision-making ends and data-absent risks begin.

Forensic DNA; Pipeline; Method Development

B85 Building Branches: Navigating Practical and Ethical Considerations for the Responsible Use of Forensic Investigative Genetic Genealogy (FIGG)

Claire L. Glynn, PhD, University of New Haven, West Haven, CT*

Learning Objectives: After attending this presentation, attendees will gain insight into the key steps in the FIGG workflow and how existing policies, guidance documents, and state legislation form responsible and ethical frameworks for conducting FIGG investigations.

Impact Statement: This presentation will impact the forensic science community by serving as guidance for those either beginning to implement FIGG or already conducting FIGG within their agencies/departments.

Abstract Text: FIGG combines the fields of forensic genetics with genetic genealogy and traditional genealogical research to generate investigative leads in criminal investigations, namely violent crimes (homicide and sexual assault) and the identification of Unidentified Human Remains (UHRs). It is estimated that FIGG has helped generate investigative leads in at least 1,000 case investigations in the United States in recent years.

FIGG traverses both the public sector and the private sector, with federal and state agencies and local departments developing in-house FIGG units/programs, and private companies offering FIGG services. Robust training and education in FIGG should equip analysts/practitioners with the core competencies necessary to interpret complex genetic data, understand genealogical methodologies, and navigate the ethical and legal implications involved. As this novel tool makes use of consumer DNA databases and goes beyond routine Short Tandem Repeat (STR) analysis of evidence samples, appropriate precautions, policies, and procedures must be developed and followed to safeguard individual privacy and uphold ethical standards when FIGG is implemented in forensic workflows. The intersection of privacy, ethics, and legal boundaries demands rigorous scrutiny, particularly as the field of FIGG continually evolves rapidly. A balance between leveraging genetic data to advance criminal investigations and respecting the privacy rights of all parties is attainable if all stakeholders make a commitment to adhering to best practices for maintaining transparency and accountability in FIGG investigations.

This presentation will address key steps in the FIGG workflow to include, but are not limited to, case qualification and triaging, use of public genetic genealogy databases, third-party DNA testing, privacy and protection of data, case reporting, and oversight. This presentation will highlight the importance of comprehensive training and education, continuous professional development, and interdisciplinary collaboration to enhance the accuracy and integrity of FIGG investigations. In addition, existing policy (e.g., the United States Department Of Justice [DOJ] interim policy), guidance documents (e.g., from the National Technology Validation and Implementation Collaborative [NTVIC] FIGG working group), and state legislation (e.g., Maryland, etc.) will be discussed. Attendees will gain insights into developing policies and procedures that protect both individual privacy and the integrity of the investigative process, ensuring that the application of FIGG adheres to sound science, legal requirements, and ethical principles.

Forensic DNA; Genealogy; Ethics

B86 Down the Rabbit Hole—Now Up a Tree: Solving Cold Cases Using Forensic Investigative Genetic Genealogy at the New York City Office of Chief Medical Examiner and the Queens District Attorney's Office

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Learning Objectives: In this presentation, attendees will gain a broader understanding of the importance of technological advancements, such as Familial Searching (FS) and Forensic Investigative Genetic Genealogy (FIGG), in solving cold cases. The lack of a hit of an unknown evidence profile to an individual or a suspect in the Combined DNA Index System (CODIS) is no longer the end of the road for a cold case but is rather another step along the path to justice.

Impact Statement: Attendees will learn the importance of collaborative efforts among law enforcement agencies to successfully solve cold cases. These collaborations are especially important for DNA laboratories, such as the New York City Office of Chief Medical Examiner (OCME), that do not currently perform FIGG testing due to limited resources. They will come to understand the challenges faced when subjecting older evidence to FIGG and FS testing and be taught strategies to overcome these obstacles.

Abstract Text: This presentation will highlight how, within the past 15–20 years, successful technological advances in forensic science have resulted in solved cold cases. Testing of crime scene evidence was once limited to serological testing. Today, however, we can associate these biological fluids with an individual through the development of a DNA profile and provide evidence to a jury that can positively associate someone to crime scene evidence or exonerate the innocent. This presentation will also demonstrate how FS and FIGG testing can be used to identify previously unidentifiable DNA profiles and enhance a prosecutor's ability to investigate and prosecute a cold case.

FIGG and FS testing requests by police departments and District Attorney's offices have steadily increased over the years, due to the volume of successful resolutions of cold cases. Many law enforcement agencies have obtained cold case grants and funding that has enabled them to seek FIGG and FS testing, even if their forensic laboratory does not have such monetary resources. The presenters will discuss the successful partnership between the Queens District Attorney's Office and the OCME that has enabled the testing to be effective. We will discuss the difficulties and complexities that arise when dealing with old cases. We will discuss the workflow and teamwork that is needed to prepare cold cases for FIGG and FS testing, both at the DAs office and in the forensic laboratory. Last, we will highlight a cold case solved using FIGG testing. This solved cold case is the first arrest and prosecution of a FIGG case in New York City and came to pass 45 years after the crime was committed. Despite what many people believe, cold cases aren't too cold or too old to investigate and prosecute.

This presentation will inspire the forensic community to continue its efforts to bring much needed answers to families and loved ones whose lives remain forever impacted by the senselessness of crime. The successful processing of cold cases illustrates how forensic science can, through the development and deployment of new technologies, continue to serve the criminal justice system and our society.

Genealogy; Forensic DNA; Collaboration

B87 The Development of Artificial Single Nucleotide Polymorphism (SNP) Data for Investigative Genetic Genealogy (IGG) Research and Training: A Preliminary Study

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Learning Objectives: After attending this presentation, attendees will understand how high-quality human SNP data, specifically concerning those markers analyzed in IGG databases, can be synthesized using artificial intelligence methods, in particular deep neural networks. Attendees will also be introduced to an alternative to human data for practitioner training as well as optimization of Identical-By-Descent (IBD) detection and matching algorithms across varying population structures.

Impact Statement: This presentation will provide the forensic community with a Convolutional Neural Network (CNN) architecture that can learn the haplotypic structure of human SNP data efficiently to generate high-quality artificial samples. With synthetic SNP profiles, IGG practitioners have a surrogate for protected human data that is flexible and can be used as a training resource, contributing to the need for the development training materials for forensic genealogical familial matching statistics identified by the Organization of Scientific Area Committees (OSAC) Human Biology subcommittee as of October 2021.

Abstract Text: IGG is a relatively new technique for identifying suspects of violent crime and unidentified human remains.[1] It employs a combination of traditional genealogical research strategies with genetic testing of SNP markers. Such markers are used to capture the genetic variation between individuals and trace familial lineages by means of IBD detection and matching algorithms.[1-3] The longer segments of shared DNA-IBD that are present, the more closely related two individuals are, following the rules of genetic inheritance.[2,3]

Our central hypothesis is that when inbreeding is found within a family tree (e.g., multiple relationships and pedigree collapse) or a population (e.g., endogamy), it will artificially elevate the background levels of IBD segments, resulting in relationship misclassification between two or more individuals.[1,4] This hypothesized effect was observed in 2020 by Thomson et al., and the measure of coancestry was the subject of numerous statistical computations described as early as the 1920s.[4,5]

Given the increase in the number of cold cases resolved with the aid of IGG, it is necessary that the methods applied accurately classify relationship states and clearly report the significance of those relationship predictions.[6-9] As far as can be determined, there is little, if any, existing literature that provides a comprehensive assessment on the effect of inbreeding on background IBD in IGG searches. To achieve such an aim, IBD detection and matching algorithms will be trained on known, non-inbred individuals. Data from an inbred population will be subsequently analyzed and the relationship classification performance assessed for different degrees of inbreeding. However, each of these objectives necessitates extensive sampling of human subjects and accurate genealogical records. Therefore, our strategy was to develop a dataset of realistic, yet artificial, human SNP data, allowing greater flexibility in downstream experimental design.

For this presentation, artificial SNP profiles were simulated in RStudio® for the length of SNPs in an Ancestry® DNA profile and used to assemble an outbred family tree spanning four generations. The family tree included twelve individual profiles categorized into two groups, viz., the two founders and the spouses of their descendants, as well as all the direct descendants of the two founders. For all union couples, direct descendants were generated by programming for genetic recombination in RStudio®. To assess a relative recombination rate for realistic fragmentation of inherited SNPs, pairwise comparisons of the proportion of shared DNA were performed for all relationship states through analysis in GEDmatch® . For all pairwise comparisons of the individuals, the measured centimorgan values were observed to fall within predicted shared IBD ranges for all relationship states. To produce realistic profiles, a dataset of up to 100 artificial human SNP profiles for a single chromosome was produced using a convolutional variational autoencoder (VAE) programmed in Python® by using PyTorch.[10, 11] The network was trained on publicly available data from the 1000 Genomes Phase 3 dataset which includes 2,504 individuals spanning 26 populations.[11-13] For each generated profile, the SNPs were pruned to include only those markers present within both the 1000 Genomes Phase 3 dataset and an Ancestry® DNA profile, preserving SNP

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Investigative Genetic Genealogy (IGG); Variational Autoencoders (VAEs); Identical-by-Descent (IBD)

B88 The Coldest Cold Case in Croatia: The Body in the Chest Freezer

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Learning Objectives: After attending this presentation, the participants will gain insight into circumstances that hamper further progress in investigation, leading to a case becoming cold. They will also understand the complexity when the case is opened with new leads and the importance of comprehensive recovery, collection, and forensic examination of sensitive evidence that may have degraded over time.

Impact Statement: This presentation will impact the forensic science community by demonstrating investigative actions in the case reopened after 14 years, particularly regarding the successful recovery and identification of a fingermark from a frozen storage environment and DNA mixtures analysis. The consideration of the case specifics and approach demonstrated this case may improve professionals' methods for managing cold cases and handling and analyzing evidence recovered many years after a crime has been committed.

Abstract Text: Cold cases are considered those cases where further progress could not be made due to a lack of evidence, leads, and/or omissions in the initial investigations, resulting in long-time inactivity.[1] As time goes by, the probability that the case will be solved decreases as evidence degrades, witnesses' memories fade, and suspects become more challenging to locate.[2] However, turnarounds, like new clues or evidence, can sometimes reopen the investigation.

This presentation will demonstrate investigative actions and forensic examinations in a case reopened with new evidence after 14 years, emphasizing the successful recovery and identification of a fingermark frozen for at least 19 years and the crucial role of mixed DNA profiling.

The case study begins in the summer of 2005, when a mother reported her daughter missing, with no contact since 2000, except for her sister, who claimed she was abroad and out of touch. Police found the missing person's mobile phone and outdated documents at the family home, casting doubt on the sister's story. Despite multiple interrogations, the case remained unsolved for years.

In February 2019, the daughter of the suspect and her partner decided to remove an old freezer in the family home that had remained unopened for years. When they opened the freezer and began emptying it, the partner cut open a black bag inside and discovered human remains, specifically legs and female genitalia, and called the police.

Upon scene examination on February 16, 2019, it was determined that the freezer had been sealed with a cyanoacrylate-based adhesive, which was confirmed by chemical and physical examination. Inside the freezer, the body was found unclothed, wrapped in a colorful sheet, along with black plastic garbage bags. Continuing the investigation at the pathology department where the freezer and the body were transported, it was found that the body was wrapped in various materials, including black nylon bags and stockings, with blood stains on the coverings and around the neck. The autopsy revealed 5–6 blunt force injuries on the left side of the face and forehead, with no defensive wounds.

In the Forensic Science Center, the victim's identity was confirmed through a fingerprint match from the right index finger of the unidentified body and the fingerprint collected to issue an ID card. Three fingermarks were developed by cyanoacrylate fuming on PVC bags and rubber glove fragments, out of which only one, found on the black plastic bag wrapped around the victim's head, was suitable for identification. Upon examination, it was proved that fingermark originated from the suspect's (victim's sister's) right thumb. DNA analysis of swab samples from the nylon stockings used to tie the plastic garbage bag around the legs revealed a mixed DNA profile that could be attributed to the deceased and her sister.

After considering all the evidence and establishing the facts, the court found the accused guilty of murder and sentenced her to 15 years in prison. In the presented case, the fingermark was recognized as the oldest one identified in Croatia and, globally, one of the rare instances where a fingermark was successfully recovered from a frozen storage environment after many years.

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Cold Case; Fingerprint Identification; Forensic DNA

B89 A 2025 Update From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

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Learning Objectives: The goal of this presentation is to provide a summary of the most recent activities and work products developed by SWGDRUG.

Impact Statement: SWGDRUG supports the development of internationally accepted minimum standards for forensic examination of seized drugs, identifies best practices within the international community, and provides resources to help laboratories meet these standards.

Abstract Text: 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 year.

The SWGDRUG core committee members met in the summer of 2024 and approved the most recent version of the SWGDRUG Recommendations, version 8.2, which includes minor revisions to 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. The most significant revision in version 8.2 of the core recommendations occurs in PART IVB (*Quality Assurance/Validation of Analytical Methods*) and associated definitions in the Annex. Revisions include information on the performance characteristics to be evaluated during the validation of both qualitative and quantitative methods. In addition, revisions to Supplemental Document SD-7 (*Methods of Analysis/Analytical Scheme for Identification of Drugs or Chemicals - Construction of an Analytical Scheme*) to include examples of analytical schemes for clandestine laboratory evidence were also completed. SWGDRUG also hosted its first webinar at the start of 2024.

Future work for the committee will involve the revision of the currently existing Supplemental Document SD-2 (*Validation of Analytical Methods*), revisions to SD-2 to incorporate sampling scenarios when negatives results are encountered, guidance on policies for cease testing and reporting procedures, structural elucidation procedures, and additional webinars and presentations.

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, practitioner and scientists from the United States; and representatives from the European Network of Forensic Science Institutes (ENFSI), the Iberoamerican Academy of Criminalistics and Forensic Studies (AICEF), the Asian Forensic Science Network (AFSN), the Australia New Zealand Forensic Science Society (ANZFSS), the Canada Border Services Agency (CBSA), and the United Nations Office on Drugs and Crime (UNODC).

SWGDRUG; Drug Analysis; Criminalistics

B90 Reference-Free Identification of Fentanyl Analogs

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Learning Objectives: In this presentation, the audience will learn details on the following: (1) limitations of the current analytical paradigm for Mass Spectrometry (MS) -based identification and quantification of small molecules (<1,000 Da): (2) a new MS-based workflow for identifying small molecules without reliance on reference materials and including advanced multi-dimensional analyses coupled with computational predictions of measured molecular properties; and (3) demonstration of the new workflow in reference-free identification of fentanyl analogs.

Impact Statement: This presentation will introduce new MS-based experimental and computational capabilities for reference-free identification of small molecules. The integrated workflow is demonstrated in reference-free identification of fentanyl analogs within a chemical space of two billion possible analogs.

Abstract Text: Fentanyl and other synthetic opioid-related deaths have outpaced all other overdose-related deaths in the United States. This situation is exacerbated by the continuous development of new compounds by clandestine manufacturers to avoid detection. Conventional MS-based methods used in forensic toxicology and criminalistics laboratories require comparing experimental data from sample analysis to similar data obtained from the analysis of pure reference compounds. With the potential for ~1,012 analogs, the reliance on authentic reference compounds is not realistic for efficient interdiction of novel fentanyl analogs.

The *m/q* Initiative at Pacific Northwest National Laboratory is developing capabilities for MS-based reference-free identification of existing and emerging fentanyl analogs, including: (1) prediction and prioritization of novel fentanyl analog structures, (2) prediction of experimental observables such as ultra-high precision molecular Collision Cross Sections (CCS), fragmentation spectra and infrared ion spectra, and (3) new multi-dimensional experimental capabilities, such as Structures for Lossless Ion Manipulations (SLIM) coupled with Orbitrap MS and SLIM coupled with cryogenic infrared ion spectroscopy and MS.[1-3] Through combinatoric analysis, we generated ~two billion fentanyl analogs that were prioritized based on predicted risk and ease of synthesis, with validation performed on a hold-out set of molecules. For these, ultra-high precision CCS calculations were performed using the HP-Ω tool, and Tandem Mass Spectrometry (MS/MS) spectra predicted using the graph neural network tool QC-GN2oMS2.[2] A mixture of the validation molecules was analyzed using the novel SLIM-Orbitrap, with experimental values compared to predicted values. Calculated CCS values showed errors ≤2% compared to measured values, and the average cosine similarity for predicted high-resolution MS/MS spectra was 0.82 when using fentanyl-focused training data. From SLIM-Orbitrap analyses, we report multi-dimensional signatures correlated to gas-phase molecular conformations diagnostic of fentanyl analogs.[3] All nine fentanyls in the mixture exhibited two IMS peaks that fragment differently, and the intensities of the two IMS peaks changed as a function of water concentration.

Together, these experimental results strongly suggest that all the fentanyls we studied exhibit two gas-phase protomers, and it seems likely all fentanyls will exhibit two protomers. Further, these multi-dimensional signatures can all be computationally predicted with high accuracy and serve as a pattern of features for confident identification of true fentanyls. Finally, to evaluate the integrated experimental and computational capabilities in a blinded analysis of fentanyl analogs, we measured a fentanyl mixture using SLIM-Orbitrap and SLIM-cryoIR-MS and compared the experimental data to predicted data for the two billion fentanyl analog chemical space. A workflow was developed to down-select the fentanyl analog chemical space based on matching predicted data to experimental data, resulting in an average of five candidate fentanyl structures per experimentally measured analog.

This work was funded by the *m/q* Initiative, under the Laboratory Directed Research and Development (LDRD) Program at Pacific Northwest National Laboratory (PNNL).

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Mass Spectrometry; Reference-Free Identification; Fentanyl

B91 Using Chemical Profiling and Chemometrics for Predictive Fentanyl Geographic Associations

Agnes D. Winokur, PhD, Drug Enforcement Administration, Miami, FL*

Learning Objectives: After attending this workshop, attendees will: (1) be able to better understand the challenges associated with associating fentanyl cases to each other and to geographic locations in seized drug analysis; (2) be able to describe the advantages and limitations of three chemometrics algorithms; and (3) be able to explain how chemical profiling and chemometrics can be used in support of forensic science intelligence investigations.

Impact Statement: This workshop will benefit the forensic science community by highlighting how algorithms can be applied to chemical profiles of fentanyl seized drug samples and build predictive models to associate and classify samples and determine potential geographic origins.

Abstract Text: Qualitative and quantitative data from Gas Chromatograph/Mass Spectrometry (GC/MS), Gas Chromatography/Flame Ionization Detection (GC/FID), and Fourier Transform Infrared (FTIR) spectrophotometry from chemical analysis of seized fentanyl samples (n=439) was used to evaluate the potential of developing chemical profiles to associate the fentanyl samples to specific geographic locations. Data from chemical compounds within these fentanyl samples (additives, excipients, contaminants, etc.) were normalized and clustered by location. The combinations of reported compounds and their relative concentrations resulting in chemical profiles for each of the fentanyl samples were insufficient to associate samples to a given geographic location for the purpose of creating a predictive model. However, the data show that chemometric models offer an approach to the classification of fentanyl samples, including limited geographic clusters, based on their chemical profiles.

An evaluation of the raw chemical data using three chemometric algorithms: the linear Partial Least Squares Discriminant Analysis (PLSDA) method, Artificial Neural Networks Discriminant Analysis (ANNDA), and Support Vector Machines Discriminant Analysis (SVMDA) were used to classify fentanyl samples to a specific geographic location. The objective of this study was to demonstrate the potential of using chemical data of fentanyl samples to build algorithms to predict geographic origin. The performance of the three algorithms were evaluated and presented for fentanyl samples (n=198). SVMDA was found to be better at dealing with non-linear relationships when compared to PLSDA. SVMDA generated the best predictive model by correctly associating 95% of the samples to the geographic location of the seizure. While the best results were for Class "C" as expected, it was surprising to see good predictive results for Class "A," which only had 13% of the data points. The best results overall were provided by the SVMDA model, not only for the calibration and cross-validation, but also for the independent prediction sample data set. For Class "C," 20 out of 22 samples were predicted correctly for a 90% accuracy using ANNDA and 21 out of 22 were predicted accurately using SVMDA (for a 95.45% accuracy). It was surprising to find good predictions for the other classes. For Class "A," there was an accuracy of 85% prediction (6 out of 7) using ANNDA and SVMDA. The samples from Class "B" were also correctly predicted using ANNDA and SVMDA. Samples for Class "G" were accurately predicted using ANNDA (100%). The results also demonstrated that chemical profile data composed of 55 different compounds found in fentanyl samples can be combined and used to associate fentanyl samples and provide intelligence about potential geographic sources.

Fentanyl; Chemometrics; Chemical Profiling

B92 Profiling Fentanyl in a Local Drug Market and Characterizing Variability Through Quantitative Chemical Analysis

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Learning Objectives: After attending this presentation, attendees will gain an understanding of the analytical and chemometric methodologies used to investigate the homogeneity and consistency of recreational drug materials sold as powdered fentanyl. The presentation will explore similarities and dissimilarities among samples, based on their branded markings and chemical compositions, providing insights into the complexity of drug adulteration and distribution within the Philadelphia, PA, fentanyl market.

Impact Statement: This presentation will enhance the forensic science community's understanding of the uniformity and variability of drug products. It will also demonstrate how inter-sample variability can be leveraged for forensic investigations and public health interventions through quantitative chemical analysis and sophisticated data processing.

Abstract Text: Understanding the consistency and homogeneity of recreational drug products is crucial for both public health and forensic investigations. Pairing Gas Chromatography/Mass Spectrometry (GC/MS) with an automated data processing algorithm allowed our laboratory to assess the homogeneity of fentanyl samples representative of Philadelphia's drug market during 2021 and 2022. Beyond simple identification, this study focused on the quantification of controlled and non-controlled substances, producing profiles of samples used for multivariate comparisons. Test groups of fentanyl samples were created for association and discrimination based upon having similar packaging and branded markings (a.k.a. logos, stamps, or labels).

At the Center for Forensic Science Research and Education, our laboratory conducts applied research and performs drug market trend surveillance and confirmatory quantitative analysis for public health and safety agencies, aiming to assess drug safety, monitor market changes, and guide harm-reduction strategies. Our current workflow includes qualitative analysis using GC/MS and externally calibrated quantitative analysis using GC/MS for fentanyl, xylazine, 4-anilino-N-phenethylpiperidine (4-ANPP), fluorofentanyl, lidocaine, cocaine, caffeine, and methamphetamine. Quantitative ranges for analytes are from ~0.5% (dependent on the amount of the prepared sample) to 100% by mass.

Quantitative data were processed using a custom algorithm, automating the processes of defining and testing pairwise equivalence among samples. The algorithm determines equivalence based on standard deviation of replicate preparation, a relative factor of the mean, and/or the absolute deviation from the mean. Our findings reveal interesting patterns of both similarity and dissimilarity among drug samples branded as a common product (i.e., identical packaging and stamp). In a case of association, two test groups of fentanyl powder, each group with a different packaging stamp, showed noteworthy inter-group similarities. Their intra-group quantitative results were: (1) 10.29 ± 0.25% and (2) 10.15 ± 0.31% for fentanyl; (1) 1.44 ± 0.05% and (2) 1.43 ± 0.07% for 4-ANPP; (1) 15.11 ± 0.68% and (2) 14.64 ± 0.51% for xylazine; and (1) 2.32 ± 0.11% and (2) 2.24 ± 0.08% for caffeine, demonstrating marked consistency in both qualitative and quantitative findings. In a case of discrimination, several samples of fentanyl powder branded with the same stamp (presumably sold as the same product) were divided into two distinct sub-populations based on the chemical differences of their contents: the first sub-group had quantitative results of 2.71 ± 0.17% for fentanyl, 0.15 ± 0.03% for 4-ANPP, 7.78 ± 0.57% for xylazine, and 21.07 ± 0.83% for caffeine; the second sub-group contained only fentanyl and 4-ANPP, with concentrations of 17.57 ± 0.58% and 0.63 ± 0.05%, respectively. Samples for each group were prepared separately and analyzed across different days.

These results demonstrate that while many similarly branded fentanyl batches exhibit consistency, other batches can vary significantly in their composition. This is especially concerning when samples are sold with consistent packaging and stamps. The potential for overdose is elevated when there is an expectation of consistency among common sample types, but the actual fentanyl content varies more than five-fold, as demonstrated by identically stamped samples ranging from 2.71% to 17.57% fentanyl by mass. The observations from this study highlight the need for comprehensive chemical analysis and refined data processing to monitor trends and uncover these variabilities. The traditional role of forensic drug chemistry in identifying controlled substances is extended here to include detailed quantitative analysis, which when coupled to the presented data processing methods, have the potential for linking and distinguishing drug samples. Such associations/discriminations would also lend vital capabilities to public health efforts to curtail the distribution of particularly harmful batches of street drugs as well as furthering strategic intelligence and public safety investigations pertaining to drug distribution.

Overdose; Fentanyl; Drug Analysis

B93 Longitudinal Insights Into the United States' Illicit Fentanyl Supply From the Characterization of Counterfeit Fentanyl Tablets

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Learning Objectives: After attending this presentation, attendees will be able to describe findings of applying various means of characterizing counterfeit illicitly manufactured fentanyl tablets and what they tell you about trends in tablet composition over a three-year period.

Impact Statement: This presentation will impact the forensic science community by raising awareness of the utility of illicit drug profiling in understanding the illicit drug threat.

Abstract Text: Illicit drug profiling of illicitly manufactured fentanyl, especially in the form of counterfeit oxycodone tablets, involves the application of a comprehensive set of analytical techniques aimed at identifying the chemical composition, origin, and distribution patterns of these exhibits. Given the significant public health threat posed by these drugs, the profiling process is an important tool for informing both law enforcement and public health initiatives about changes in the drug supply, from manufacturing and precursor supply to drug potency and harm reduction efforts.

Fentanyl, a potent synthetic opioid, and counterfeit oxycodone tablets are often clandestinely produced with varying degrees of purity and potency, containing potent principal component drugs such as fentanyl and fluorofentanyl, adulterants, reaction by-products, and unreacted precursors. This can lead to increased risks of overdose and death. Profiling these drugs involves both qualitative and quantitative analyses. Using previously described and validated workflows, we present the results of three years of monitoring the composition and signature of counterfeit oxycodone tablets seized adjacent to the Southwest border between the United States and Mexico.

Tablet exhibits were grouped based on their physical appearance, color, dimensions, and monogramming. Subsamples of each exhibit were selected and subjected to qualitative Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography quadrupole Time-Of-Flight/Mass Spectrometry (LC-qTOF/MS), and quantitative Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) analysis. These methods enable the identification of active ingredients, cutting agents, and impurities, which can provide insights into the manufacturing process and origin.

For this report, we describe the analysis of data from 262 counterfeit drug exhibits consisting of 2,058 tablets seized between Q3 2020 and Q1 2024, which were tested qualitatively to identify drugs and adulterants, and of which 1,684 pills were also tested quantitatively for fentanyl. Across seven different sites, with numbers of tablets ranging from 15 to 1,167, the fentanyl content of the tablets was in aggregate consistent but ranged from 1.0±0.3mg to 1.9±0.3mg. However, in terms of individual tablet variability, there were significant outliers, with some tablets containing as little as 0.012mg of fentanyl and as much as 6.6mg. There were also seasonal fluctuations in the fentanyl content of the tablets, with the most recent quantitative trend being a decline in fentanyl purity from a high of 2.4mg/tablet in Q4 2022 to 1.1mg in Q3 2023. Overall, the potency of fentanyl in 2023 was slightly higher than in 2022. This unpredictable variability in potency, especially at the higher end, is a likely contributor to accidental overdose and other adverse events. The other constituents of the tablets were also consistent, irrespective of their origin of seizure, consisting of fentanyl, 4-ANPP, acetaminophen, metamizole, and mannitol. Only 2% of the tablets contained other opioids, principally para-fluorofentanyl. By and large, tablets containing both fentanyl and para-fluorofentanyl contained more of the latter than the former. Para-fluorofentanyl has a potency estimated to be 2.5 times less potent than fentanyl, while other studies suggest that the two drugs are equipotent. Unreacted precursors and reaction by-products (e.g., para-fluorophenethyl-4-ANPP, N-BOC-norfentanyl, N-BOC-4-ANPP, and others) were also identified, as were other adulterants, including tramadol, xylazine, methamphetamine, lidocaine, levamisole, and pentobarbital.

Illicit drug profiling not only aids in law enforcement efforts to dismantle trafficking networks but also supports public health initiatives by providing data that can inform prevention and intervention strategies. By understanding the specific characteristics of illicitly manufactured fentanyl and counterfeit oxycodone tablets, agencies can better predict and respond to emerging threats, ultimately aiming to reduce the prevalence and impact of these dangerous substances. The data above demonstrate that greater insights into the nature of the illicit drug threat can be achieved through more comprehensive testing.

Drug Analysis; Signature Identification; Mass Spectrometry

B94 The Optimization of Soft Ionization by Chemical Reaction in Transfer Coupled to a Time-of-Flight Mass Spectrometer for Rapid Seized Drug Screening

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Learning Objectives: After attending this presentation, attendees will better understand the analysis of seized drugs via Soft Ionization by Chemical Reaction in Transfer (SICRIT) Mass Spectrometry (MS). In addition, attendees will discover the optimization of the above system for the application of general seized drug screening.

Impact Statement: This presentation will impact the forensic science community by discussing a recently developed Dielectric Barrier Discharge Ionization (DBDI) source, SICRIT, and its optimization for the general screening of seized drugs. This rapid analysis requires no auxiliary gases and provides an alternative to other gas-dependent Ambient Ionization (AI) sources like Direct Analysis in Real Time (DART).

Abstract Text: Increasingly, AIMS is being employed for forensic applications, including seized drug analysis. Given the increased presence of emerging drugs and novel psychoactive substances amidst backlogs, AI sources, such as DART, provide a rapid analysis. SICRIT allows for further minimization of reliance on auxiliary gases while maintaining this rapid analysis, holding the potential to be fieldable and more accessible.

This research employed a JEOL AccuTOF LC-Express connected to a recently developed in-line DBDI source (Plasmion SICRIT) operated in positive polarity mode.[1] A Gas Chromatography (GC) inlet heating attachment was coupled to the SICRIT for sample introduction. Samples were introduced using a stainless-steel Swagelok holder for glass microcapillaries. An aerodynamic-assist interface (IonSense Vapor Interface) was used to help entrain the sample through the heater and source. Optimization of the method focused on identifying the Vapor Flow Rate (VFR) (rate at which ambient air enters the SICRIT source), the temperature of the heater, the plasma generation voltage, and the plasma generation frequency. The MS method parameters, including the In-Source Collision Induced Dissociation (IS-CID) voltages, were replicated from previous work.[2]

Various common seized drugs, adulterants, and diluents of interest were included within this optimization: fentanyl, heroin, cocaine, N-N-Dimethylpentylone (DMP), methamphetamine, and alprazolam. Increasing the VFR increased the peak area of compounds, with the exception of heroin that showed no general trend. This increase was substantial from 1.5L/min to 1.75L/min with a significant reduction in background due to the improved transport of the compounds through the heater, SICRIT, and AccuTOF inlet. Increasing the temperature of the heater increased peak areas for fentanyl and alprazolam while that of methamphetamine decreased. Cocaine and heroin plateaued after 220ºC and DMP showed no general trend. Increases in the plasma generation voltage increased peak area up to 1,800V. Methamphetamine, alprazolam, and DMP peak areas remained consistent after 1,800V while those of heroin and fentanyl decreased. With increased voltage, a greater charge for ionization is present, which must be compromised with increased fragmentation of present compounds and the stability of the plasma. Increasing SICRIT frequency increased peak areas until 15MHz, after which values plateaued. Optimized values for this general seized drug screening method were found to be a VFR of 1.75L/min, an introduction temperature of 240ºC, a SICRIT voltage of 1600V, and a SICRIT frequency of 15MHz. These parameters were chosen to maximize peak areas while minimizing fragmentation and the presence of dimerization within spectra, notably for alprazolam, heroin, and fentanyl. Similar to DART and other AI sources, the spectra primarily feature that of the protonated molecular ion peak with limited fragmentation. While many compounds have similar spectra to that of DART-MS, some show a difference in observed fragmentation and dimerization of compounds is observed at lower concentrations. Notably, fentanyl observes a fragment at *m/z* 150 in SICRIT-MS as opposed to *m/z* 189 that is seen in DART-MS.

After method optimization, a validation was conducted using a panel of single component solutions of common seized drugs at IS-CID of 30V, 60V, and 90V. This focused on obtaining a greater understanding of the potential challenges and limitations for the implementation of SICRIT-MS for qualitative seized drug analysis. This presentation will address the proposed methodology accuracy, precision, reproducibility, specificity, limits of detection, environmental factors, robustness, and potential for use in casework. Additionally, this research compares obtained SICRIT-MS spectra to that of DART-MS to increase understanding of this AI source and its fragmentation. To investigate the applicability to seized drug analysis, realistic samples were obtained and analyzed following the optimized method. Results were compared to those obtained by GC/MS.

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Seized Drugs; Dielectric Barrier Discharge Ionization; Time-of-Flight/Mass Spectrometry

B95 The Development of an Analytical Scheme for the Identification of Nitazene Compounds

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Learning Objectives: An analytical scheme to qualitatively identify nitazene compounds using various types of instrumentation, including Gas Chromatography/Flame Ionization Detection (GC/FID), Gas Chromatograph/Mass Spectrometry (GC/MS), Infrared (IR), Gas Chromatography coupled with Vapor Phase Infrared Detection (GC-IRD), Direct Analysis in Real-Time Mass Spectrometry (DART-MS), and Direct Analysis in Real-Time-Tandem Mass Spectrometry (DART-MS/MS), with each compound and also identify any method limitations.

Impact Statement: Nitazenes have entered the illicit drug market and have raised public health concerns. Due to their structural similarities, forensic identification has proven to be a challenge. In this study, we will identify an appropriate analytical scheme using various instrumentation to support the identification of these nitazene compounds.

Abstract Text: Nitazene compounds (2-benzylbenzimidazole) are a class of synthetic opioids that have recently emerged as a significant public health concern due to their potency compared to other opioids. The development of an analytical scheme for identifying nitazene compounds is crucial to law enforcement and their forensic counterparts. This study aimed to determine an appropriate qualitative analytical scheme to differentiate and identify nitazene compounds. As with any emerging substance, it is critical for forensic analysts to know the limitations associated with the instrumentation used and the minimum number of methods required for an appropriate analytical scheme to support the differentiation and identification of each substance.

A series of nitazene reference materials were analyzed using various types of instrumentation, including GC/FID, GC/MS, IR, GC-IRD, DART-MS, and DART-MS/MS. For each compound, we were able to identify method limitations associated with each technique and determine a final analytical scheme to support the identification. Most forensic laboratories analyzing seized drug evidence use GC/MS with Electron Ionization (EI) to identify nitazenes, but many of these compounds have very similar EI spectral patterns due to their structural similarities. Since additional confirmatory data was needed, the reference materials were analyzed using GC-IRD to determine if the vapor phase infrared spectra could provide structural confirmatory data for isomers. Although not part of this study, this technique would also provide adequate separation from the complex matrices these nitazene compounds tend to accompany in seized drug evidence. GC/FID data was also obtained; although it lacks confirmatory data, it did provide variations in retention time, which is critical when trying to differentiate compounds that have similar EI spectral data. The presumptive data provided the required information for a proper qualitative identification. DART-MS and DART-MS/MS provided soft ionization mass spectral data that allows for both mass separation and confirmatory identification. With these techniques, the soft ionization, extremely low limits of detection, and speed of analysis posed valuable additions to the analytical scheme. Finally, due to the high purity of the materials and the accessibility of IR for forensic laboratories, IR data was also collected. The IR data that was collected provided structural information for these compounds; however, the practicality of using this technique while analyzing seized drugs in casework may pose an issue due to the low purity and complex combinations of substances found in samples containing nitazenes. A combination of these techniques is required to provide complimentary data to make an accurate identification.

Nitazenes; Forensic Chemistry; Analytical Chemistry

B96 Novel Approaches to Increase Specificity for Difficult Isomeric Separations With Ultra-High Performance Liquid Chromatography (UHPLC) for the Analysis of Seized Drugs

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Learning Objectives: After attending this presentation, attendees will understand different approaches to achieve UHPLC separations for complex isomeric compounds as evidenced by JWH-018 positional isomers. In addition, attendees will understand novel approaches of enhancing specificity when compared to more traditional drug analysis separation techniques such as Gas Chromatography (GC).

Impact Statement: This presentation will impact the forensic science community by displaying evidence of enhanced separation specificity of positional isomers using UHPLC. These methods aim to demonstrate alternative approaches to methods currently used within the field for resolving complex isomeric mixtures.

Abstract Text: GC, the standard separation technique for forensic drug analysis, is problematic for thermally degradable, polar, and non-volatile analytes, which can require sample preparation steps such as derivatization and/or liquid-liquid extraction. Conversely, UHPLC, which affords separations complementary to GC, is amenable to the dilute-and-shoot analysis of seized drugs. For both GC and UHPLC, extensive overlap exists for the separation of positional isomers, which are highly prevalent for Novel Psychoactive Substances (NPS). In this vein, various reverse phase chromatographic approaches were investigated to increase retention time specificity for UHPLC for 11 JWH-018 positional isomers, including the use of serial columns, use of different organic modifiers, or employing separate runs using different stationary phases with either acetonitrile or methanol modifiers. For all chromatographic runs, formic acid was used as the mobile phase additive with single quadrupole mass spectrometric and/or diode array Ultraviolet (UV) detection. Columns investigated included classical silica and silica hydride phases (C18, C30, Bidentate C8, UDC Cholesterol, PFP-Pentafluorophenyl, Phenyl Hexyl, and Diamond Hydride).

For the UHPLC separation of 11 positional isomers of JWH-018 extensive co-elution was obtained for both classical silica and Silica-Hydride (SiH) columns under Reverse Phase (RP) conditions using an acetonitrile modifier, with at best only 3 out of 11 satisfactorily resolved. Utilizing serial columns with an acetonitrile modifier at best provided satisfactory resolution for 5 out of 11 positional isomers. However, 9 positional isomers were satisfactorily resolved using a Cogent RP PFP column with a methanol modifier. The additional two positional isomers were resolved using a Cogent RP C18 column with a methanol modifier.

The range of stationary phases using both acetonitrile and methanol modifiers demonstrated considerable differences in selectivity in both single and serially coupled column trials, thus indicating the ability of both mobile phase and combined orthogonal columns to increase specificity of chromatographic separations, improving the separation of synthetic cannabinoid positional isomers.

UHPLC Separations; Emerging Drugs; Positional Isomers

B97 Determining the Utility of Gas Chromatography/Derivative Vacuum Ultraviolet Spectroscopy for Seized Drug Analysis Through a Comparison to Gas Chromatography/Mass Spectrometry Capabilities

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Learning Objectives: This presentation will demonstrate the utility of a Gas Chromatography-Vacuum Ultraviolet Spectroscopy (GC-VUV) method for seized drug analysis. After attending this presentation, attendees will know the capabilities of the GC-VUV method, the value of second derivative analysis of VUV spectra, and how this method compares to the favored Gas Chromatography/Mass Spectrometry (GC/MS) for forensic drug analysis in terms of specificity.

Impact Statement: This presentation will impact the forensic science community by providing a method framework using an emerging technology in forensic drug analysis, Gas Chromatography-Derivative Vacuum Ultraviolet (GC-DVUV) spectroscopy, to identify 14 different commonly seen illicit compounds from all major drug classes that is comparable to, if not better than, GC/MS capabilities. Forensic scientists will learn the value of GC-DVUV application to general drug screening that may exceed the current capabilities of the commonly employed workflow in forensic drug analysis laboratories.

Abstract Text: GC/MS has long been praised as the gold standard in forensic drug analysis due to its accuracy and precision in identifying and differentiating between illicit substances. However, GC/MS has inherent limitations, such as the lack of a molecular ion and difficulties in distinguishing between positional isomers and diastereomers. Our study aims to compare the specificity and repeatability of GC-VUV, GC-DVUV, and GC-MS drug identification.

GC-VUV is an emerging technology that shows great promise in drug detection. GC-VUV excels in the areas in which GC/MS falls short with its ability to deconvolute spectra and its potential to distinguish between positional isomers and diastereomers. Our study aims to demonstrate that GC-VUV provides more specificity in drug identification as compared to GC/MS by employing a derivative analysis of VUV spectra.

Fourteen illicit drugs from various major drug classes, including opiates, opioids, stimulants, cannabinoids, hallucinogens, depressants, and anabolic steroids, were analyzed using GC-VUV. Spectral data were processed to determine the first and second mathematical derivatives of the spectra to increase identification specificity. Principal Component Analysis (PCA) was used to analyze raw data, first derivative, and second derivative spectra from five repeats, and the results were compared to those obtained from GC/MS data.

PCA demonstrates that increasing the derivative of the VUV spectra enhances the distance between clusters, leading to better identification specificity. PCA comparisons between GC-MS and second derivative GC-VUV data revealed that GC-VUV identification was more repeatable. Points representing runs of the same compounds were clustered closer to each other in the VUV data, whereas in the MS data, there was more spreading, showing more variability between runs. Run-to-run repeatability was also evaluated by comparing each run to an in-house spectral library, resulting in an R2 value representing match probabilities. Our study shows that higher derivatives of the VUV spectra significantly decrease the likelihood of misidentifications by increasing the difference between the most likely probable match R2 value and the second and third closest matches, resulting in a more definitive identity of the compound.

Overall, increased specificity in identification is attained by using both DVUV and MS data in analysis, and repeatability is greatly improved in VUV analysis compared to MS. The use of GC-DVUV could be particularly advantageous for the more accurate identification of current and emerging psychoactive substances, better equipping forensic drug laboratories to analyze novel substances.

Gas Chromatography-Vacuum Ultraviolet Spectroscopy; Derivative Spectroscopy; Seized Drug Analysis

B98 Chemometrics as a Tool for Transformation: Addressing the Limitations of Gas Chromatography/Infrared Spectroscopy for Synthetic Cathinone Analysis

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Learning Objectives: After attending this presentation, attendees will understand the limitations associated with using Gas Chromatography solid deposition Infrared Spectroscopy (GC/IR) for the analysis of synthetic cathinones and how chemometrics may be the key to addressing these challenges.

Impact Statement: This presentation will impact the forensic science community by showcasing situations in which the expert visual assessment of spectra in combination with a typical library searching algorithm may not be sufficient for confident compound identification. In these instances, chemometric analysis may be better suited to assist the forensic scientist in reaching a sound conclusion.

Abstract Text: Novel psychoactive substances pose a significant analytical challenge for forensic laboratories. Many structurally similar compounds produce visually indistinguishable mass spectra, thus leading forensic scientists to investigate other methods of analysis. One commonly proposed instrumental technique is the combination of GC/IR. Typically, this alternative is presented as producing distinct spectra that can be easily differentiated with the combination of a library search and an expert visual examination. However, it is important to acknowledge that this is not the case for all compounds. In this study, we showcase three synthetic cathinone analogs (methylone, N-ethylpentylone, and pentylone) that produce extremely visually similar solid state IR spectra to one another.

A primary dataset of these three cathinone analogs was generated at the Amsterdam Police Laboratory over the course of three weeks. Libraries were created in tandem using two different sample preparation methods. In addition, the same compounds were analyzed at the Virginia Department of Forensic Science (DFS) using the same sample preparation methods. It was observed that changes in either instrument or sample preparation were enough to pose challenges to both the visual assessment and the library matching algorithm. Additionally, week-to-week variation was observed within the primary dataset. Using Principal Component Analysis (PCA), the efficacy of a chemometric model in combination with Mahalanobis distances for objective comparison was assessed. Optimization of preprocessing methods and wavenumber selection showed that the second derivative of the full spectrum resulted in the best separation of the three compounds in principal component space. This method successfully identified three replicates of two real case samples as well as validations samples not utilized in the training of the PCA model.

Chemometrics showed particular improvement over the library-searching algorithm for samples analyzed at an alternative laboratory from that which prepared the library. In addition to producing higher match scores (suggesting lower similarity) for all samples, the pentylone samples analyzed at DFS were mis-identified as N-ethylpentylone when using the library matching algorithm. However, they were successfully identified using the chemometric method.

This study showed how the application of chemometrics to spectral GC/IR data can improve and substantiate the differentiation capabilities of the instrument and provide valuable objective support to the expert when making a compound identification. In addition, limitations in the consistency of GC/IR spectra over time and across instrumental conditions were observed, which could affect how the forensic community utilizes these techniques in the future.

Drug Analysis; Machine Learning; Spectroscopy

B99 An Additional Analysis of Drug Test Strips by Mass Spectrometry for Rapid Comprehensive Drug Checking

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Learning Objectives: After attending this presentation, attendees will be introduced to a method for extracting compounds from used Lateral-Flow Immunoassays (LFIs) that can then be analyzed by mass spectral techniques to provide complementary comprehensive information for rapid drug checking.

Impact Statement: This presentation will impact the forensic science community by providing information on a rapid drug screening method that takes advantage of an existing technique. This method would allow for rapid onsite testing using LFIs followed by additional laboratory-based analysis resulting in complementary information that can be used by forensic science laboratories and public health agencies to triage samples, monitor drug trends, and identify emerging threats.

Abstract Text: Over the past five years, there has been a 70 % increase in opioid-involved drug overdose deaths.[1] Many of these overdoses occur because People Who Use Drugs (PWUD) are often unaware of what is in their drug products. To help combat the opioid epidemic, public health agencies provide point-of-care testing using a range of techniques including LFIs (herein referred to as test strips) as well as comprehensive laboratory-based testing (e.g., direct analysis in real time – mass Spectrometry (DART-MS) through programs such as the Rapid Drug Analysis and Research (RaDAR) program at the National Institute of Standards and Technology (NIST).[2]

Test strips were initially designed for urine analysis, but studies have shown their effectiveness in testing drug material.[3] Public health agencies provide these tests to PWUD to test their drugs prior to consumption, but they are also used by forensic science agencies as part of their standardized analytical scheme to conduct presumptive testing. These tests are cheap and highly sensitive but are highly selective, resulting in rapid non-comprehensive information. In addition to onsite testing, there are programs, such as RaDAR, which provide rapid laboratory-based drug checking through the analysis of trace drug residues collected from used paraphernalia by DART-MS. The information provided through laboratory-based techniques is more comprehensive than onsite testing but has longer turnaround times and requires programs to be in place that can sample used paraphernalia.

This project hypothesized that the methods used for point-of-care/presumptive testing (test strips) and laboratory-based testing (DART-MS) could be combined to provide rapid comprehensive information for drug checking programs and forensic science laboratories. For this to be possible, an optimized extraction process needed to be developed to remove the compounds from the used test strips to then be analyzed by DART-MS. This study presents an optimized method that evaluated several variables, including test strip type, target compound, and extraction solvent, time, and volume as well as identified limits of detection for a range of compounds (fentanyl, xylazine, cocaine, carfentanil, methamphetamine, heroin, and isotonitazene). Following optimization of the extraction method, real-world samples were tested using the developed method. Test strip samples were submitted alongside 100 traditional RaDAR samples. Compounds were extracted from the test strips then analyzed using the same method used to analyze traditional RaDAR samples. The results were then compared to the traditional samples and showed 95% agreement between the test strip and traditional samples. These results show that test strips can be used to rapidly test samples onsite by PWUD or law enforcement, then be submitted to a laboratory for analysis by mass spectral techniques providing rapid comprehensive information. This information can then be used to triage samples for forensic science laboratories or help track drug trends and emerging threats across the United States.

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Test Strips; Rapid Drug Screening; Mass Spectrometry

B100 Detector Dogs: Advancing Drug Detection Operations Using Science at the United States Customs and Border Protection

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WITHDRAWN

B101 The Development of Fentanyl Training Aids to Advance Canine Detection Strategies

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WITHDRAWN

B102 Canine Training Aid Development of Novel Target Odors to Advance Strategies to Combat Fentanyl Precursors at the Border

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WITHDRAWN

B103 The Evolution of VOC Profiles During Early Human and Animal Decomposition in Application for Canine Detection

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Learning Objectives: This presentation will examine and advise attendees on the changes in odor profiles during early decomposition using varying analogs for human remains—human blood, pig skin, and mouse carcasses. The transition from the odor profile of fresh remains to decomposed will be demonstrated both chemically, by examining the Volatile Organic Compounds (VOCs), and via canine trials to display how this shift in the odor profile may impact canine detection in the field.

Impact Statement: The results in the presentation will differentiate distinct odor profiles of the various analogs, with a focus on the first 48 hours after death/collection, utilizing chemometric analysis and canine detection. This has applications to Search And Rescue (SAR) and Human Remains Detection (HRD) detection canines and their abilities to discover crucial evidence in fresh crime scenes or disaster scenarios.

Abstract Text: HRD canines provide a crucial role in locating missing persons and in revealing valuable evidence for criminal investigation. Previous research has demonstrated that the odor profiles of remains, whether human or animal, change significantly over time. This evolution of VOC profiles can affect canine detection accuracy, requiring that handlers train their dogs on a variety of materials of different ages.[1-4] This study aims to address the gap in understanding the changes in decomposition within the first 48 hours after death—a critical timeframe in SAR operations. Anecdotal findings from HRD canine handlers suggest a perceptible change in odor to canines about two to four hours after death, highlighting a need for frequently sampling in the first 24 hours, a task not previously applied to human remains research. Due to difficulties in obtaining human remains immediately postmortem, this work aims to begin with a simpler human remains matrix and full animal carcasses: human blood, pig skin samples, and then on to full mouse carcasses.

Odor analyses of blood, skin, and carcasses were conducted using Headspace/Solid Phase Microextraction (HS/SPME) for sample collection and Gas Chromatography/Mass Spectrometry (GC/MS) for VOC separation and detection. This allowed for detection of the compounds in the odor profiles and tracked their changes over time. Chemometrics techniques, including Principal Component Analysis (PCA), hierarchical cluster analysis, and Multivariate Curve Resolution using constrained Alternating Least Squares algorithm (MCR-ALS), were applied to reveal statistically significant changes in the odor profiles. Results indicate an early change in the VOC profiles of all samples between Hour 4 and Hour 8, with a fresh odor profile present from Hours 1–4. After Hour 8, there was a decrease in compounds characteristic of the fresh profile and an increase in those of a later odor profile, with a notable a shift to an aged odor profile between Hours 24 and 40. HRD canine trials with blood of varying ages support these findings, with minimal detection of blood one to two hours old and the optimal detection at 34–36 hours old. These results implied that early changes in odor profiles of numerous remains samples impact canine detection, providing valuable insights to the training of HRD canines.

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Headspace Analysis; Human Remains; Search and Recovery

B104 Assessing the Accuracy and Reliability of Canine Explosives Detection Using OSAC Training and Certification Standards

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Learning Objectives: Attendees will learn the level of knowledge and general awareness that operational canine-handler teams have of the existing training and certification standards produced by the Dogs and Sensors subcommittee at the Organization of Scientific Area Committees (OSAC). In addition, attendees will receive information about the recommended training and certification protocols specified in the American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 092, which outlines a training program to develop the necessary skills and provides guidance for testing, explosive detection canine-handler teams performance on baggage/parcel, vehicle, and building searches that mimic operational contexts. Attendees will learn about current efforts that are underway to validate the protocols outlined in ANSI/ASB Standard 092 and the performance data collected to date.

Impact Statement: This presentation will impact the forensic science community by demonstrating the reach of OSAC standards among operational canine handler teams. The presentation will also detail the practical and scientific challenges associated with validating standard protocols and can serve as an example for other groups of researchers or practitioners seeking ways to develop empirical support for their procedures. Finally, this presentation will include a first look at important data demonstrating canine handler team performance in classic testing scenarios and circumstances designed to mimic operational contexts.

Abstract Text: Canines have played a formal role in criminal investigations, emergency response, security, and other important fields in the United States for decades. There is no question that canines have superior abilities when it comes to detecting, tracking, and identifying odors, and that humans can train canines to perform myriad useful tasks. That said, as is the case for most forensic disciplines, there is a lack of empirical data to support protocol, procedures, and programs that canine handler teams know to be effective through experience.

The development of dog detection training and certification standards by the OSAC Dogs and Sensors Subcommittee has made a significant contribution in this area by developing and publishing detailed protocols with the goal of improving consistency in the field. However, consistency of methods—*method conformance*—is only one piece of the puzzle. To provide empirical evidence that a discipline is reliable, the performance that results from the use of a particular method also must be demonstrated empirically, under conditions that are controlled and for which ground truth is known—*method performance*.[1] The canine will exhibit normal variations in behavior and skill, and a person is responsible for subjectively interpreting the canine's behavior using their experience and expertise. Hence, there will be some variation in both method conformance and performance, but variation can be measured using empirical methods and accounted for in real cases.[2]

Although canine detection practitioners worldwide are actively working on disseminating, developing, and promoting canine detection standards in both training and operational contexts, the utility of the standards has not yet been evaluated. Thus, the extent to which canine handler teams are training and certifying their canines according to OSAC standards remains an open research question. In addition, the OSAC standards have consensus support among experienced canine handlers and researchers, but the protocols have not yet been assessed in terms of the resulting performance that can be expected if a canine handler team trains and certifies in line with this method. This presentation will describe a recent effort to assess how widespread knowledge and use of OSAC standards are among the canine-detection community. In addition, the same research team is conducting a proof-of-concept black box study that seeks to objectively assess the OSAC National Registry Standard 092, *Standard for Training and Certification of Canine Detection of Explosives* (ANSI/ASB Standard 092).

In this presentation, we will present survey results demonstrating how far-reaching the Standards produced by the OSAC Dogs and Sensors subcommittee have been in the canine-detection community. We will also present a first look at the data from operational canine assessments that align with the certification testing paradigms developed in Standard 092. This is the first black box study to assess canine detection and has followed a similar approach to black box studies conducted to validate other forensic disciplines. Future directions will be discussed as well as a discussion about the challenges that have been overcome to get this research project underway.

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Canines; Sensor Dog; Validation

B105 Developing a Universal Smokeless Powder Training Aid

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Learning Objectives: Participants will gain insights into the development of a universal Smokeless Powder (SP) training aid. Attendees will learn about the challenges posed by the diverse chemical compositions of SPs and the strategies used to overcome these obstacles.

Impact Statement: This research addresses the challenges posed by the diverse chemical compositions of SPs by developing a universal training aid for detection dogs. By overcoming these obstacles, the study aims to standardize SP training aids, potentially enhancing security measures by improving the reliability and effectiveness of canine detection teams, improving security measures.

Abstract Text: SPs, extensively utilized as propellants in firearms and artillery ammunition, are readily accessible and hence frequently exploited for illicit purposes. Given their involvement in firearm and explosive-related criminal activities, the analysis of SPs is of forensic significance. The detection of trace levels of SPs is challenging due to the extensive diversity in their chemical compositions and the plethora of available brands. Previous headspace analyses of pre-blast SPs have indicated volatiles such as 2,4-Dinitrotoluene (2,4-DNT), Diphenylamine (DPA), ethyl centralite, and nitroglycerin, among other compounds, as compounds of interest in the vapor phase pertinent to the trace-level detection of SPs.[1]

Addressing the complexities associated with the SPs, this study aimed to develop a universal SP training aid intended for the training of detection canines. During the current study, comprehensive analysis of SPs was conducted to quantify the abundance of 2,4-DNT, DPA, ethyl centralite, and nitroglycerin in the headspace, utilizing Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS). Subsequent to the headspace analysis, training aids were formulated to represent the average abundance of these volatile compounds prevalent in commonly available SPs.

The formulated SP training aid underwent a rigorous evaluation employing an innovative approach involving murine models to monitor brain receptor activity. This phase of the study was conducted in collaboration with Canaery, an organization pioneering the development of the world's first Nose-Computer Interface (NCI).[2] The empirical data derived from this investigation demonstrated that the SP training aid developed in this study is proficiently generalized across diverse SP variants. The brain receptor activity tracked in the murine models furnished compelling evidence corroborating the efficacy of the training aid in emulating the olfactory profile of a broad spectrum of SPs.

The results of this study indicate that the developed SP training aid holds significant promise for enhancing the capabilities of detection dogs. This advancement has the potential to improve the accuracy and reliability of forensic investigations involving SPs. The universal SP training aid offers a practical solution to the complex challenge of detecting trace levels of SPs across various compositions and brands.

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Gas Chromatography; Smokeless Powders; Detection

B106 OSAC/ASB Standards Development Activities in Firearm and Toolmark Examination

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Learning Objectives: 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 (CB) for Firearms and Toolmarks and to update the status of those discipline-specific standards.

Impact Statement: The 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.

Abstract Text: The OSAC for Forensic Science was created by 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 CB was created by the ASB. The Firearms and Toolmarks CB 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 CB 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 originate from the Firearms OSAC, which promulgates draft standards. The CB reviews the proposed draft standards, submits them for rigorous discussion and review by the relevant committee members and by the CB 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; Toolmarks

B107 A Quantitative Topography Comparison of Toolmarks Made by Tongue-and-Groove Pliers

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Learning Objectives: Attendees will learn about measuring the three-dimensional (3D) surface topography of impressed toolmarks made by tongue and groove pliers and the application of algorithms for objective quantitative comparison. Experimental results characterize similarity scores of toolmarks obtained under different conditions and the effects of possible subclass characteristics.

Impact Statement: This presentation provides foundational support for the objective identification of impressed toolmarks made by hand tools.

Abstract Text: Toolmarks made by hand tools are encountered in various crime scenes (e.g., murder, burglary, theft, arson, and bombings). The forensic comparison of these toolmarks can help solve criminal cases by linking a suspect tool to a crime scene or by connecting related crime scenes. In this study, we evaluated the application of objective comparison methods developed for forensic firearm identification to impressed marks made by tongue-and-groove pliers.

We made toolmarks under different conditions relevant to casework using 12 consecutively manufactured pliers and 15 non-consecutively manufactured pliers. The pliers were made from drop forged steel, and the teeth were machined using a milling process. Each jaw of the pliers has a flat tooth at the front and back, and 10 teeth in between that have a triangular cross-section. After several heat treatment operations, including laser hardening of the teeth, the pliers were bead blasted and coated with a transparent lacquer for rust protection. Impressed toolmarks were generated on metal strips of different materials (lead, tin, aluminum, copper, galvanized steel, and brass).

The 3D surface topography of the tools' working surfaces and toolmarks were measured using focus variation microscopy. The analyzed regions of interest were toolmarks generated by the front flat tooth and the triangular teeth. After trimming the topography images of toolmarks to the region of interest, we applied a bandpass filter to attenuate noise and highlight individualizing features. The images were then compared algorithmically to obtain two similarity scores: the maximum value of the normalized Areal Cross Correlation Function (ACCF) and the number of Congruent Matching Cells (CMCs). Experimental distributions of the toolmark similarity scores were generated for Known Matching (KM) and Known Non-Matching (KNM) comparisons to evaluate individuality and reproducibility. To investigate possible subclass characteristics, we compared similarity score distributions for toolmarks made by consecutively and non-consecutively manufactured tools. We also characterized the effects of potential influence factors on similarity scores, including substrate material, change of the tool's working surface after use, and contact angle.

The KM and KNM score distributions for both ACCF and CMC showed complete and large separation for all conditions. No significant effects of subclass characteristics were observed. Variations due to differences in substrate material and contact angle were reduced by automatically limiting the compared topography to the bottom area of the tool mark. Persistence tests showed changes in the similarity of KM marks, mainly due to the accumulation of debris.

The results indicate that the two algorithmic comparison methods of topography data (3D images) have utility in the identification of impressed marks made by tongue-and-groove pliers.

3D Technology; Toolmarks; Quantitative Evidence

B108 The Interoperability of 3D Firearm Toolmark Measurements

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Learning Objectives: A set of 120 cartridge cases originating from four different sets of consecutively manufactured firearms were used in a round robin style interoperability study. The research goal was to evaluate the effect of different measurements methodologies and laboratory protocols on the National Institute of Standards and Technology (NIST) similarity metrics Areal Cross Correlation Function (ACCF) and Congruent Matching Cells (CMC).

Impact Statement: 3D surface topography measurements can be generated by different measurement methodologies, each of which results in slightly different reconstructions of the surface. In order for the interoperability and exchange of 3D measurement data between different agencies, there is a critical need to understand the measurements limitations. The research demonstrates that there were significant differences observed when applying the NIST similarity algorithms. However, once these differences are documented, correction factors and uncertainty estimates can be generated to account for these differences. The research can help facilitate interoperability between different laboratories and instruments in the future.

Abstract Text: 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 future goal for these instruments is to generate measurements used to report on the statistical weight of evidence of comparison in casework.

For impressed toolmarks, there is no comprehensive study that characterizes the resulting differences in 3D data obtained using different instrument types 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. Twelve laboratories participated in this study to generate a total of 18 datasets across 9 different 3D instruments.

To quantify the differences between labs and technologies, each lab's measurements were analyzed using two well-established similarity scores: the normalized Areal Cross Correlation Function (ACCFMAX) and the number of Congruent Matching Cells (CMC).[1,2] The results were used to generate Known Matching (KM) and Known Non-Matching (KNM) score distributions, which were used to statistically analyze for differences between labs and systems. Results will facilitate improvements in the consistency of measurement results while providing the foundational research data required to defend the future use and interoperability of 3D measurements in casework.

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3D Technology; Interpretation; Firearms

B109 The Generation and Interpretation of the Similarity Score Distribution for Matched and Non-Matched Bullets and Cartridge Cases

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Learning Objectives: After this presentation, attendees will understand: (1) how Automatic Ballistic Identification Software (ABIS) is used to identify possible matches in database correlations; (2) the basis of histogram generation for matched and non-matched similarity scores; and (3) how correlation results can be interpreted. This will include reviewing how sample types included in correlations can have a bearing on the interpretation of correlation results.

Impact Statement: This presentation will impact the performance of examiners using ABIS by increasing the efficacy in interpreting correlation results by better understanding the relationship between matched and non-matched samples, in addition to factors that can contribute to software presenting possible matches that are not matched or class exclusions (false positives).

Abstract Text: ABIS systems use computer algorithms to derive a similarity score that represents how alike two bullets or cartridge cases are as a numerical result—falling into one of two categories: matched samples or non-matched samples. This presentation reviews how matched and non-matched similarity scores are represented in histograms; how these histograms are related to each other; how sample features can affect the correlation scores generated by algorithms; and how datasets can create bias in correlation results.

By providing a fundamental understanding of how sample features relate to generated similarity scores, including class characteristics, examiners will better be able to interpret ABIS system results to reduce the number of possible matches that need to be compared, or the number of samples that are on the top rankings of their correlation results, to ensure that matched samples do not get missed in addition to understanding how class characteristics can be used to prevent false positives (the indication of possible match for class exclusions).

Comparison tools, such as Leeds Evofinder, are designed with an advanced, automated identification system to automatically correlate sample images to other images stored in your database, providing a numerical similarity value between two representative images. These similarities can then be compared to each other using Virtual Comparison Microscopy tools in the Evofinder or under a comparison microscope to identify possible matches. The Leeds Evofinder System compares land engraved areas/land engravings, groove engraved areas/groove engravings, and slippage marks on bullets and breech face impression and striker mark/firing on cartridge cases. Total process time from receipt of sample to correlation result on a 9mm bullet or cartridge case face is approximately 4 minutes. It scans cartridge case, bullets, and bullet fragments; performs VCM gun sorting correlations, and requires no annual licensing. Your lab owns the image files and there are no consumables, no critical cleaning is needed, and is portable for field use.

Firearms; Accuracy; Forensic Imaging

B110 Quantitative Assessment of Barrel Wear From Solid Copper Bullets

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Learning Objectives: This presentation will utilize algorithm comparisons to demonstrate the effect of solid copper bullets on the persistence of barrel rifling characteristics. By attending this presentation, attendees will also learn about three-dimensional bullet scans and how they can be used in comparisons.

Impact Statement: This presentation will impact forensic science because it highlights the use of three-dimensional scans and algorithms to conduct bullet comparisons. Furthermore, this research shows the effect of solid copper bullets on the enhanced polygonal rifling of Glock Marksman Barrels, which are present in some of the most common pistols in the United States.

Abstract Text: The persistence and wear of barrel individual characteristics is a common research topic in forensic firearm analysis; however, most studies are conducted on conventional ammunition such as full metal jacket. While solid copper bullets are not yet as common, calls for lead-free ammunition are leading to a rise in the use of solid copper bullets. As they become more widespread, crime laboratories will see an increase in the number of solid copper bullets submitted as evidence. For firearm examiners to better understand the effects of solid copper bullets on barrel wear, more research is needed.

This study included the successive firing of 500 solid copper bullets through a new Glock 19 Gen 5 with a Glock Marksman Barrel. To profile the barrel at the beginning and end of the study, the first ten and last ten bullets were collected. Every tenth bullet was collected to track any changes in the features. The Cadre Forensics Versa Scanner was used to scan the land engraved areas on each collected bullet. These scans were used to compare the individual characteristics left on the bullets by the barrel rifling.

Compared to similar studies that involved qualitative comparison methods, this study focused on quantitative data. The Cadre Forensics system includes a built-in algorithm that compares the bullet scans and generates a similarity score for each comparison. The algorithm is Cadre Forensics' implementation of the Congruent Matching Profile Segments algorithm developed at the National Institute of Standards and Technology. The data were analyzed using the Kwiatkowski-Phillips-Schmidt-Shin time series test to determine the statistical significance of differences observed in the similarity scores. Results from the algorithm comparisons were supported by visual examinations of the depth profiles generated from the bullet scans.

The results from this study demonstrate that solid copper bullets have a statistically significant effect on the individual characteristics of firearm barrels. This conclusion is supported by the algorithm results and the visual examinations. There is a decreasing trend in the similarity scores, and the depth profiles of later bullets are less detailed than those of bullets fired earlier in the sequence. These results will inform firearm examiners that they may need to be more conservative when using individual characteristics to reach an elimination.

Solid Copper Bullets; Three-Dimensional Scanning; Algorithm Comparison

B111 A Quantitative Determination of Muzzle-to-Target Distance in Shooting Cases

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Learning Objectives: This presentation demonstrates a method to quantitatively determine muzzle-to-target distance using Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES) by the Inorganic Gunshot Residue (IGSR) profile of common ammunition. The main outcome of this study works to create a reliable mathematical relationship between elemental composition and shooting distance to be applied in shooting cases.

Impact Statement: This presentation will expand the scope of both the firearms and trace evidence sections in the forensic science community by utilizing instrumental analyses that focus on the target material rather than the shooter-end of firearm-related evidence. By developing this technique, we would also be able to improve current techniques by creating quantitative, less subjective, and more specific conclusions to be used in court.

Abstract Text: In the years between 2014 and 2022 alone, firearm-related deaths in the United States nearly doubled, increasing conversations among the public surrounding gun control. Gun regulations vary between states; several states, including Mississippi, not only have relaxed gun control laws but also have the highest overall firearm-related deaths. Currently, some gun-control laws are related to the type of firearm a person can purchase, concealed carry laws and processes, registration laws, and other aspects. However, many Americans believe these laws are still not enough to reduce and prevent gun violence, calling for more research surrounding the analysis of firearm-related evidence in shooting cases.

Research involving IGSR typically focuses on the shooter end rather than the target material. This work seeks to create a mathematical relationship between the concentration of well-known elements, namely lead, barium, and antimony, on target materials and muzzle-to-target distance with an attempt to estimate shooting distance quantitatively. Cloth samples containing IGSRs were obtained by shooting Remington UMC 9mm Luger ammunition from a Smith & Wesson M&P9 Shield semi-automatic pistol in a controlled indoor environment at varying distances. The IGSR was extracted from samples using an acid digestion procedure and analyzed using ICP/OES to quantify barium, lead, and antimony. A mathematical model was developed for the interpretation of barium, lead, and antimony concentrations in both ammunitions analyzed. Each inorganic metal produced a unique polynomial function with statistical value at an R2 of 0.9886, 0.9748, and 0.992, respectively. This research provides evidence that a relationship between muzzle-to-target distance and the concentration of metal on the target material not only exists but is a polynomial relationship. This relationship can be used to create mathematical models for varying metals in specific ammunition types and provides a quantitative method for distance determination in shooting cases.

Ballistics; Firearms; Gun Shot Residue

B112 Close Non-Matches and Database Searches: How Often Can We Expect to See a True Match Among the K Highest Correlations?

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Learning Objectives: This presentation will explore close non-matches and the probability of a true match appearing in the top *k* search results in database searches involving bullets and cartridge cases. Participants will gain insight into the reliability of database searches when close non-matches may be present.

Impact Statement: This presentation will have an impact on the forensic science community by raising awareness about the challenges inherent when conducting searches of large databases and the potential for increased contextual bias that may result from those searches. Forensic practitioners should be aware of the interplay between database size, size of largest candidate correlations, the presence of close non-matches, and the number of true matches in the database.

Abstract Text: 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 the National Integrated Ballistics Information Network (NIBIN) to correlate a piece of evidence to potentially similar bullets or cartridge cases recovered from other crime scenes. Investigators upload images of the crime scene item(s) onto the system, request a list of the top *k* most similar items from the database and then decide whether any of the *k* candidates are similar enough to the questioned items to warrant a physical comparison.

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. One 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 detect similarities that are not really there. This is not an unreasonable concern; after a database search that included a close non-matching print, three senior latent print examiners from the Federal Bureau of Investigation (FBI) incorrectly identified Mr. Mayfield as the perpetrator of a 2004 train bombing in Madrid that resulted in over 200 casualties.

To explore this question, we carried out a simulation where we varied the potential number of correlations (*N*), the size of the list of candidates (*k*) when the number of true matches in the database (t) is exactly one. In a small state such as Iowa, the size of the local NIBIN database is likely to be small, perhaps with *N* around 500 potential correlations. In large states, the potential number of correlations might be in the order of a few hundred thousand. We have reported on earlier findings that relied on the use of beta probability models to represent the distributions of same and different source (SS, DS) similarity scores, with parameter values that closely reproduced scores obtained by Fadul and Weller (Song et al.) after scaling.[1]

We have extended the study to the more realistic cases where the number of actual true matches t among the *N* possible correlations is larger than 1. We have also investigated the use of alternative probability models for the distributions of scores. How does the probability that the top *k* correlations include 0, 1,…,t true matches vary when *N*, *k*, and t vary? What if t = 0 but *N* includes close matches to the questioned item? We explore the similarity scores that may be obtained for the *k* most similar candidates and discuss how this may affect the chances of a false positive lead.

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Bullet; Probability; Data

B113 Deep Ocean Recovery and DNA Identification of Human Remains From the Titan Submersible

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Learning Objectives: After attending this presentation, attendees will be informed of the DNA identification of human remains from the Titan submersible implosion and of environmental factors of the deep ocean that may help preserve DNA for forensic examination.

Impact Statement: This presentation will impact the forensic science community by providing an overview of oceanic environmental factors affecting DNA identification of recovered human remains and demonstrating the impact of those factors in the context of the Titan submersible implosion event in the deep ocean.

Abstract Text: There is a great deal of practical experience in the forensic biology community with obtaining DNA from human remains recovered from the ocean at relatively shallow depths. Analysts are familiar with considering how the remains, and ultimately the DNA, have been affected by predation, salt water, light, and other factors at these depths. There is very little experience, however, in the forensic community with DNA recovery and analysis from human remains recovered from ocean depths greater than approximately 650 feet (200 meters).

The OceanGate Expeditions submersible vessel, Titan, had five men aboard for a deep-sea dive in the North Atlantic Ocean to the wreckage of the Titanic when it imploded on June 18, 2023. Missions to recover vessel debris from approximately 12,500 feet (3,800 meters) were conducted in late June 2023 and early October 2023. Possible human remains were discovered among the vessel debris and delivered to the Armed Forces Medical Examiner System (AFMES) for examination and sorting. Selected samples were then delivered to the Armed Forces DNA Identification Laboratory's–Current Day Operations section (AFDIL-CDO) for DNA processing. Sixteen bone and tissue samples from the June recovery and fifty bone, teeth, nail, and tissue samples from the October recovery were tested and yielded five distinct autosomal Short Tandem Repeat (STR) DNA profiles and four Y-chromosomal Short Tandem Repeat (Y-STR) haplotypes. Family reference samples for each decedent were also collected and processed. DNA profiles generated from the Titan evidence samples and the family references were compared and subjected to multiple kinship analyses, resulting in identification of all five decedents from the Titan submersible.

Deep ocean recovery of disaster victims and forensic DNA identification of human remains will be compared from the Titan submersible implosion and the Air France 447 crash of June 1, 2009, in the South Atlantic Ocean. Factors that may contribute to preservation of human remains at ocean depths of approximately 12,500 feet will be presented.

Identification; Search and Recovery; Ocean

B114 An Evaluation of DNA Recovery From Chemically Treated Human Remains

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Learning Objectives: This presentation will offer foundational insights into the quantity and quality of DNA recovered from human remains treated with household chemicals, highlighting the potential for successful human identification from these compromised remains.

Impact Statement: This study is the first of its kind to expose intact large segments of human cadavers to common household chemicals, providing realistic samples for examining DNA recovery. The alterations in the samples' appearance, integrity, and recovered DNA will be assessed, offering guidelines for sampling these challenging remains. Our findings emphasize the need for authentic samples when studying chemically damaged remains.

Abstract Text: The identification of human remains has always been a complex task but can be further complicated when extreme methods of disposal are implemented. Offenders may resort to household chemicals to dissolve human remains, aiming to completely dispose of them or prevent identification. Once primarily associated with large criminal organizations, these practices have become more common, influenced by popular television shows like *Breaking Bad*.'

Despite the growing frequency of such cases, research on DNA recovery from chemically treated human remains is limited. Most studies focus on the visual effects these chemicals have on tissues, while the few that examine DNA recovery rely on animal proxies or focus solely on DNA recovery from human teeth, leaving a gap in research using realistic samples.[1-6] Our study addresses this issue by recreating the most authentic case scenarios, exposing intact segments of human cadavers to readily accessible household chemicals available at local hardware stores.

Whole heads, forearms, and hands were obtained from five cadavers provided by the Southeast Texas Applied Forensic Science (STAFS) facility at Sam Houston State University. These body segments were exposed to one of five chemicals: bleach, Rid-X septic treatment, lye drain cleaner, sulfuric acid drain opener, and hydrochloric acid pool cleaner. Tissue samples, including bone, teeth, muscle, fingernails, and hair, were collected at days 0, 1, 3, 5, 7, and 28. Subsequent DNA analysis included traditional Short Tandem Repeat (STR) typing and mitochondrial DNA analysis.

Although full STR profiles were recovered from remains after exposure to all five chemicals, hydrochloric acid was the fastest acting chemical, limiting identifications to only 3 days of exposure. In contrast, full STR profiles were recovered after 21 days of exposure to sulfuric acid and 28 days of exposure to bleach, Rid-X, and lye. Furthermore, comparisons to a pilot study using porcine rib samples highlight the need for authentic samples when examining these compromised remains, as the effects of certain chemicals varied drastically.

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Chemical Damage; Skeletal Remains; Human Identification

B115 The Use of Mitochondrial DNA to Provide Ancestry of Skeletal Remains Discovered in the East Marshall Street Well (EMSW)

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Learning Objectives: This presentation will demonstrate how ancestry markers are used to gather information on human remains when there are limited avenues of DNA analysis and show the importance of mitochondrial DNA in predicting ancestry in samples that are severely degraded.

Impact Statement: This presentation will demonstrate how phenotypic and ancestral data can be obtained and used to provide life histories for severely degraded samples.

Abstract Text: During the construction of a building on the Medical College of Virginia campus of Virginia Commonwealth University in Richmond, VA, the East Marshall Street Well was discovered. The contents of the well included a commingled assemblage of human remains for upward of 50 individuals dating back to the mid-19th century. Of these individuals, anthropologists estimated there were at least nine juveniles.[1] These individuals were victims of grave robbing and were utilized for both dissection and amputation practice by the medical school before ultimately being disposed of in the well.[2]

With the exception of one juvenile, previous nuclear genetic research failed to re-associate the subadult remains into discrete individuals. In an effort to deepen understanding of the life history of the juveniles found in the well, mitochondrial-DNA analysis was performed to obtain mitochondrial haplotypes from targeted regions of the genome. Select samples were chosen to be sequenced on the MiSeq FGx to obtain autosomal Short Tandem Repeat (STR), Y-chromosomal Short Tandem Repeat (Y-STR), X-chromosomal Short Tandem Repeat (X-STR), and ancestral and phenotypic Single Nucleotide Polymorphism (SNP) information. The ForenSeq DNA Signature Prep Kit was used to prepare the library and sequence.

Of the remains sequenced thus far, African ancestry has been demonstrated for most of the juveniles. Two femora, one fibula, and one humerus were predicted as Sub-Saharan African haplogroup L1c2b1c through the sequencing of HVI; results from other remains are pending. The MiSeq FGx sequencing showed two more femora and one tibia clustering toward African ancestry, and another femur exhibited mixed ancestry. Phenotypic expression of hair and eye color was obtained for two femurs through the MiSeq FGx sequencing. One femur contained markers indicating the individual had black hair and brown eyes; the other had brown eyes and dark hair, with no hair color able to be predicted.

The results are consistent with the data obtained from the adult nuclear DNA sequencing, adult mitochondrial DNA sequencing, and anthropological work that has been done on the skeletal remains recovered from the well since its discovery. The majority of the remains in the well were assumed to be of African origin, due to a dark history of grave robbers targeting the burial sites of enslaved people, although DNA also demonstrated that several adult individuals of European ancestry were also present. Mitochondrial analysis of the juveniles has, thus far, narrowed the maternal line origin point to West-Central Africa, which echoes historical accounts of the origin of the enslaved in Richmond, VA.

Ongoing analysis of the ancestry of the juvenile remains will continue to broaden our knowledge of the life histories of these young individuals.

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Mitochondrial DNA; Ancestral; Human Remains

B116 An Optimized Demineralization Method Using Bone Slices for Improved DNA Recovery of 19th-Century Human Skeletal Remains

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Learning Objectives: This presentation will introduce a novel processing method for obtaining endogenous DNA from compromised skeletal samples and describe how it can improve Short Tandem Repeat (STR) profile quality from challenging biological samples. Attendees will understand how to integrate this modified approach into the standard forensic DNA workflow and the applications of powder-free processing methods of skeletonized remains for human identification.

Impact Statement: This presentation will positively impact the forensic community by providing a unique approach to recovering endogenous DNA from degraded skeletal remains. This research aims to improve front-end processing methods for isolating genomic material from compromised skeletal samples, which will have broad applications across numerous fields of study.

Abstract Text: Current processing techniques for harvesting DNA from osseous elements are destructive of potentially limited samples, and success rates vary widely. Few studies have explored whether the pulverization of bone contributes to DNA damage observed in STR profiles obtained from skeletal remains for human identification. Grinding the hard tissue into a fine powder exposes a greater surface area of the bone matrix to the various chemicals utilized in the extraction process and is suggested to release DNA molecules from the hydroxyapatite mineral matrix.[1,2] However, such homogenization of skeletal elements can introduce inhibiting components and exogenous DNA to the sample, competing with endogenous DNA during amplification.

In this work, we have developed and optimized an alternative front-end processing method using aged, weathered bovine cortical bone as a model for aged and degraded human skeletal remains. Given its dimensions, collagen composition, and comparatively reduced ethical restrictions, bovine bone was used as an experimental model for normal human bone.[3] After sample preparation, complete demineralization of small cortical bone fragments was performed, and samples were sliced manually with a razor blade before isolation with silica-based DNA extraction chemistry. For comparison, an organic extraction method was performed on a subset of pulverized samples following the protocol described by Edson and McMahon.[4] Bovine mitochondrial DNA quantification data and STR profile quality were assessed for powdered bone samples and demineralized slices. Analysis of Variance (ANOVA) was used to determine significant means differences for total bovine mitochondrial DNA yields across the treatment types. Bovine profiles generated from demineralized slices yielded a higher average percentage of alleles detected ($p < 0.05$) and had greater retention of larger STR loci than pulverized samples. In conclusion, using demineralized slices resulted in more complete, balanced STR profiles, higher peak heights, and less degradation than powdered samples.

Using 19th-century human cortical skeletal samples, the efficacy of the developed methodology was assessed to investigate the concordance of genetic markers through traditional and emerging genotyping applications, including length-based and targeted sequencing technologies with Capillary Electrophoresis (CE) and High-Throughput Sequencing (HTS) to maximize the recovery of degraded DNA for human identification. Total endogenous DNA yields and degradation indices of powdered samples and demineralized slices were evaluated. The average total DNA yields for pulverized and demineralized slices were 0.1391ng and 0.9043ng, respectively. The Internal Positive Control (IPC) Cycle threshold (Ct) values were assessed for each processing method, and 52% of pulverized samples and 73% of demineralized slices samples had IPC Ct values within the acceptable range (27–31). The average degradation index for pulverized samples could not be calculated due to failed large autosomal amplification. Demineralized slices ultimately resulted in greater recovery of amplifiable DNA than traditional homogenization methods of skeletal remains for DNA analysis.

By eliminating the powdering step of bone and utilizing modified silica-based extraction chemistry, the susceptibility to inhibitory and competitive effects from native and foreign components often found in degraded human skeletal remains can be minimized when prolonged demineralization of small cortical bone fragments and subsequent slicing is performed before lysis.

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Bone; DNA; Skeletal Remains

B117 Biparental and Uniparental Ancestry of the Unidentified Human Remains From the East Marshall Street Well

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Learning Objectives: Attendees will learn about advanced genetic techniques for analyzing historical human remains, including the use of Insertion/Nulls (INNULS), Short Tandem Repeat (STR) markers, and sequencing technologies to determine ancestry and phenotypic traits. They will also gain insights into the ethical considerations and community involvement necessary for handling ancestral remains and how these methodologies can be applied to contemporary forensic casework.

Impact Statement: This presentation will impact the forensic science community by demonstrating the successful application of advanced genetic analysis techniques to historical human remains, thereby setting a precedent for similar future investigations. It will highlight the importance of integrating historical context with modern forensic methods to uncover ancestry and phenotypic traits, which can enhance the accuracy of identification efforts in forensic casework. Additionally, the research underscores the ethical considerations and community involvement necessary for handling ancestral remains, promoting a more inclusive and respectful approach in forensic practice.

Abstract Text: In April 1994, during the construction of the Kontos Building at the Medical College of Virginia on East Marshall Street, a well was discovered to contain artifacts and human remains, mainly of African American descent. Archaeologists were given a limited time to recover the remains and artifacts discarded over numerous years. The improper excavation of the well resulted in a commingled assemblage of skeletal remains and material. Later anthropological analysis indicated that remains from at least 44 adults and 9 children were in the well. The well was dated back to the mid-19th century when grave robbing and the use of cadavers of African descent in medical schools were common practices.

Addressing one of the recommendations of the Family Representative Committee (a committee of community individuals representative of the descendant family of the ancestral remains), genetic reassociation of the human bones recovered from the East Marshall Street Well have been performed using INNULS and STR markers. A total of 15 individuals were assembled.

Aiming to obtain biparental and uniparental ancestry information on the individual groups, one or two samples each from 15 adult individual groups were genotyped using Primer B of ForenSeq DNA Signature Prep Kit on the MiSeq FGx sequencing system. Universal analysis software was used to predict the biological sex, hair color, eye color, and ancestral origin of the 15 individuals. 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 haplotypes were obtained for the HV1 segment, through Sanger sequencing.

Most individuals exhibited primarily African autosomal ancestry. Nonetheless, European and admixed ancestry was also detected for other individuals. Brown eye color was observed in all the individuals typed, with predominantly black hair color and dark brown observed in some samples. Three of the four Y-STR profiles obtained were predicted as African haplogroups, while the other one was predicted to be European. Maternal haplogroups obtained were of African origin, except for the individual with autosomal European ancestry that exhibited a European lineage. The findings complement the historical records regarding the slave trade and human trafficking, to Richmond, VA. Information obtained on the ancestry and phenotypic traits will enable the family representative committee, and the African American community in Richmond, VA, to know about their ancestors and to ensure a proper burial ceremony for the ancestral remains. This research will also provide insights into future genetic identification of human remains.

Forensic Science; Ancestral Remains; Genetic Ancestry

B118 Non-Destructive Brand Classification From Automobile Tire Rubber Traces Using ATR/FTIR Spectroscopy and Machine Learning for Forensic Investigations

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Learning Objectives: This study presents a novel approach for the classification of tire rubber samples using Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) spectroscopy and machine learning. By combining these advanced techniques, the study offers a powerful tool for the non-destructive identification of tire traces in forensic investigations. The findings demonstrate the potential of this method to accurately and efficiently determine the brand of tires from rubber evidence, thereby providing critical information that can link suspects to crime scenes. As forensic science continues to evolve, the integration of machine learning and spectroscopic techniques will play an increasingly important role in ensuring that justice is served swiftly and accurately.

Impact Statement: The non-destructive nature of ATR/FTIR spectroscopy ensures that valuable evidence is preserved, allowing for further analysis if needed. By providing a systematic, rapid, and accurate method for identifying tire brands from trace evidence with the efficiency of machine learning, this study enhances the capability of forensic experts to quickly and confidently narrow down the range of suspects. This is particularly important in cases where time is of the essence, such as in hit-and-run incidents or abductions. Furthermore, the integration of advanced spectroscopic techniques with machine learning represents a growing trend in forensic science toward more sophisticated and automated methods of analysis that could lead to significant improvements in the efficiency of forensic laboratories, reducing backlogs and enabling faster turnaround times for evidence processing.

Abstract Text: The identification of tire rubber traces is a crucial aspect of forensic investigations, particularly in cases involving traffic accidents, hit-and-run incidents, abductions, hostage situations, and the unauthorized transit of prohibited items. These traces can appear as skid marks on road surfaces, imprints on clothing, or residues left on victims or other objects after sudden stops or abrupt movements. Such evidence plays a vital role in narrowing down suspects by establishing a connection between the crime scene, the getaway vehicle, and the perpetrator through the through the identification of the brand, manufacturer, or origin of automobile tire. There is a growing need for efficient, non-destructive, and accurate methods to examine these traces, as traditional approaches can be both time-consuming and destructive. This study proposes an innovative approach that utilizes ATR/FTIR spectroscopy, combined with machine learning algorithms to classify tire rubber samples from various brands sold in India and throughout the world.

ATR/FTIR spectroscopy is a non-destructive analytical technique that provides detailed spectral information on the molecular composition of tire materials, making it ideal for forensic applications. Its rapid analysis and minimal sample preparation requirements further enhance its applicability. The study involves the classification of 220 tire rubber samples from multiple brands, creating a comprehensive database for forensic comparisons. The main focus of this study is to evaluate the accuracy of classifying tire rubber samples from Light Motor Vehicles (LMVs) and Heavy Motor Vehicles (HMVs) and to identify the most effective machine learning algorithm for discriminating various rubber tires in accordance with brands. The spectral information obtained from ATR/FTIR is subjected to several preprocessing steps, including baseline correction, smoothing, derivatization, and normalization, to reduce noise and enhance reliability of the subsequent machine learning analysis by minimizing the effects of noise and other inconsistencies in the data.

Further, several machine learning models were evaluated, with the Extra Tree (ET) classifier emerging as the best-performing algorithm, achieving over 90% classification accuracy in distinguishing between LMV and HMV automobiles. After fine-tuning, it provided 73% accuracy in identifying three specific tire brands, namely JK Tires, Apollo Tires, and MRF Tires. The ability of machine learning algorithms to handle large datasets and identify complex patterns within the data makes it an ideal tool for forensic applications by providing valuable evidence that can link a suspect to a specific vehicle.

Tire Rubber; Trace Evidence; FTIR

B119 The Development of a Protocol for the Elemental Analysis of Tire Marks in Road Crime Forensics

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Learning Objectives: Attendee's will learn about different methods of skid mark particle lifting and analysis, with their comparison to known tires. They will also learn about its application to Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) analysis for forensic association of tire skid marks.

Impact Statement: This presentation will impact the forensic science community by providing another form of trace evidence analysis, especially helpful in traffic accidents and hit-and-run accidents.

Abstract Text: With nearly 33,000 hit-and-run fatalities occurring in the United States between 2004 and 2022, extracting detailed information from accident scenes is crucial. Identifying who is at fault is essential for delivering justice to the victims. Traditionally, tire mark analysis relies on physical reconstruction of the accident, but analyzing the rubber left from braking events can provide additional evidence. By examining tire particles and linking them to their specific tire source, crucial evidence can be gathered to perhaps identify the perpetrator to the hit-and-run accident.

Tires vary by model and brand, each with a unique chemical composition designed for specific characteristics. Additionally, tires have trace elemental contaminants introduced through manufacturing that help in the association with their source. While some studies have shown the use of rubber analysis for the classification of tires, elemental analysis can be a very sensitive approach to use the contaminants as signatures of manufacturers and models of tires. To that end, ICP/MS has been explored in this study, and we show its success to classify tires with over 90% accuracy based on their elemental profile, distinguishing among 39 different tire models.

While the unicity of tire models is a necessary demonstration, the association of tire particles from skid marks to the tire itself is the goal of such application. Effective sampling of tire marks from the road is then essential for capturing as many tire particles as possible while minimizing contamination from road surfaces and the sampling method itself. Various techniques were explored for sampling and extracting tire rubber from road surfaces while being optimized for ICP/MS analysis. Statistical analysis shows that the association between skid marks and their source tires can be confirmed or reveal no connection to unrelated tires.

The results presented in this work will show how tire particles and tire rubber can be analyzed quantitatively with minimal to null contamination from the road materials, and we will discuss how this novel analysis could help road crime forensics practitioners.

Trace Analysis; Tire Marks; Accident

B120 The National Institute of Standards and Technology (NIST) Mass Spectral Reference Libraries

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Learning Objectives: During this presentation, attendees will learn about the types of mass spectral libraries available from NIST and how they are made (how compounds are chosen, how spectra are evaluated, etc.). Attendees will also learn about the software tools freely available from NIST.

Impact Statement: This presentation will impact the forensic community by informing them of how high-quality mass spectral libraries are made and how to use the tools available to them. This talk will also demonstrate the utility of using retention indices in coming to the correct identification.

Abstract Text: The NIST Mass Spectrometry Data Center (MSDC) maintains several mass spectral libraries. These libraries include the Electron Ionization (EI) library (collected at 70eV), the tandem (electrospray) spectral library, and the Direct Analysis in Real Time (DART) library. The EI and tandem libraries are commercial products distributed through vendors and are on a three-year update cycle, with 2026 being the next release. The MSDC has an extensive compound selection process to ensure new additions to the library are relevant. The libraries are carefully curated by a professional staff to ensure the spectra are of the highest quality. The MSDC has developed data processing software tools to aid the extraction, search, and evaluation of spectra, and the tools are freely available. The software available includes Automated Mass Spectral Deconvolution and Identification System (AMDIS) for mass spectral deconvolution, MS Search for spectral searching, and MS Interpreter (to aid in interpretation).

The NIST23 EI mass spectral library contains 394,000 spectra of which 347,100 are unique compounds.[1] The library has 492,000 experimentally determined retention index values for a variety of column polarities. Each library entry also has an artificial intelligence-predicted as well as a group additivity model-predicted retention index value for a semi-standard non-polar column. Each library entry contains meta data that includes common name, molecular formula, molecular mass, chemical structure, InChIkey, Chemical Abstract Service registration number (when available), and a link to search the internet for more information. The EI library contains compounds of interest for the forensic community, including, but not limited to, over 250 fentanyl-related compounds, over 125 tryptamine-related compounds, over 125 benzodiazepine-related compounds, and over 500 synthetic cannabinoid-related compounds.

The 2023 tandem mass spectral library contains 2.4 million spectra from 51,501 compounds. The library contains ESI high resolution accurate mass spectra for 49,590 compounds, ESI low resolution spectra for 49,865 compounds, and high resolution APCI spectra for 561 compounds. The tandem library has low resolution spectra for over 220 fentanyl compounds, over 50 tryptamines, over 75 benzodiazepines, and over 350 synthetic cannabinoids. The library also has high resolution spectra for over 225 fentanyl compounds, over 50 tryptamines, over 65 benzodiazepines, and over 350 synthetic cannabinoids.

The DART mass spectral library is a freely available library tailored to compounds of forensic interest. Each of the 1,371 compounds in the library consists of a set of three different in-source collision-induced dissociation mass spectra. The DART Data Interpretation Tool (DIT) utilizes an Inverted Library Search Algorithm (ILSA) to look for confirmation in higher fragmentation energy spectra of a component found in low fragmentation energy spectra.[2]

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Mass Spectrometry; Spectral Libraries; NIST

B121 The Development and Evaluation of a Contrastive Learning Framework for Source Identification: Technology for Transformation in the Interpretation of Forensic Data

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Learning Objectives: Participants will learn about the contrastive learning strategies from artificial intelligence and a specific framework applicable for using these algorithms to assist examiners in interpreting data for source identification.

Impact Statement: The forensic science community will be impacted through the understanding of the proper usage of machine learning methods, which examiners can apply to comparative analyses.

Abstract Text: To interpret the value of forensic evidence that results in data from a pair of items, the common source identification framework asks the question: do the items share a common unknown source or come from two different unknown sources?[1] This question can be addressed using a variety of forensic statistics techniques, including the usual Two-Stage, Likelihood Ratio, and Bayes Factor approaches.[2,3] Contrastive learning methods address the question using two major components: a method for quantifying the similarity (or dissimilarity) of pairs of evidential items and a method for determining the best separation between within-source or between-source comparisons.[4] Contrastive learning methods are particularly useful when the data derived from the evidence is high-dimensional or complex. In this case, the contrastive learning algorithms take advantage of high-performing artificial intelligence and machine learning tools to avoid specifying complicated probability models for the usual forensic statistics approaches. In this presentation, a contrastive learning algorithm framework is developed for complex evidence and applied to data from aluminum (Al) powders.

In comparing Al powder particles recovered from two pre-blast Improvised Explosive Devices (IEDs), the goal is to determine whether the powder sources are associated, potentially providing investigative between-case linkages. Unfortunately, the complex nature of the resulting data creates difficulties in applying the usual statistical approaches in a straightforward manner. For characterizing features of the Al powder, we take several subsamples from the bulk Al powder, several aliquots from each subsample, several fields of view are imaged on each aliquot, and then multiple micromorphometric parameters are measured for each particle in a field of view. This means that the multiple measurements on each single object can be aggregated at different substructure levels when applying statistical techniques for interpreting the evidential value.

During this presentation, we apply contrastive learning techniques with the ultimate aim of assigning an interpretable value of evidence, and we explore different methods of summarizing the data, quantifying pairwise similarity, and classifying pairs as within- or between-source comparisons. The contrastive learning methods in this presentation can also be applied to other complex data encountered in the analysis of trace and pattern evidence.

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Scores; Machine Learning; Trace Evidence

B122 The Curious Case of the Elusive Generalist

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the need to establish a structured and universal generalist course of study to serve as the foundation of forensic science education in order to enforce a shared understanding of forensic science, regardless of individual discipline or specialization.

Impact Statement: This presentation will impact the forensic science community by challenging individuals to rethink existing forensic science education with the goal of viewing the generalist approach as a foundation on which to build one's knowledge as a forensic scientist. This approach focuses on principles outlined in the Sydney Declaration and includes content such as understanding the nature of traces within the event scene; logic and reasoning; meaningful evaluation and interpretation of findings in context; and communicating scientific information. It is this fundamental generalist education on which later specializations should be built, and it is the properly educated and trained generalist that can serve as advisor as traces move from the scene to the court.

Abstract Text: Forensic science has advanced considerably with technology. Many scientists have welcomed this development but bemoaned the lack of guiding principles from which scientific problem-solving should proceed. This presentation will focus on the need for the generalist to support the specialist role.[1] Some technologies will be explored to illustrate the risk of technology becoming the master and the scientist its subordinate. An obvious difficulty arises when we are not in a position to interpret and explain the output of technology applied to forensic science problems. How to manage this will be explored.

Different jurisdictions have addressed the application of new and emerging technology in different ways, primarily relying on Standard Operating Procedures (SOPs) and (traceable) standards. This can be rendered more complex when statistical methods are arbitrarily applied to forensic science problems. This presentation suggests that SOPs, standards, and "statistics" alone are insufficient, and a meaningful, structured education in forensic science principles and philosophies is essential to ensure the correct use and application of science within the criminal justice system.

Within myriad fields, the specialist employs a narrow but deep focus, while the generalist has a wider, more shallow focus. As reflected in the medical model, these two should be complimentary. The specialist in the laboratory has no material to examine unless it is recovered from the scene. This statement is obvious but not enough attention is devoted to the need for a generalist to facilitate this process. More is needed beyond blindly utilizing technology without applying reasoning to ensure the right questions are being asked in the context of the case. The assumption may be that the crime scene examiner is knowledgeable about the technology employed and thus will be well-versed in how and what to collect in order to maximize the "evidential potential" within the laboratory. In extreme situations, this can lead to overlooking other traces that could meaningfully address the questions that need to be answered in an investigation and reconstruction—*who, what, where, when, why,* and *how*. As such, the authors argue that the generalist role is needed to support forensic science within the broader criminal justice system.

Once laboratory results become available, someone is needed to interpret them within the context of the case. This has been left primarily to the specialist in which their views have been developed according to different norms for different evidence types, leaving decision makers to differentiate between the values of various outcomes. The inclination is to focus on the specialist field to define the problem. The growing tendency is to assume that digital forensic science will be the answer to all forensic science problems, as human nature seeks to find a simple answer or silver bullet to address issues that may arise during an investigation. Forensic scientists are no different in seeking useful outcomes with minimal effort and faster turnaround times.

Would the situation be better if an educated generalist was present at the starting point for all forensic science problems and if all forensic science was built on foundational principles regardless of trace type or technology? We argue, yes. The key is a generalist program serving as the foundation of forensic science education, enforcing a shared understanding of forensic science—one that extends beyond forensic science history and terminology provided in introductory textbooks. The generalist education requires: knowledge of traces and how they behave (e.g., creation, transfer, persistence); logic and reasoning; the proper application of statistics; meaningful evaluation of findings; and the ability to clearly communicate findings.[2] The Sydney Declaration defines forensic science and the fundamental principles that underpin it, thus establishing a foundational knowledge for all forensic scientists regardless of discipline or specialization.[3,4]

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Generalist; Education; Sydney Declaration

B123 The Implementation of a Uniform Approach to Determine Measurement Uncertainty in Routine Chemical Forensic Casework

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Learning Objectives: After attending this presentation, attendees will have knowledge of a new uniform approach to determine Measurement Uncertainty (MU) in routine chemical forensic casework.

Impact Statement: This presentation will impact the forensic community by illustrating how reliable and uniform results may be obtained when determining MU in routine chemical forensic casework.

Abstract Text: Results obtained from quantitative research inherently involve uncertainty. At every step of the analytical process, from sampling to the final measurement, deviations from the true value in question will occur. The concept of MU is used to represent this imperfection. Without information on MU, measurement results may be misinterpreted, leading to incorrect conclusions. Determination of the MU basically entails describing how to convert an observed measurement into an interval that, with a certain level of confidence, contains the true value.

MU determination is a common issue in routine chemical forensic casework, where measurements are taken on, for example, seized drugs or whole blood samples. In these cases, quality control measurements are routinely performed and recorded on control charts to monitor and quantify measurement process variation. Control measurements are often performed on either Certified Reference Material (CRM), uncertified reference material (prepared in-house), or in Proficiency Tests (PTs).

Principles of the methodology for determining MU are laid down in guidelines.[1-4] Typically, the statistical model assumes that any measurement equals a real value plus a systematic bias, plus a random error. In forensic chemistry measurements, this model is often applied in a relative manner, meaning relative to the true value.

The approaches for MU found in the literature may have some drawbacks. For example, the formulas used to quantify MU involve implicit double counting of variance components, and the formulas vary considerably depending on the type of reference material used. Moreover, compensation terms for bias may be quite conservative.

A method was developed to determine MU in a uniform manner, for any type of reference material, based on formal statistical analysis. The system corrects measurements for observed bias, after which the combined MU is only determined by two factors: the relative standard deviations under reproducibility conditions within the lab and of the assumed true value of the reference material. Estimations of these factors are based on a top-down approach because of the complexity of the chemical analysis. This method has been implemented at our institute.

In conclusion, MU that was determined in guidelines could be quite conservative, differed heavily on the type of reference material used, and was not always exact as to calculation rules. Therefore, we see the new approach as a step forward in these respects. It may also be useful in a broader area than forensic casework.

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Measurement Uncertainty; Forensic Chemistry; Uniform Approach

B124 A Study of Standards Implementation Across the Forensic Sciences: Notes, Trends, and Future Directions

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Learning Objectives: Attendees will learn about efforts to standardize forensic practices from the national point of view, how standards are created and published, and the extent to which these standards are being deployed in forensic units throughout the United States. The manner in which implementation data is being used to improve the standards development process will also be emphasized.

Impact Statement: This presentation will impact the forensic community by demonstrating the extent to which standards are being used throughout the forensic sciences. It is expected that additional forensic science service providers will be encouraged to implement standards, provide data on that implementation, and further support the efforts to increase and improve forensic standardization across the forensic landscape.

Abstract Text: In 2009, after much anticipation by the forensic science community, the National Academy of Sciences' National Research Council released their Report, *Strengthening Forensic Science in the United States: A Path Forward*.[1] The Report outlined 13 recommendations to strengthen scientific rigor across the forensic landscape. Among these recommendations was a call to increase standardization throughout forensic practice with particular emphasis on the scientific analysis of evidence and the reporting of results. To address this need, the National Institute of Standards and Technology (NIST) in conjunction with the United States Department of Justice established the Organization of Scientific Area Committees (OSAC) for Forensic Science in 2014.

Over the past ten years, OSAC has built processes to evaluate, review, draft, and improve forensic standards with the goal of placing them on a highly vetted repository known as the OSAC Registry. Through the diligent efforts of hundreds of forensic practitioners, laboratory managers, scientific academicians, defense attorneys, prosecutors, judges, statisticians, and human factors experts, the Registry has grown to include almost 200 published and proposed standards.

The growth of the Registry over this period is certainly a significant accomplishment. However, without data to demonstrate the extent of implementation of these standards, it is difficult to evaluate the impact of these efforts. To this end, the National Institute of Standards and Technology's (NIST's) Special Programs Office has been collecting standards implementation data from forensic science service providers willing to voluntarily provide this information since 2021. With the initiation of an "open enrollment" concept in 2023, in conjunction with the launch of a new electronic survey tool in 2024, NIST has gained greater ability to look more critically at implementation data to better evaluate and improve the state of forensic science standards implementation.

This presentation will provide a brief overview of NIST's initiative to collect standards implementation data and discuss the importance of these efforts. Through various illustrations, this presentation will also reveal the current extent of standardization in the forensic sciences, highlight implementation related trends, and outline how these data can be used to improve the national forensic science standards landscape.

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Standards; OSAC; Data

B125 Problematic Exclusionary Differences: The Tyranny of Universal Standards for Determining Exclusionary Differences

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Learning Objectives: Attendees of this presentation will gain an understanding of the statistical issues with the use of the American Society for Testing and Materials (ASTM) -style comparison methods for trace element profiles when source-dependent thresholds for declaring an exclusionary difference exist. Attendees will learn about novel developments for testing if multiple thresholds are warranted.

Impact Statement: The use of statistically rigorous methods for characterizing the applicability of standards for identifying exclusionary differences will be explored. The implications of differing rates of false exclusions among sources (resulting from the inappropriate use of a single threshold) will be discussed relative to the concept of algorithmic fairness and the fidelity of evidence interpretation to assist in the administration of justice.

Abstract Text: Within the forensic science community, there is a need for a statistically rigorous determination of whether an exclusionary difference exists. With respect to the ASTM glass interpretation standards, this difference is determined to have occurred if any one of the standardized differences between the measured trace element concentrations is greater than four.[1-3] In this work, we will present strategies for assessing the validity of using a common threshold for all sources when determining if an exclusionary difference exists.

The determination of when an exclusionary difference exists is integral to the Kirkian Two-Stage approach to evidence interpretation.[4] If an exclusionary difference is observed when comparing questioned v.s known source objects, then the known source is excluded as having given rise to the questioned object at the first stage of the approach; however, if the known source is not excluded as the source of the questioned object, then the examiner must determine the rate at which competing alternative sources would be excluded. These methods/algorithms are typically constructed to ensure that the false exclusion rate for each source is equal. However, if the underlying score function that the algorithm is based on has a distribution that varies by source, then the corresponding thresholds for determining an exclusionary difference will need to vary as well.

In this work, we will review various strategies for identifying when the within-source distribution of scores varies between sources; methods for estimating the thresholds; remedial approaches such as pooling subsets of the sources together; and implications of this type of variability among the sources to the classical Kirkian approach to evidence interpretation. Although we will focus on the Kirkian approach, these issues are even more problematic for Likelihood Ratio (LR) -based methods due to the need to estimate a likelihood function from just a few observations from a specified source; the discussed remedial methods will apply equally to the LR paradigm for evidence interpretation.

We will illustrate these methods with trace element concentrations in glass and Aluminum (Al) foil as well as micromorphometric data from small arms propellants.

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Exclusionary Difference; Algorithmic Fairness; Evidence Interpretation

B126 Standards Development Activities Related to Friction Ridge Examination

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Learning Objectives: 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.

Abstract Text: 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 American Academy of Forensic Sciences (AAFS) in 2015 as an 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

B127 Forensic Lab's Phoenix Rise: The Remarkable Redemption of a Lost Latent Fingerprint Unit

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Learning Objectives: Attendees will learn about the comprehensive strategies and methodologies employed to restore the accreditation of the Latent Fingerprint Unit (LFU), including the overhaul of Standard Operating Procedures (SOPs) and the implementation of a specialized training program. They will gain actionable insights into quality control measures and continuous improvement practices that can be applied to elevate standards and achieve excellence in forensic identification.

Impact Statement: The loss of accreditation and quality issues within the LFU had significantly impacted the credibility and reliability of our forensic processes, eroding trust within the community and among stakeholders. Implementing robust SOPs and specialized training programs is essential to restoring our accreditation and elevating our operational standards. These measures will ensure accuracy and integrity in our analyses, helping to rebuild our reputation and regain confidence in our forensic capabilities.

Abstract Text: In recent years, the Department of Forensic Sciences (DFS) faced a significant setback with the loss of accreditation and quality issues within its LFU. This adversity prompted a critical evaluation of existing practices and a call for comprehensive reforms. In response, Alice White, a renowned expert from Evolve Forensics, conducted a thorough gap assessment and specialized training sessions for LFU personnel. This initiative aimed to address deficiencies and equip the LFU team with the latest techniques and methodologies in latent print examination.

The challenges faced by the LFU were multifaceted, including outdated procedures, lack of standardized training, and gaps in quality control measures. The loss of accreditation served as a wake-up call, highlighting the urgent need for systemic changes. The collaboration with Evolve Forensics marked the beginning of a transformative journey. Alice White's expertise was instrumental in identifying critical gaps and areas for improvement, setting the stage for a comprehensive overhaul of the unit's operations.

The gap assessment revealed issues in the foundational skills (minutiae mark-up, distortion, latent isolation, and suitability) that required immediate attention. These included the need for updated SOPs, enhanced training programs, and the implementation of rigorous quality control measures. The specialized training sessions conducted by Alice White were designed to address these deficiencies, providing LFU personnel with the skills and knowledge necessary to perform their duties to the highest standards. The training covered various aspects of latent print examinations, from incorporating an explicit suitability criterion to expanding the conclusion scale, ensuring that the team was well-equipped to handle the demands of their work.

Following the completion of the training, the LFU Manager played a pivotal role in implementing the recommended changes. This included the overhaul of SOPs to align with best practices in the field, as well as the development of a robust training manual tailored to the specific needs of LFU personnel. The new SOPs provided clear guidelines for applying the suitability criteria appropriately and consistently, ensuring consistency and accuracy in the unit's work. The training manual served as a valuable resource for both new and experienced team members, offering comprehensive guidance on all aspects of latent print examination.

The proactive measures taken by the LFU Manager also included the establishment of a continuous improvement program, aimed at maintaining high standards of quality and efficiency. Regular audits and reviews were instituted to monitor compliance with the new procedures and identify areas for further enhancement. These efforts have been instrumental in restoring the unit's reputation and regaining accreditation.

Through these concerted efforts, the LFU has not only elevated its standards but also positioned itself as a beacon of excellence in forensic identification. The journey of the LFU serves as a testament to the power of collaboration, expertise, and unwavering commitment to quality. The LFU's experience provides valuable lessons for other forensic units facing similar challenges, highlighting the importance of continuous improvement and the pursuit of excellence in the field of forensic science.

Accreditation; Quality Assurance; Latent Prints

B128 Assessing the Performance of Fingerprint Laboratories Through Collaborative Exercises: The ENFSI Fingerprint Working Group Experience

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Learning Objectives: By attending this presentation, attendees will learn how to design an interlaboratory testing scheme (i.e., collaborative exercise), including (1) how to define the aim of the test; (2) how to design the test in its phases (selection of the type(s) of item, preparation of the pilot test, evaluation of the pilot test); (3) how to prepare and distribute the test; and (4) how to collect and evaluate the information collected.[1] Moreover, attendees will benefit from a series of examples of collaborative exercises run between 2004 to 2024.[2-6]

Impact Statement: This presentation will impact the forensic science community by setting practical rules on how to organize an interlaboratory comparison in the fingerprint domain, in conformance to the published regulatory literature (European Network of Forensic Science Institutes [ENFSI], National Institute of Standards and Technology-Organization of Scientific Area Committees [NIST-OSAC]). Additionally, major outcomes and lesson learned from the organization of interlaboratory testing schemes in the past 20 years will be highlighted, thus demonstrating their use in the assessment of the performance of forensic science providers.

Abstract Text: According to the document "Council conclusions on the Action Plan for the European Forensic Science Area 2.0," there is a need to "ensure continuous development of key forensic science capabilities in order to secure and strengthen its current and future relevance within the judicial system."

Friction ridges are still the most used biometric used in the criminal justice system. To guarantee the validity of the friction ridge examination, since the end of 2015, all European fingerprint services laboratories have to be International Organization for Standardization **(**ISO) 17025 accredited to be able to exchange the results of their expertise within European Union (EU) countries. Therefore, the conduct of proficiency tests and collaborative exercises at the international level has to be seen as a mandatory activity for the forensic fingerprint laboratories. The availability and use of proficiency tests is limited in the friction ridge domain. There are only a few companies providing proficiency tests in the field of friction ridge visualization, imaging, and comparison, and these tests show a modest level of difficulty, according to practitioners.

"Demonstrating reliability in forensic results" imposes a robust quality and competence assurance, which can be achieved not only by measuring the outcomes through proficiency testing, but also by collecting all the information needed to ensure a root cause analysis in case of failure, which is granted only by means of collaborative exercises. Interlaboratory exercises are aimed to: (1) provide a clear picture of the performance of fingerprint laboratories participating in the scheme; (2) identify shortcomings or failures in the practice; (3) suggest remedies to the measured errors;(4) detect the needs for specific training related to the application of the current forensic methods; (5) disseminate the results, facilitating educational sessions through ad hoc workshops; (6) contribute to the standardization of the methods and of the evaluative reporting, through the benchmarking of laboratories' performance; (7) provide with the European Conformity (CE) organizers (i.e., ENFSI ATACH Working Group on Financing [FIN-WG]) a robust procedure to design, validate, realize, and evaluate CEs in the friction ridge field to assess the performance of the member laboratories.1

ENFSI FIN-WG started in 2004 to provide free-of-charge CEs to the WG members both in the visualization of friction ridge marks and in the comparison of marks with a known source.[2-6] The outcomes of these CEs showed a heterogeneous situation across Europe: failures in CEs are not strictly related to the accreditation status of laboratories.

As stated by European Food Safety Authority (EFSA) 2.0, "The ability to extract, use and exchange biometric data is one of the cornerstones of forensic science. Securing the current and future use of biometric procedures, including final human verification of results, within forensic science is essential for the ability to support forensic science at large". The data collected through CEs in the past 20 years, and only partially made available to general public via scientific publications, demonstrates that there is a consistent gap to reach this objective.[2-6] The use of commercially available PTs does not provide the assurance of a reliable performance since such tests are not sufficiently representative of the average difficulty of routine examinations. Moreover, the collected results show a relevant inconsistency in the examination and in the interpretation of the data, thus reducing the reliability of the friction ridge evidence. The main objective of the action is to highlight the impact of the different examination methods and evaluation on a homogeneous (or identical) testing sample, thus highlighting the limits of local procedures. A robust quality and competence assurance is the only path to ensure the reliability of forensic results. By constantly demonstrating with factual data the limits of some of the current procedures, a standardized approach to friction ridge examination will be reached in the long term.

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Fingerprint; Interlaboratory Comparison; FSPs Performance

B129 An Investigation of Fingermark Residue Components and Differentiation From Contaminants

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Learning Objectives: After attending this presentation, attendees will be able to identify exogenous cosmetic contaminants and key endogenous volatile compounds present in fingermark residue.

Impact Statement: This topic will impact the forensic community by providing a proof-of-concept method study for the non-targeted analysis of fingermark residue using new instrumentation. Baseline fingermark residue analysis will provide a foundation for the analysis of trace contaminants found in fingerprint residue.

Abstract Text: The ridge characteristics from a deposited fingerprint are routinely used by forensic specialists to individualize a pattern to a suspect. Partial fingerprints that lack a complete ridge pattern are often not fit to undergo the identification process, although their residue may still contain valuable chemical information about a suspect or a crime scene. These incomplete prints, called fingermarks, consist of sweat and oil originating from the sebaceous glands of their donor. There have been initial studies analyzing fingermarks using Gas Chromatography (GC) that have demonstrated their residue contains biomarkers, including alcohols and lipids, that can distinguish between sex and age of donors. However, there has been little research in the full, non-targeted characterization of fingermark residue using advanced chromatographic methods to allow: (1) full comprehensive profiling of all fingermark residue components simultaneously without derivatization, and (2) simultaneous differentiation of fingermark components from external contaminants. This is largely due to the fact that the number of components can be extremely high when considering both endogenous fingermark components and other residues present through contact, and, therefore, traditional methods for component analysis can be limited in their ability to separate, identify, and visualize all analytes within a single method.

The goal of this study was to develop a method for the collection and non-targeted analysis of fingermark residue using comprehensive Two-Dimensional Gas Chromatography/Time-Of-Flight/Mass Spectrometry (GC×GC/TOF/MS). Fingermarks were collected on a microscope slide after hand washing and regeneration of residue. The residue was dissolved in solvent and analyzed using GC×GC/TOF/MS. Peaks for common fingermark compounds such as cholesterol and squalene were identified and exhibited peak areas with Relative Standard Deviation (%RSD) of approximately 56.53 % and 33.21 %, respectively, over a sample set of 18 fingermarks. Fatty alcohols such as palmityl alcohol (C16) and stearyl alcohol (C18) were identified as well as various saturated fatty acids from C_8-C_{34} such as octanoic acid (C8:0), myristic acid (C14:0), and palmitic acid (C16:0).

Several cosmetic contaminants were resolved from fingerprint residue. Octocrylene and avobenzone, common sunscreen ingredients, were respectively present in 44 % and 28 % of samples analyzed. In addition, α-tocopheryl acetate, the cosmetic form of Vitamin E, was resolved from fingermark compounds. The use of GC×GC subtraction plots yielded effective visualization of endogenous and exogenous components that may yield value for presentation in expert witness testimony. The presence and identification of analytes outside of expected fingermark compounds provide a baseline for future probing of residue with additional external contaminants such as explosives, gunshot residue, drugs, or other personal care products that may be relevant in forensic casework.

Fingerprint Residue; Trace Analysis; Chromatography

B130 Developing Reproducible Protocols and Definitions for Selecting Combined Minutiae: Applications in Research and Practice

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Learning Objectives: Attendees will learn about the protocols developed to assess the content of latent print examiners' responses to questions about use of minutiae frequencies in case work as well as the process through which a repeatable and reproducible protocol was created for standardizing the collection of data about combined minutia types. Attendees will see new data regarding the way in which latent print examiners use minutiae rarity in their Analysis and Comparison judgments and what sources of information informed their estimates of minutiae rarity. Attendees will see new information about objective minutiae frequencies and the protocol developed to establish objective frequencies for combined minutiae in research.

Impact Statement: This presentation will impact the forensic science community by providing fingerprint examiners with information about how latent print examiners weigh the presence of minutiae according to the perceived rarity of those minutiae and their placement as well as the sources of the information used to make these judgments. The presenters will also give an update regarding the collection of objective frequencies for combined minutiae. This presentation will also include a description of the development of the repeatable and reproducible protocol created for standardizing the collection of data about combined minutia types and the benefits of using this protocol in future research.

Abstract Text: 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 given that 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 examiner, as is their ability to communicate their analytic process and the reasons for their expert opinion.

Although we know that some minutiae are rarer than others, there exists very little systematic research about minutia base rates and latent print examiners' perceptions of minutiae rarity.[1] Research on judgment and decision-making shows, though, that people generally struggle with the task of estimating how often events or features occur, and that different contexts can alter how data are perceived, interpreted and evaluated.[2] Thus, we can expect variation in latent print examiners' estimates of minutia frequency and their classification of friction ridge events within minutia types. We can also expect that latent print examiners' beliefs about minutia rareness or commonness will affect how much weight is given to each feature during analyses.[3]

Last year, we presented data on latent print examiners' perceptions of minutia frequency.[4] With these data, we were able to provide consensus-based evidence of minutia frequency in general, within different friction ridge patterns (loop, arch, whorl), and by location within the fingerprint impression (i.e., center versus periphery). In addition to consensus data on minutia rarity, we also presented research-based suggestions for appropriate, popular minutia labels to help standardize communication in the field.[5]

This year, there are two recent, significant data updates to share. First, we will present the results of a content analysis that involved extracting themes from latent print examiners' explanations of how they use their perceptions and knowledge of minutia frequency in their casework. We will present information about the most common ways minutia frequency is factored into latent print examiners' Analysis and Comparison judgments, as well as their reports concerning the origin of their knowledge about minutia base rates (e.g., experience, formal training, scientific articles).

The second major update concerns the creation and implementation of a repeatable and reproducible protocol created for standardizing the collection of data about combined minutia types. As was the case for other phases of this research project, the development of this protocol was an interdisciplinary endeavor, and relied on an iterative process to achieve the goal of a useable, practical protocol that could be adopted with ease by other research groups and in casework. We will present some updates on the objective minutia frequency database and discuss the benefits of using a rigorous, research-based protocol like the one developed for this project in future research.

Taken together, these data provide new insights into latent fingerprint examiners' use of perceived minutia frequency in their casework. Our data provide significant advances in the standardization of latent print examiners' use of minutiae in casework and will help to standardize future research so that data across studies can be synthesized. These studies can also serve as a model to other forensic disciplines seeking to increase consistency and reliability.

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Latent Prints; Standards; Human Factors

B131 Likelihood Ratios for Fingerprint and Palm Impressions

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Learning Objectives: Attendees will learn how likelihood ratios can be calculated from human examiner data from error rate studies. They should be able to describe the limitations of source conclusions and the advantages of likelihood ratios. Finally, they should be able to communicate how current practice overstates the strength of the evidence by wide margins in some cases, which can lead to clear miscarriages of justice.

Impact Statement: Articulation language must be calibrated against the strength of the evidence or fact finders will overinterpret the results of forensic comparisons. We find that in many cases the likelihood ratios for image pairs are much lower than what would be expected based on published verbal equivalency scales. This demonstrates that the terms Identification or Source Identification as currently defined are inappropriate, and the present work provides the data to allow for community-approved changes that bring the terms in line with the actual strength of the evidence.

Abstract Text: The results of fingerprint and palm comparisons are communicated using posterior language such as "Identification" and "Source Identification." Recent efforts have recast these to strength-of-support statements such as "extremely strong support for common source."[1] However, none of these terms have been calibrated against the actual strength of the evidence, except indirectly in error rate studies.

In this talk we use an ordered probit model to reanalyze error rate study data to compute likelihood ratios for each image pair in an error rate study.[2-4] The distribution of subjects who made conclusions on each pair are converted to an underlying latent scale that expresses the support for the same source proposition as determined by the distribution of responses. For example, if 12 examiners say Identification and 4 say Inconclusive, this offers more support for the same source proposition than an image pair where 3 examiners say Identification and 13 say Inconclusive. The ordered probit model is an extension of signal detection theory to individual image pairs, and, when combined with additional assumptions of independence between the examiners and image pairs, can be used to compute the probability of the observation (the latent value), given the same source and different sources propositions. The ratio of these two probabilities is the likelihood ratio.

We find that in many same source pairs the likelihood ratios are less than 100. Likelihood ratios are less than 10 for some comparisons for which a majority of examiners reached an Identification decision. Given that phrases such as "extremely strong support" are typically associated with likelihood ratios of 10,000 or a million, we find that current articulation language overestimates the strength of the conclusion by up to five orders of magnitude.[5]

The results help calibrate the existing source conclusion articulation scale. Likelihood ratios have additional benefits: They explicitly acknowledge the support for both the same-source and different-sources propositions, do not require knowledge of priors or the costs of errors, and are readily integrated with other likelihood ratios through Bayesian updating. We explore possible extensions to active casework and discuss the potential problems with moving to a strength-of-support reporting style.

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Score Likelihood Ratios; Accuracy; Fingerprint Identification

B132 One Size Does Not Fit All: Variations in Documenting the ACE-V Process Among Latent Print Examiners

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Learning Objectives: Attendees will gain an understanding of the range of current technological tools and approaches utilized by latent print examiners to document the Analysis, Comparison, Evaluation, and Verification (ACE-V) methodology within the examination process and how these strategies have progressed over time. Examples of agency circumstances, associated workflows, and practical insights will be discussed, and attendees will be able to identify factors that latent print units must consider prior to updating procedures or implementing new technological solutions.

Impact Statement: Based on the need for transparent and comprehensive documentation during the latent print examination process, it is important to understand what technological tools (e.g., commercial and forensic software products) are utilized to achieve this goal. It is also necessary to understand how these tools are effectively integrated into case workflows based on diverse agency circumstances. Research has been conducted on what latent print examiners document during the examination process, but there are limited resources describing how the information is documented.

Abstract Text: Latent print examination is commonly conducted using the ACE-V methodology. A variety of manual and digital tools, such as magnifying equipment and software, are routinely utilized by examiners in the examination process to better visualize and annotate observed features in latent and known prints. For example, documentation in the Analysis phase may include details of image acquisition and processing (e.g., color enhancement, calibration, magnification, and annotation of observed friction ridge features), as well as written notes (e.g., free text and dropdown entries) to describe various attributes of a latent print such as the depositing matrix or receiving substrate, clarity, anatomical source, and pattern type. The Comparison, Evaluation, and Verification phases of the examination may include side-by-side depictions of annotated latent and known prints, along with written notes.

Latent print examination notes have historically been criticized as being deficient in reflecting observations from this process. The 2009 National Academy of Sciences (NAS) Report, 2016 President's Council of Advisors on Science and Technology (PCAST) Report, and various emerging best practice documents set forth by the Friction Ridge Subcommittee of the Organization of Scientific Area Committees (OSAC) have called for more transparent and comprehensive latent print examination documentation.[1,2] To accomplish this, examiners and agencies have attempted to provide more thorough written observations and visual aids utilizing varied approaches based on their capacity and organizational infrastructure.

This study will inform the forensic community and provide an overview of the technological tools and strategies currently in use by latent print examiners to document the latent print examination process, with emphasis on the ACE-V methodology. Approaches used by agencies will be categorized into three high-level areas: agency-specific (homegrown) approaches, which include software not specific to forensic applications, such as Adobe Photoshop or Microsoft Office products; customizable laboratory information management systems; and forensic software specifically designed for latent print examination and documentation. Of note, some agencies use a combination of these approaches, which will also be discussed. Based on data collected through end-user experience, details on current agency latent print documentation workflow processes will be elucidated. This study will also cover other current challenges and considerations when implementing a certain documentation approach.

Overall, this study will provide a timely update on the strategies and technology latent print examiners are utilizing, considerations for technology implementation, and recommendations for improving workflow efficiency.

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Latent Prints; Technology Transition; Casework

B133 Visualizing and Enhancing Latent Fingerprints Using Different Floral Powders: An Eco-Friendly Approach

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WITHDRAWN

B134 Enhanced Genetically Variant Peptide Profiling From Human Hair Using Keratinase and Trypsin

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Learning Objectives: The goal of this presentation is to increase the confidence of Genetically Variant Peptide (GVP) identification using the National Institute of Standards and Technology (NIST) -developed hair protein extraction, sample preparation, and data analysis pipeline—at present only traditional, non-targeted, error-prone proteomics methods are available. A freely available, comprehensive hair peptide library covering GVPs will be made available to the forensics community. This work will be of direct use to forensic colleagues for making confident GVP identifications.

Impact Statement: Our long-term goals are to: (1) build a comprehensive and in-depth hair/skin peptide mass spectral library, including all identifiable peptides, a diverse array of experimental, introduced artifacts, and all known hair/skin GVPs; and (2) build a pipeline to generate a useful report of GVPs regarding quality control.

Abstract Text: **Introduction:** Trypsin is usually the default digestion enzyme in proteomic experiments. GVPs derived from human hair proteins have been studied as evidence for human identification in forensics.[1,2] We hypothesized that the use of different proteases can expand sequence coverage in the hair proteome and therefore increase the chance of a variant peptide being identified as well as confirm the findings from tryptic digests. Since keratins and keratin-associated proteins are the main components of hair proteome, we examined keratinase alone and combined with trypsin to explore their ability to generate additional identifiable regular or variant peptides, and this led to both an increase in number of identified GVPs and their confidence of identification.

Methods: Human hair proteins were extracted from individual 5cm-long hair shafts of a male donor, reduced, and alkylated, followed by an optimized SP3 method.[3,4] Keratinase (K, repeated 3 times) was tested alone with enzyme-to-protein ratio 1:20 for 2 hours at 50°C or followed by trypsin (KT, repeated 3 times) with enzyme-to-protein ratio 1:20 for 18 hours at 37°C. K or KT was compared to trypsin (T, repeated 3 times). Peptides were cleaned up with MonoSpin C18 columns for Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) on an Orbitrap Fusion Lumos mass spectrometer. The FASTA file was expanded by adding mutations reported by BioMuta5 for 54 keratins with 7,554 proposed mutations and 92 keratin-associated proteins with 4,534 proposed mutations. We used Sequest (Proteome Discoverer) for database searching.

Preliminary Data: Preliminary analyses led to the following findings: (1) using keratinase alone, the average number of GVPs identified was 31; (2) using trypsin alone, the average number of GVPs identified was 106; (3) using keratinase combined with trypsin, the average number of GVPs was 140; (4) the significant increase of numbers of GVPs found in the combined group (140 vs. 31, $p = 0.0002$; 140 vs. 106, $p = 0.02$) suggests that (a) the addition of keratinase pretreatment facilitated downstream trypsin digestion by making it more efficient, and (b) the combination performed slightly better than by summing results of independent digestions; (5) we identified approximately two times as many peptides in the combined group when compared to trypsin or keratinase alone (average peptides/spectral counts for K, T, and KT: 696/2,034, 680/7,942, and 1,962/16,066); (6) the observation that a variant site was identified in different GVP sequences generated by different enzyme digestions increases the confidence of identification of a single variant site; (7) keratinase led to unique sequence coverage in some hair keratins and keratin-associated proteins compared to trypsin, demonstrating the complementary nature of keratinase and trypsin; and (8) each enzyme resulted in the identification of unique variant sites, suggesting improved GVP discovery. In addition, we are in the process of testing the effect of combining trypsin and other enzymes on protein identification and GVP profiling from human hair.

Novel Aspect: Improved variant peptide identification and confidence by combining keratinase with trypsin for digestion of human hair.

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Hair; Proteomics; Mass Spectrometry

B135 Developing Digital Polymerase Chain-Based Assays for the Detection and Speciation of Foodborne Illness

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Learning Objectives: After attending this presentation, attendees will be informed about a novel digital Polymerase Chain Reaction (dPCR) multiplex analysis for detection of contaminated agricultural products using Bacteroides DNA detection and the use of vertebrate mitochondria DNA (mtDNA) for sourcing the contamination. The results of this work should prove valuable for border protection (Homeland Security) and tracking of foodborne illness.

Impact Statement: This research will impact the forensic science community by informing attendees on the use of nanoplate digital PCR for rapid detection and sourcing of fecal contamination in casework involving foodborne illnesses.

Abstract Text: It has been estimated by the United States Center for Disease Control and Prevention (CDC) that consumption of foods, especially fruits and vegetables, contaminated with feces can lead to about 48 million cases of foodborne illness in the United States yearly, which causes 128,000 hospitalizations and 3,000 deaths.[1] Fruits and vegetables have a significant risk of microbial contamination due to an array of environmental factors that might affect their growth, harvest, and distribution. The bacteriological quality of farm products is commonly affected by fecal contamination.

The routes of fecal contamination of agricultural produce include human excreta on land run off and sewage contamination of oceans, rivers, lakes, and groundwater.[2] Criminal activity or neglect may also be a factor. For example, absence of sanitation barriers and contamination of water sources used on farmlands can lead to enteric bacteria infections. Similarly, contamination from livestock, wild animals, and birds as well as food industry wastes can result in illness or death.

Conventional methods such as the use of enrichment-plating-based detection are considered the most reliable method for the detection of fecal contamination; however, it is time consuming, expensive, and difficult to automate. More recent techniques utilize real-time or quantitative PCR-based primers and probes to detect specific bacteria; however, the complex matrices of these samples can sometimes limit precision, sensitivity, and specificity. A potential alternative to these methods is nanoplate dPCR. This procedure is a more rapid, precise, and sensitive method that partitions the samples into thousands of individual wells (8.5K or 26K), improving specificity and limiting problems with PCR inhibition. The results obtained are then quantified using a Poisson distribution that is based on the target gene's presence or absence in each discrete well.

The goal of this study is to develop a method for use by homeland security using dPCR to detect fecal (Bacteroides) contamination on agricultural produce (i.e., strawberries) at border crossings and to compare these results with standard real-time PCR-based methods.[3] Fecal samples from cattle, rodents, poultry, and other animals were collected and bacterial DNA (Bacteroides) from these samples was collected and analyzed. To determine the origin of the fecal contamination, mitochondria DNA (mtDNA) primers and probes were also developed to detect individual vertebrate species. Multiplex amplifications were developed and validated, and the resultant procedures were tested on inoculated and unwashed fruit. The results indicated the procedure was capable of detecting femtogram levels of bacteria in a sample and the mtDNA probes were species specific and useful in determining the origin of the contamination.

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Bacteroides; Mitochondrial DNA; Digital PCR

B136 Statistical Analysis of Fairness in Forensic Source Identification Problems

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WITHDRAWN

Fairness; Likelihood Ratio; Algorithmic Bias

B137 Enhancing the Number of Contributor Inference Using mtDNA Haplotypes for Mixture Deconvolution

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Learning Objectives: By attending this presentation, participants will learn about using the control region of the mitochondria to complement STR analysis in determining the number of contributors (NoC) to a DNA mixture.

Impact Statement: One of the biggest challenges in forensic DNA analysis is accurately determining the number of contributors in a complex mixture. Although probabilistic genotyping tools have improved mixture analysis, they still rely on the correct NoC input, which is determined by the analyst. This study demonstrates how mtDNA sequencing can support DNA analysts when evaluating STR mixtures for NoC determination.

Abstract Text: With the advent of probabilistic genotyping, more complex DNA mixtures with a greater NoC have become easier to deconvolute and interpret. Despite this, determining the NoC present in a mixture remains a significant concern to accurate mixture deconvolution, as this still requires manual interpretation by the analyst. Entering the wrong NoC into probabilistic genotyping software can drastically alter the accuracy of the deconvolution.[1,2] NoC is usually inferred with STRs by using the Maximum Allele Count (MAC) method, where the locus with the greatest number of alleles is counted to determine the minimum NoC and infer the actual NoC, along with considerations from the rest of the profile. When using MAC with STRs, it can be difficult to accurately infer the NoC in mixtures with four or more contributors due to allele masking. Allele masking occurs when an allele of one contributor is obscured by the allele of another (more abundant) contributor.[3] Preferential amplification, sister allele peak height imbalance, and allele dropout represent additional challenges that may result in underestimating the NoC of a mixture. MtDNA is less affected by this masking when sequencing the hypervariable regions, which are the most polymorphic regions of its genome. Given its abundance and greater resistance to degradation, it is not affected by preferential amplification in the same way STR alleles are.[4] Additionally, there is usually only one haplotype per contributor, rather than one or two STR alleles, reducing the uncertainty around how many markers should be present in a mixture of a given NoC and making the evaluation of allele balance to determine whether two alleles are from the same contributor mute. These factors make mtDNA a promising tool to aid analysts in accurately inferring NoC, complementing the information provided by the STR profile. Despite these potential advantages, mtDNA mixture interpretation has not received much attention due to the difficulties of mtDNA mixture interpretation using Sanger Sequencing; however, the popularization of Massively Parallel Sequencing (MPS) has placed renewed focus on mtDNA mixtures.[5]

In this study, the Precision ID mtDNA Control Region Panel was used to target the mtDNA hypervariable regions, followed by library preparation with the Precision ID Library Kit, sequencing on the Ion GeneStudio™ S5, and analysis using Integrated Genomics Viewer, Qiagen CLC Workbench, and Converge™ Software. Twenty-three individuals were typed to determine the amplicon from the Control Region Panel with the highest discrimination power between the tested individuals. Individuals were then selected that could be differentiated at the region of this amplicon, and 19 mixtures were created between them, representing from 2 to 14 different haplotypes. Contributors were differentiated in the mixture by examining the haplotypes at the chosen region in phase, and the NoC was inferred by counting the number of different haplotypes present. In the mixture with 14 different haplotypes, 12 were successfully identified, and all haplotypes were successfully differentiated in the mixtures with individuals with 9 and 12 different haplotypes. A set of extremely imbalanced mixtures was also tested, and the lowest contributor was detected down to a 1:77 ratio.

Heteroplasmic individuals and maternally related contributors present in a mixture represent limitations to this assay as they could appear as more or fewer contributors, respectively. Determining the extent of these limitations, as well as developing an assay targeting a larger amplicon for increased discrimination, represent the next steps of this research.

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Mixture Analysis; Mitochondrial DNA; Next Generation Sequencing

B138 Different TrueAllele Users, Same DNA Answer: A Multi-Center Proficiency Study

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Learning Objectives: After attending this presentation, attendees will understand how TrueAllele results are independent of source, laboratory, or analyst.

Impact Statement: This presentation will impact the forensic science community by evaluating TrueAllele software across multiple DNA Short Tandem Repeat (STR) data types, DNA laboratories, and forensic analysts. It also shows how analyst software proficiency can be demonstrated within the forensic community.

Abstract Text: For two decades, interlaboratory studies have highlighted considerable variation in DNA mixture interpretation outcomes.[1-3] The same DNA evidence can produce widely different results—one laboratory may calculate a match statistic that connects a suspect to the mixture, another lab may exclude him, while a third can't reach any conclusions. This variability diminishes confidence in forensic DNA science.

Cybergenetics conducted a study of crime laboratories that use its TrueAllele technology. A TrueAllele computer applies Bayesian inference and statistical search to derive genotypes from DNA mixture data. Each "probabilistic" genotype corresponds to one contributor to the mixture. These single-contributor genotypes are compared with reference profiles to calculate a Likelihood Ratio (LR) match statistic. The LR quantifies the statistical support for a person having left their DNA (or not) in the evidence.

Our study had two goals: assessing the TrueAllele proficiency of participating analysts, and examining the concordance of their reported results. Each TrueAllele laboratory sent us electronic data from one mixture item, along with a matching reference profile. The labs produced data using five different Polymerase Chain Reaction (PCR) kits and four different genetic analyzers. The DNA mixtures contained three to five contributors; 70% were four-contributor mixtures. The comparison person comprised 18% to 90% of the mixture.

We sent anonymized data from ten mixtures to 32 analysts across ten participating laboratories. Each TrueAllele analyst processed every item. Once an analyst had completed their TrueAllele processing, we sent them reference profiles for LR comparison. This two-stage data distribution assured objectivity—TrueAllele did not need or use reference information to interpret mixture data.

The lab analysts used TrueAllele comparisons to first determine which reference was associated with which mixture sample. They then calculated LR match statistics for the DNA associations. The analysts returned their match statistics to Cybergenetics, who collated their results and conducted Analysis of Variance (ANOVA) statistical tests. The ANOVA grouped the LR results by mixture item, laboratory, and analyst.

The study showed analyst proficiency in using TrueAllele—all were able to process DNA mixture data and produce match statistics. The ANOVA results demonstrated no statistical difference in LR outcomes between laboratories (p-value = 0.273 > 0.05) nor between analysts (p-value = 0.856 > 0.05).

The TrueAllele laboratories derived reliable results using STR data from other laboratories. No PCR kit or genetic analyzer calibration was needed, since TrueAllele learns this information directly from evidence data. It made no difference where the DNA data came from, nor what lab technology was employed to generate the data.

The study showed that TrueAllele results do not depend on where, when, who, how, why, or what DNA mixture data is generated and interpreted. The LR results are invariant across person and laboratory, DNA complexity and analysis procedure, motivation and bias, or time and space. The answers are the same regardless.

With TrueAllele mixture analysis, all laboratories and analysts get the same output LR answer from the same input DNA data. All qualified experts will report the same answer.[4] This cross-laboratory consistency improves on other approaches that showed high inter-laboratory reporting variation. Reporting concordant LR results increases confidence in forensic DNA science and human identification.

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Proficiency Testing; Validation; Crime Laboratory

B139 The Advancement and Practical Application of a Kinship Identification System Through the Exploration of Genetically Shared Chromosomal Regions Using High-Density SNP Data

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Learning Objectives: After attending this presentation, attendees will understand a novel approach in kinship determination that extends beyond conventional methods by measuring genetically shared regions of chromosomes using high-density Single Nucleotide Polymorphisms (SNPs). Additionally, they will gain insights into the potential applications of this approach in identifying kinship among distant relatives and complex familial relationships.

Impact Statement: This presentation will impact the forensic science community by presenting a promising kinship approach validated through SNP analysis using DNA samples from Korean families. We explored various influencing factors crucial for practical forensic application and demonstrated the effectiveness in resolving real kinship cases across diverse scenarios.

Abstract Text: Relatives share wider regions of chromosomes compared to non-relatives, with closer relatives sharing more regions. While previously theoretical, recent advancements in high-throughput SNP genotyping technologies have facilitated their practical implementation. We have developed a novel kinship determination method that measures shared chromosomal regions between individuals using approximately 250K SNP data generated from the Axiom Korean Biobank Array (Affymetrix Axiom KORV1.1-96 Array). The measurement is quantified in centimorgan (cM) and is referred to as the "GD-ICS" value in the study. This method has been validated on independent SNP datasets generated using real DNA samples from Korean families, providing robust evidence of its effectiveness.[1] Our findings demonstrated the efficacy of this method in distinguishing distant relationships up to the fifth degree of kinships.[2] It has proven effective in distinguishing unrelated pairs from true relatives. Additionally, we examined several factors influencing forensic applications, including the quality of samples and DNA for the array, and the number of SNPs analyzed in the GD-ICS calculation. We also considered the performance of various SNP microarray platforms and ethnic differences beyond the Korean population.

Furthermore, we have successfully applied this method in actual kinship cases where traditional STR and lineage marker testing proved inconclusive. This method provided clarity not only in cases involving distant or complex relationships but also in scenarios where multiple STR mutations posed challenges, even in close relationships. It also firmly supported the absence of genetic relatedness between individuals, proving its applicability in various types of practical casework.

To enhance the practical usability of this method, we are developing a Next Generation System (NGS) -based SNP sequencing panel and calculation system specialized for efficient GD-ICS value analysis. This NGS panel is expected to streamline the process, making it more time and labor efficient. We anticipate this customized panel analysis will provide more practical and efficient solutions for the forensic and broader scientific communities.

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Forensic DNA; Families; Data

B140 DNA Metabarcoding: Can It Reliably Be Used to Characterize Pollen From Surface Soils?

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Learning Objectives: After attending this presentation, attendees will understand: (1) the wet-laboratory steps for completing DNA metabarcoding of pollen, and (2) reproducibility between morphology and DNA metabarcoding for qualitative and quantitative characterization of pollen.

Impact Statement: This presentation will impact the forensic science community by demonstrating of the value of DNA metabarcoding for the characterization of pollen. This method, if implemented into casework, could provide useful information in criminal and intelligence cases.

Abstract Text: Geologic materials such as surface soils are important types of trace evidence as they are transferred during criminal acts, possess unique characteristics, and are often overlooked by perpetrators. Pollen grains are ubiquitous in the environment year-round and are nearly always present in surface soils. In casework, pollen can provide valuable probative information on sample origin and assist in sample-to-sample comparisons. Despite the utility, pollen analysis is not routinely completed on evidence, as it is time consuming, requires specialized expertise, and the quantity of material needed varies on sample type. High throughput DNA sequencing approaches, such as DNA metabarcoding, have been successfully applied to characterize pollen in bulk environmental samples, but have yet to be applied in a forensic context.

Before DNA metabarcoding could be implemented into casework, a side-by-side comparison with results generated using traditional morphological approaches is needed. This study focused on completing this comparison using 250 diverse surface soils with respect to pH, sand/silt/clay composition, surficial material, and location within the contiguous United States. For each sample, pollen was characterized two ways: (1) sequencing two commonly used plant DNA metabarcoding regions (ITS2 and p6 loop of the *trnL*-UAA intron), and (2) using traditional morphological techniques.

We will present the results we have generated to date, focusing on assessing whether DNA metabarcoding is comparable to morphological results at two levels: (1) *qualitative*, whether the same plant taxa are identified using both methods (i.e., taxa presence or absence), and (2) *quantitative*, whether the relative abundance of identified plant taxa is comparable between methods (i.e., proportion of pollen grains compared to proportion of sequencing reads). Additionally, we will outline a cost- and time-effective protocol to purify pollen grains from archived herbarium material, which provides a straight-forward approach to creating mock standards for proficiency testing or inclusion as positive controls in casework.

Pollen; Morphology; DNA Metabarcoding

B141 An Analysis of DNA Preservation in Human Remains Embedded in Solid Building Materials

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Learning Objectives: This presentation will compare the quantity and quality of DNA extracts from human remains that have been embedded in solid building materials such as concrete. The goal is to assess tissue preservation and DNA conservation levels over different timepoints.

Impact Statement: Embedding human remains into solid building structures is a documented practice of body disposal by criminal and narcotic trafficking organizations. This study's findings will help laboratories understand challenges in the recovery process of human remains embedded in solid building materials, preservation differences of different tissue, as well as DNA conservation levels in those tissues over differing timepoints.

Abstract Text: Embedding human remains into building structures, such as concrete foundations, wells, walls, and other construction filled with concrete has been documented as one means of body disposal used by criminal and narcotic trafficking organizations. The recovery of DNA from hard and soft tissue of remains embedded in these materials for human identification has not been well documented. Components of concrete are sand, silica, quartz, cement, and other potentially DNA-damaging substances. The aim of this experiment was to assess tissue and DNA preservation levels over different time points and selected body parts embedded in standard commercially available concrete mix and other building materials such as alternative concrete mixes, lime, asphalt, and mortar. Replicate sections of right forearms submerged in commercial concrete mix were sampled for skin swabs, hard and soft tissue, and surrounding concrete once a month for the period of six months. Furthermore, to assess the effect of different building materials (concrete mix, lime, asphalt, and mortar), replicate fingers were embedded separately and recovered at two three-month intervals to take skin swabs and soft and hard tissue samples as well as samples from the surrounding material. Sections of left forearms and replicate fingers decomposing on the ground surface were sampled over the same time period to serve as controls. DNA was extracted, quantified, and analyzed for Short Tandem Repeats (STRs) using standard forensic methods and kits.

Phase one of the experiment was to analyze decomposition and DNA content of humans remains embedded in concrete using forearms submerged in commercial concrete mix for up to six months. The samples taken consisted of a skin swabs, tissue, bone, and surrounding concrete. Phase two analyzed decomposition and DNA content of whole fingers embedded in other solid building materials and alternative concrete mixes, lime, asphalt, and mortar over six months. The samples taken at each time period will consist of a skin swab, nail, tissue, bone, and surrounding concrete. Two methods of extraction (Investigator chemistry automated on the EZ2 platform and the manual Dabney method) were used to evaluate any differences in the DNA yield.

Human Remains; Forensic DNA; Concrete

B142 A Decade of ForenSeq in Forensics: Where Are We Now?

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WITHDRAWN

B143 Microhaplotypes: A Double-Edged Sword in Forensic Genetics

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Learning Objectives: This presentation focuses on the complex implications of this technology, with an emphasis on evaluating both its advantages and disadvantages in the context of technological advancement.

Impact Statement: This presentation will shed light on the scientific basis of microhaplotypes, their practical applications in forensic genetics, and challenges associated with their routine implementation.

Abstract Text: Microhaplotypes, defined as short DNA sequences with high variation, hold immense potential for revolutionizing forensic identification. This presentation will focus on the complex implications of this technology, emphasizing the evaluation of both its advantages and disadvantages and the promises and perils in the context of technological advancement. Using multiple microhaplotypes could yield superior discrimination power over traditional methods, such as Short Tandem Repeat (STR) typing or Y-chromosomal Short Tandem Repeat (Y-STR) typing, which could aid in resolving complex caseworks and forensic individualization. Due to their high resistance to postmortem degradation, they are an invaluable tool for challenging forensic samples consisting of highly degraded tissues and bruised bone samples in mass disaster identification. Specialized expertise, resources, and software are necessary for analyzing them, but they may not be available in all situations or at local laboratories in every country. Additionally, it is important to carefully consider the privacy and ethical concerns that come with using them.

This presentation will shed light on the scientific basis of microhaplotypes, their practical applications in forensic genetics, and challenges associated with their routine implementation. Not only will it address ethical issues, how to prevent violation of the personal privacy along with national securities, but also propose frameworks for proper applications and more equitable use. This presentation aims to promote a comprehensive discussion about the importance of this methodology in case work analysis and shaping the future of forensic genetics, taking into account its diverse aspects. National/international guidelines must be approved for clarification of the use and misuse of micro-haplotypes in forensic genetic case works analysis and for which category of caseworks it must not to be used due to ethical considerations.

Forensic DNA; Genetics; Microhaplotype

B144 Investigating a Different Forensic Workflow for DNA Paternity Testing

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Learning Objectives: Attendees will learn about a novel forensic workflow for DNA paternity testing that employs microFLOQ direct (MF) subsampling from buccal swabs that were stored long-term at Room Temperature (RT). The study explores how this method, which bypasses traditional DNA extraction and quantification steps, can streamline the testing process and preserve original samples for future use. Key insights include the effectiveness of the MF subsampling approach in generating full and consistent DNA profiles from buccal swabs stored at RT for extended periods as well as the potential for this method to simplify and enhance forensic workflows.

Impact Statement: This study will impact the forensic science community by offering a streamlined alternative to traditional paternity testing workflows. By demonstrating that the MF subsampling method can successfully generate complete DNA profiles without the need for preliminary DNA extraction and quantification, it introduces a more efficient and cost-effective approach for forensic analysis. This innovation not only accelerates the testing process but also preserves valuable samples for potential future use.

Abstract Text: **Introduction:** In legal contexts, establishing paternity hinges on obtaining full DNA profiles from all parties involved, which are crucial for providing definitive genetic evidence necessary for judicial decisions. Copan buccal 4N6FLOQSwabs (4N6FS) are extensively utilized in the forensic field for collecting buccal DNA due to their non-invasive nature and high efficiency. This study investigated an alternative method using MF for subsampling from buccal 4N6FS that had been stored at RT for over five years, across five simulated paternity cases. The study aimed to streamline the paternity testing process by eliminating the steps of DNA extraction and quantification, thus preserving the primary 4N6FS samples for potential future use. Additionally, the study evaluated whether complete DNA profiles could be successfully obtained through this subsampling approach from buccal samples that were stored long-term at RT.

Materials and Methods: Starting in 2018, five simulated paternity cases were set up. For all cases, two buccal 4N6FS were collected from each person of interest; one was analyzed shortly after collection (T_0), and the second was subsampled using two pre-wet MFs at the Time To Subsampling (TTS) interval as described below:

- Case 1 (T_0: 07/2018 – TTS: 5 years and 8 months): Daughter, Presumptive Mother
- Case 2 (T_0: 01/2020 – TTS: 4 years and 2 months): Son, Mother, Presumptive Father
- Case 3 (T_0: 09/2020 – TTS: 3 years and 6 months): Son, Mother, Presumptive Father
- Case 4 (T_0: 07/2021 – TTS: 2 years and 8 months): Daughter, Presumptive Father
- Case 5 (T_0: 10/2021 – TTS: 2 years and 4 months): Son, Presumptive Father

The first MF was processed immediately following the subsampling procedure and the second after four weeks of storage at RT. Each MF was directly amplified according to a workflow, which included: profiling with GlobalFiler Express kit on Veriti 96-well thermal cycler; capillary electrophoresis using AB 3500 Genetic Analyzer and GeneMapper ID-X v1.6 software. The profiles obtained with the two MFs were compared to those obtained at T_0 from 4N6FS.

Results: Compared to the results obtained at T_0 from 4N6FS, the MF alternative workflow provided full and matching profiles for the majority of individuals, whether the MF was analyzed right after subsampling or after being stored at RT for four weeks prior to analysis. Although there was some variability in average peak heights, peak height ratios, and intralocus balances, obtained profiles for most of the individuals were complete and consistent. However, partial profiles were observed from the MFs of one individual in Case 4. These partial profiles may result from low sample quantity on the initial 4N6FS or from subsampling with the MF from areas of the swab with little sample content.

Conclusion: This study supports the effectiveness of the MF approach in accelerating the paternity testing process, regardless of whether the samples are analyzed right after subsampling or after being stored at RT for four weeks. By eliminating the need for traditional DNA extraction and quantification, this method not only simplifies the workflow but also keeps the original 4N6FS samples available for subsequent analysis. Remarkably, complete profiles were generated for the majority of individuals from subsampled 4N6FS that had been collected several years earlier and stored at RT.

Buccal Swab; Backlogs; DNA

B145 Enhancing Rapid DNA Programs: Implementing a Metrics Tracker to Improve Performance and Measure Success

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Learning Objectives: Understand the Functionality of the Rapid DNA Metrics Tracker: Attendees will learn how to utilize the Excel-based Rapid DNA Metrics Tracker to monitor and evaluate the effectiveness and outcomes of Rapid DNA technology.

Evaluate Performance Metrics: Attendees will gain insights into how to assess sample success rates, identify optimal techniques for resource allocation, and enhance operational efficiency through the tool.

Analyze Operational Efficiency: Attendees will learn how to monitor individual operations to pinpoint training needs, ensure quality, and maintain consistency in processing.

Assess Program Impact: Attendees will be equipped with methods to compile and analyze data on case outcomes to evaluate the impact of Rapid DNA technology on the criminal justice system.

Utilize Data Management Features: Attendees will explore the tool's data management capabilities, including menu selections and visualization options for effective data entry and reporting.

Apply Strategic Planning Techniques: Attendees will learn how to use the metrics tracker to compare data with broader crime statistics, aiding in strategic decision-making and policy development.

Impact Statement: This presentation will advance the forensic science community by introducing a practical, data-driven tool designed to optimize Rapid DNA technology usage. The Rapid DNA Metrics Tracker enables Law Enforcement Agencies (LEA) and Forensic Science Service Providers (FSSPs) to systematically monitor and enhance their Rapid DNA programs, leading to increased operational efficiency and more informed decision-making. By offering detailed insights into performance metrics, operational workflows, and program impact, this tool supports evidence-based strategies and strategic planning. As a result, the forensic science community will benefit from more effective integration of Rapid DNA technology into forensic practices, ultimately strengthening the overall effectiveness of forensic operations.

Abstract Text: This poster presentation will showcase a Rapid DNA Metrics Tracker. an Excel-based tool developed to help LEAs and fFSSPs monitor the effectiveness and outcomes of Rapid DNA technology. By providing a data-driven approach to both the implementation and continuous improvement of Rapid DNA programs, this no-cost tool offers tangible benefits to LEAs and FSSPs looking to optimize use of this technology. Drawing from experience assisting multiple Bureau of Justice Assistance Byrne Discretionary Grants Program grantees in developing Rapid DNA programs, this poster presentation will detail how this tool is currently applied and highlights how a strategic, data-driven approach can enhance the value of Rapid DNA technology.
As the use of Rapid DNA technology by LEAs and FSSPs expands, effective metrics tracking is critical for evaluating program performance, assessing impact, and supporting decision-making. The Scientific Working Group on DNA Analysis Methods is developing standards to facilitate the future integration of Rapid DNA data into the Combined DNA Index System (CODIS). As these standards are implemented, the applications of Rapid DNA will broaden, making comprehensive metrics tracking even more important for optimizing its use. The Federal Bureau of Investigation's document, Non-CODIS Rapid DNA Considerations and Best Practices for Law Enforcement Use, emphasizes the value of tracking key metrics such as the volume and types of cases submitted, the number and types of samples processed, the types of profiles generated (e.g., partial, complete), and the impact on case outcomes (e.g., arrests, prosecutions, convictions, exonerations). [1]By collating these key metrics, the Rapid DNA Metrics Tracker will assist LEAs and FSSPs with:

- Performance Evaluation: Provides insights into sample success rates and techniques, optimizing resource allocation and improving workflows, policies, and procedures.
- Operational Efficiency: Monitors individual instrument usage to identify training needs and ensure quality and consistency.
- Outcome Analysis: Compiles data on case outcomes to assess the impact of Rapid DNA on the criminal justice system.
- Data Management: Provides customizable menu selections and visualization tools for consistent data entry and reporting.
- Program Impact and Strategic Planning: Analyzes program metrics against broader crime statistics to inform strategic decision-making, resource allocation, and policy development.

As part of this poster presentation, attendees will be able to download the no-cost, Excel-based Rapid DNA Metrics Tracker, which can be further customized to meet their agency's specific policies and needs.

Reference:

1. Federal Bureau of Investigation, Non-CODIS Rapid DNA Considerations and Best Practices for Law Enforcement Use, 2019.

Rapid DNA; DNA; Metrics

B146 Upgrading to STRmix v2.11: A Chance to Do It All Again

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Learning Objectives: This presentation will highlight additional validation studies incorporated into the version upgrade of STRmix and the reasons why the additional studies were considered.

Impact Statement: This presentation will impact the forensic science community through the evaluation of additional validation studies meant to test the limits of STRmix and the impact it can have on interpretation.

Abstract Text: In life, second chances may not always come along but in the field of forensics, where new or improved technology continues to expand the limits of DNA testing, that is routinely not the case. One such technology is probabilistic genotyping, specifically STRmix, which has undergone various version upgrades since its original introduction to forensic DNA testing laboratories. With each new version release, laboratories must consider which additional validation studies should be undertaken prior to implementation in casework. Standards and guidelines are in place for the validation of new methodology in the laboratory to assist in forming a basis for establishing reliable methods, but it is ultimately up to the laboratory to determine which studies are relevant for a given method and to identify additional studies that can further assist in defining method limitations/parameters.

Signature Science (SigSci), a private Forensic DNA Laboratory based in Austin, TX, initially validated STRmix v2.5 in 2018; however, validation efforts have been ongoing. Several validation addendums have been completed as well as an upgrade to STRmix v2.11. A thorough validation was initially performed prior to casework implementation to include the examination of known and non-probative evidence samples and investigations into reproducibility and precision, sensitivity, and stochastic effects, and mixture data. As is often the case with newly implemented methodology, SigSci identified a need to explore and characterize additional parameters of the STRmix software functionality prior to any version upgrades. Interpretation guidelines were updated to evaluate unintuitive comparisons as a result of potential familial relationships in casework mixture data. A validation addendum was also performed to evaluate drop-in and stutter modeling.

In addition to the original and addendum studies that were performed when validating v2.5, the v2.11 validation studies included the characterization of additional parameters. Familial relationships, specifically relationship likelihood ratios generated, were explored to establish a familial diagnostic range for use in casework. Additional challenging samples affected by degradation and/or inhibition were included to further assess features within STRmix modeling. Finally, a wider range of mixture samples were prepared and analyzed for the integration of interpretation thresholds in the laboratory. These validation efforts ultimately establish more robust and definitive guidelines for DNA profile interpretation, specifically regarding samples and/or contributors not suitable for comparison.

DNA; Validation; Probabilistic Genotyping

B147 The Application of Deep Learning in Latent Fingerprint Enhancement

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Learning Objectives: The presentation will demonstrate the application of deep learning for latent fingerprint development and enhancement. The attendees will understand the use of Artificial Intelligence (AI) tools to assist in latent fingerprint visualization and comparison. The attendees will also understand the general process of developing AI tools for latent fingerprint examination.

Impact Statement: This study will impact the forensic science community by introducing an AI agent to improve the efficiency and quality of latent fingerprint examination and comparison. The AI agent can also shorten the training time to improve the quality of young and experienced fingerprint examiners. The application of AI to fingerprint comparison and development can support crime scene investigators and fingerprint examiners in enhancing the overall efficiency of the workflow.

Abstract Text: Deep learning is a subfield of AI that utilizes Multilayer Neural Networks (MLNN) to learn from data. A MLNN can learn complex patterns that simple linear models cannot solve, and it is a type of model that can classify the given input based on what has already been learned. Among the models of MLNN, the Fully Convolutional Network (FCN) and U-net are used in this research work because both are technologies commonly used for medical image processing and image segmentation. Segmentation technology detects changes in pixel values to find boundaries and then divides the image based on those boundaries to extract meaningful data.

Visualizing latent fingerprints at crime scenes is considered one of the most important tasks in obtaining crucial pieces of physical evidence, just like detecting and collecting DNA evidence. However, latent fingerprint development requires highly skilled investigators with forensic expertise and experience. In the case of partial fingerprints, an additional single minutia can lead to the determination of whether the fingerprint can be identified or not. Various optical technologies are used to complement this, but most of them still rely on analysts' proficiency. Due to this limitation, using AI-enhanced fingerprinting techniques for assisting determination are worth attempting. Information invisible to the human eye but detectable by optical devices can be enhanced through AI. AI trained on data similar to that of experienced professionals could process images captured by optical devices in place of human eyes. Using AI technology could lead to savings in time and resource.

In this work, latent fingerprints were visualized by ethyl cyanoacrylate fuming method on red envelopes made of glossy film materials that are water resistance. The vitalized latent fingerprints were enhanced with fluorescent powder and photographed for subsequent analysis and comparison. Moreover, synthesized fingerprint images were used to simulate the red envelope fingerprints collected at the crime scene. A qualified latent fingerprint examiner was engaged to annotate the simulated images and assess their accuracy in comparison to collected fingerprints.

The experimental outcomes demonstrated the possibility that AI technology can correct distorted and blurring fingerprint images. When this technology was used to correct distorted friction ridges in fingerprints, it resulted in more valuable images for analysis and comparison. With the advancement of AI recognition technology alongside other techniques, a significant portion of fingerprints that cannot be identified using current methods may become valuable as investigative leads. In summary, when compared FCN and U-net as a tool for comparison of incomplete fingerprints, U-net shows higher performance in every performance benchmark, including accuracy, Jaccard Index (JI), Dice Similarity Coefficient (DSC), sensitivity, and specificity.

Artificial Intelligence; Latent Prints; Crime Scene Investigation

B148 Elucidating the Mechanism of Unwanted and Uncontrolled Water Adducts in the Tandem Mass Spectra of Synthetic Cannabinoids

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Learning Objectives: After attending this presentation, attendees will understand how uncontrolled ion molecule reactions can interfere with the mass spectrometric analysis of synthetic drugs. Audience members will leave with the knowledge of how common molecules, like water and methanol, can adduct to certain Synthetic Cannabinoid (SC) fragments when stored for extended periods of time, such as in Orbitrap mass spectrometers.

Impact Statement: This research will help drug analysts and forensic toxicologists identify novel SCs in seized drugs and body fluids, respectively.

Abstract Text: SCs are modeled after cannabis, which is a Schedule I drug in the United States that has recreational and medicinal uses. SCs have recently been identified as Novel Psychoactive Substances (NPS), meaning that certain new analogs have not yet been scheduled or controlled under United States federal law. Since 2008, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has reported 224 new SCs.[1]

When SCs are analyzed on different types of mass spectrometers, some subtle differences are expected in the resulting tandem mass spectra. However, when SCs with an indazole core—like the FUBINACA and PINACA families of SCs—are analyzed on certain tandem mass spectrometers, the fragmentation patterns can be dramatically different. This presentation focuses on elucidating the fragmentation mechanisms that lead to the major fragments observed in several types of indazole and indole SCs. This presentation also provides an explanation for the occurrence of certain fragments that have so-far defied explanation.

Ten different SCs, including three isotopically labelled versions, containing indole and indazole-cores were analyzed using an LTQ Velos Pro linear ion trap mass spectrometer with a Heated Electrospray Ionization (HESI) ion source in positive mode. When particular fragment ions from indazole-containing SCs were isolated and stored for tens of milliseconds, fragments containing an acylium or ketene group on the indazole ring gained +18 Da and +32 Da with rates following first order kinetics. These two gains in mass correlate to the uncontrolled adduction of water and methanol to the fragments. When this same procedure was applied to indole-containing SCs, no such adduction was observed. Therefore, the indazole core adjacent to the acylium group is crucial for the adduction of these background reagents. The adduct product ions could be isolated at MS3 level, and the adducts were easily reversed with low-energy CID, which is indicative of a non-covalent reaction. These findings are supported by high-level Density Functional Theory (DFT) calculations conducted using Gaussian 16 and GaussView, which showed large free energies of formation for the water adducts. The proclivity for water adduction of certain ions in the tandem mass spectra of SCs helps explain why spectra collected on certain ion trap instruments—that store ions for prolonged periods before mass analysis—are more likely to contain these unusual "fragments." In addition to helping drug analysts identify novel cannabinoids in seized drugs, this project could also help forensic toxicologists identify emerging SCs in the emergency room or during autopsies.

Reference:

1. European Monitoring Centre for Drugs and Drug Addiction. *European Drug Report 2022: Trends and Developments* (2022). Publications Office of the European Union, Luxembourg.

Novel Psychoactive Substances; Cannabinoids; Mass Spectrometry

B149 Utilizing a Field-Deployable, High-Resolution Mass Spectrometry System for Screening of Seized Drugs by DART-MS Analysis

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Learning Objectives: After attending this presentation, attendees will understand how an existing workflow for the screening of seized drugs by Direct Analysis in Real Time (DART) Mass Spectrometry (MS) can be adapted for a field deployable, High-Resolution Time-Of-Flight/Mass Spectrometry (TOF/MS) system.[1] Attendees will be presented with optimization and validation results for seized drug analysis by DART-TOF/MS.

Impact Statement: This poster will impact the forensic science community by providing the foundational work for the utility of a recently developed, mobile, high-resolution TOF/MS system. The optimization and validation of this MS system will allow for field-deployable analytical screening with the ability to obtain rapid, high-quality mass spectral measurements.

Abstract Text: The ever-changing drug landscape continues to highlight the need for rapid, on-site analytical techniques to assist public health, public safety, and customs personnel to accurately identify compounds in the drug supply. Existing on-site techniques (Raman, Fourier transform infrared spectroscopy, ion mobility, lateral flow immunoassays, etc.), benefit from portability and/or low cost, but often suffer from lack of sensitivity and selectivity. TOF mass spectrometers have the necessary sensitivity and selectivity but are limited by extensive laboratory requirements. However, a high-resolution TOF mass spectrometer that is mobile, and therefore deployable to sites of interest, has recently been developed. The ability to obtain rapid, high-quality mass spectral measurements is realized when this TOF mass spectrometer is coupled with a widely utilized ion source, such as DART.

This work investigated the adaptation of a laboratory-based seized drug analysis DART-TOF/MS workflow to a field-deployable, high-resolution mass spectrometer.[1]
A Tofwerk Vocus TOF/MS was coupled with a Bruker DART-SVP ion source. Initial studies investigated optimal instrument parameters to maximize the mass spectral response. Specifically, the depth of the ceramic tube, distance between the ceramic tube and DART ceramic cone, flow rate of the external Vapur pump, and SSQ pressure were investigated. Fragmentation spectra from in source-Collision-Induced Dissociation (is-CID) voltages ranging from 10V to 50V were then compared to existing spectral libraries.[2] Once the instrument parameters were optimized, the field-deployable, high-resolution mass spectrometer was then validated for the screening of seized drugs. Single- as well as multi-component standard solutions of compounds across multiple drug classes were used to investigate mass accuracy, fragmentation pattern precision (for mid-voltage spectra), specificity, reproducibility, carryover, limits of detection, solvent effects, and robustness.

The performance of the field-deployable, high-resolution DART-TOF/MS system was determined to be appropriate for the screening of seized drugs. The protonated molecular ions for a 15-component solution were used to determine a mass accuracy within ±0.020 Da. Single-component solutions were analyzed across multiple is-CID voltages to ensure correct and repeatable responses for mid-voltage spectra, as compared to an existing DART-TOF/MS library. Both the low- and mid-voltage library spectra were found to have comparable fragmentation patterns when using 15V and 30V for is-CID for this DART-TOF/MS system. The mass accuracy of the mid-voltage spectra, as determined by the major fragment ion, was within ±0.020 Da. The DART-MS Data Interpretation Tool was used to determine scores for reverse match factor and fraction of peak intensity explained for the 15V spectra as compared to a down-selected DART-MS low-voltage library, and the 30V as compared to a down-selected DART-MS mid-voltage library.[2] Reproducibility was then investigated for ten replicates for seven analyses across a 21-day period, and the mass assignments for the 15V (low-voltage) were monitored to test the mass tolerance of ±0.020 Da. Carryover was monitored through methanol blanks that were analyzed between replicate samples. The limits of detection for most compounds, as investigated by the protonated molecular ion for single-component solutions, were determined to be in the single nanogram range. A second analyst, familiar with the system, also completed the reproducibility study, to determine the robustness of the system. Current work is focused on determining additional limits of detection and investigating solvent effects for common organic solvents. The ability for the system to identify compounds in complex, real-world mixtures will also be investigated.

Once validated, this system will be deployed in a mobile laboratory for on-site analysis at public safety and public health venues.

References:

1. M.G. Appley, E.L. Robinson, A. Thomson, E. Russell, E. Sisco. An analytical platform for near real-time drug landscape monitoring using paraphernalia residues, *Forensic Chemistry* 34 (2023) 100489. https://doi.org/10.1016/j.forc.2023.100489.
2. https://www.nist.gov/programs-projects/methods-software-tools-and-resources-forensics-laboratories-dart-ms-or-other-ai-ms.

Field Deployable; Seized Drug Analysis; Mass Spectrometry

B150 The Effect of Mass Spectral Selection on Discrimination of Fentanyl Analog Positional Isomers

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Learning Objectives: After attending this presentation, attendees will be familiar with a statistical method to compare mass spectra and understand the effect of mass spectral selection on the ability to discriminate spectra of positional isomers.

Impact Statement: This presentation will impact the forensic science community by providing further evaluation of a statistical method to compare mass spectra of seized drug samples and demonstrate the ability to distinguish positional isomers.

Abstract Text: The number of new *novel psychoactive substances* (NPS), specifically novel synthetic opioids, has increased over the past decade, increasing from 450 new substances reported in 2013 to 618 in 2021.[1] Submitted samples are typically analyzed by Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS) for identification. However, with the emergence of structural and positional isomers, definitive identification based on the EI mass spectra is challenging due to the high spectral similarity.

In our previous work, a method was developed to compare two mass spectra in an objective, statistical manner. The method uses an unequal variance t-test to compare ion intensity at all m/z values of two mass spectra.[2] The null hypothesis (H0) states that the difference in ion intensity is equal to zero while the alternative hypothesis (Ha) states that the difference is not equal to zero. If H0 is accepted at all m/z values, the two mass spectra are statistically indistinguishable, and the association of the spectra is confirmed. In contrast, if Ha is accepted for at least one m/z value, the two mass spectra are statistically distinguishable, and the m/z values responsible are defined as discriminating ions.

In our ongoing work, the robustness and ruggedness of the statistical comparison method are being evaluated. To this point, the effects of concentration and inherent instrument variation on the ability to associate and discriminate spectra of positional isomers have been evaluated.[3] The focus of the current work is to evaluate the effect of mass spectral selection on the ability to discriminate spectra of positional isomers.

The ortho (o-), meta (m-), and para (p-) positional isomers of sets of fentanyl analogs, including methyl fentanyl, fluorobutyryl fentanyl, fluoroisobutyryl fentanyl, and fluoro methoxyacetyl fentanyl, were used in this work. Each isomer was analyzed in triplicate by GC/EI/MS and spectra were selected in two ways. First, spectra were collected from the apex of the chromatographic peak, and second, spectra were averaged across the peak width at half height. For each fentanyl analog, spectra of the positional isomer were statistically compared, based on both the peak apex spectra and the average spectra.

Discrimination of positional isomers was achieved at the 99.9% confidence level for both the apex and average spectra, albeit with some differences in the number of discriminating ions. For example, m-methyl fentanyl and p-methyl fentanyl were distinguished with seven discriminating ions. Many of these ions (e.g., m/z 107, 164, 176, 236, and 280) were present in the spectra at low intensity, which is defined in this work as less than 5% of the base peak intensity. Based on the average spectra, discrimination of m-methyl fentanyl and p-methyl fentanyl was also achieved although with four ions responsible for discrimination. Further, all ions identified as discriminating in the average mass spectra were also identified as discriminating in the apex mass spectra.

Similar trends were observed for positional isomer comparisons of the other fentanyl analogs considered, all of which were distinguished at the 99.9% confidence level. In this presentation, these trends will be presented in more detail and the chemical relevance of discriminating ions will be discussed.

References:

1. UNODC Early Warning Advisory Dashboard. https://www.unodc.org/LSS/Page/NPS/DataVisualisations (accessed 2024-07-22).
2. Bodnar Willard, M. A.; Waddell Smith, R.; McGuffin, V. L. Statistical Approach to Establish Equivalence of Unabbreviated Mass Spectra. *Rapid Communications in Mass Spectrometry.* 2013, 28 (1), 83–95. DOI:10.1002/rcm.6759.
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Fentanyl Analogs; Mass Spectrometry; Statistical Comparison

B151 Structural Characterization of Nitazene Analogs Using Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS)

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Learning Objectives: After attending this presentation, attendees will have learned about Collision-Induced Dissociation (CID) fragmentation trends and characteristic product ions observed for 38 nitazene analogs using ESI-MS/MS. The audience will learn about conserved fragmentation pathways between analogs as well as the impact of different types and locations of substitutions to the core nitazene structure on the resulting fragmentation behavior. This information will provide attendees with additional tools to help identify novel nitazene analogs.

Impact Statement: This presentation will impact the forensic science community by providing a comprehensive fundamental understanding of nitazene analog fragmentation behavior under ESI-MS/MS conditions. The identification of characteristic product ions as well as trends in the CID fragmentation behavior for different substitutions will aid with the development of screening and targeted Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) methods for nitazene analogs. The identified trends in fragmentation behavior will also assist the toxicology and seized drug communities in identifying novel nitazene analogs using ESI-MS/MS methods.

Abstract Text: Nitazene analogs are a class of Novel Synthetic Opioids (NSOs) that have become prevalent on the illicit drug market in recent years due to increasing legislation of fentanyl analogs in both the United States and China. Although several nitazene analogs have been categorized under Schedule I of the Controlled Substances Act, novel analogs continue to emerge to circumvent legislation. This novelty provides a challenge for forensic scientists when it comes to identifying nitazene analogs. Therefore, there is a need to understand the behavior of nitazene analogs under various analytical conditions. ESI-MS/MS is often utilized with liquid chromatography instrumentation, which is routinely applied in toxicology, and is an emerging technique for seized drug analysis. This research provides a comprehensive understanding of nitazene analog behavior under ESI-MS/MS conditions. The central hypothesis of this research is that the type and location of substitution to the core nitazene structure will impact the resulting ESI-MS/MS fragmentation behavior and thus, enable the differentiation of nitazene analog subclasses.

This research utilized 38 representative nitazene analogs, including compounds with various substitutions at the amine moiety, the benzyl ring, and the benzimidazole core. This selection of analogs includes every type and location of substitution that has been observed on the illicit drug market to date, as well as several novel substitutions that have not yet been observed in drugs of abuse. All nitazene standards were prepared at a concentration of 10ppm and analyzed using an Agilent 6530 quadrupole Time-Of-Flight (qTOF) mass spectrometer, using direct injection. CID activation was achieved using collision energies of 15, 25, 35, and 45eV. All standards were analyzed individually, and three replicates of each compound were collected to understand the natural variability in the observed CID fragmentation behavior.

The results indicate that there are many similarities between the product ion spectra of most nitazene analogs, with a few key differences that enable the identification of certain substitutions. For example, nitazene analogs with a diethyl substitution at the amine moiety have a base peak of *m/z* 100, analogs with a desethyl substitution have a base peak of *m/z* 72, analogs with a pyrrolidine ring have a base peak of *m/z* 98, and analogs with a piperidine ring have a base peak of *m/z* 112. These fragments are all formed through an even-electron cleavage involving the nitrogen of the amine group, but the observed *m/z* value shifts based on the substitution present. Another indication of a diethyl substitution is a product ion 73 Da less than the protonated molecule. Additionally, desethyl compounds produce a product ion that is 71 Da less than the protonated molecule, which is likely formed through a 4-center elimination at the β-hydrogen of the nitrogen in the benzimidazole core. This information will be helpful for the identification of novel desethyl nitazene analogs. Additionally, the length of the alkoxy chain attached to the benzyl ring can be determined based on the presence of product ions, such as those observed at *m/z* 121 for methoxy substitutions and *m/z* 135 for ethoxy substitutions. Interestingly, desnitazene analogs displayed the unique formation of doubly charged precursor ions. This behavior is extremely helpful for identifying desnitazene compounds, which otherwise have very similar, if not identical, product ion spectra to nitazene analogs with a nitro group at the benzimidazole core. The comprehensive CID fragmentation behavior and characteristic product ions identified in this study enhance our understanding of the behavior of nitazene analogs using ESI-MS/MS and equip the toxicology and seized drug communities with knowledge that can help to differentiate existing nitazene analogs and identify novel nitazene analogs.

Novel Synthetic Opioids; Analogs; Tandem Mass Spectrometry

B152 Fingerprint Evidence Analysis of Fraudulent Receipts in Taichung City, Taiwan

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Learning Objectives: Through this field study, fingerprint examiners will gain insights into where criminals are likely to leave fingerprints on fraudulent documents and recognize that the 1,2-IND method is significantly more sensitive and effective than the ninhydrin method in real cases. Additionally, the study highlights how the time elapsed between the crime and evidence collection impacts the likelihood of obtaining usable fingerprints. This information enables fingerprint examiners to evaluate and choose the best methods for processing such evidence in their own laboratories.

Impact Statement: This study, viewed from the perspective of Taichung City, Taiwan, analyzes fraud schemes involving fake receipts used by criminal organizations. It summarizes various aspects of fingerprint evidence collection and provides valuable insights for forensic laboratories worldwide to effectively assess and improve their methods for handling such evidence.

Abstract Text: Taiwan has experienced a significant increase in online fraud cases in recent years. Since 2023, fraud rings have frequently collected cash from victims in person to make financial flows harder to trace. To build trust with their victims, these fraudsters provide paper receipts as proof of payment. When victims eventually realize they have been scammed and report the incident, these receipts become crucial evidence, potentially containing fingerprints of the criminals involved.

This study, conducted with the Taichung City Police Department in Taiwan, analyzed 122 fraudulent receipts to examine fingerprint evidence.[1] The results revealed that the IND-Zn method detected an average of 2.97 fingerprints per receipt, with 2.03 matching suspects in the criminal database, 0.34 matching victims, and 0.60 with no matches to either. In comparison, the ninhydrin method detected an average of 2.13 fingerprints per receipt, with 1.37 matches in the database, 0.31 matching victims, and 0.44 with no matches to either. Remarkably, fingerprints from suspects were detected more frequently than those from victims. The IND-Zn method proved to be significantly more sensitive than ninhydrin, providing a higher probability of matching fingerprints to suspects.[2,3]

This study also examined the effect of the time between the crime and evidence collection on fingerprint recovery. The likelihood of obtaining usable fingerprints diminished as the interval increased.[4] For collections within 30 days of the crime, an average of 2.97 matching fingerprints per receipt was found. This number dropped to 1.36 for collections between 31 and 60 days, 1.67 for 61 to 90 days, and only 0.22 for periods exceeding 91 days. Further analysis of fingerprint locations showed distinct patterns: suspects were more likely to leave fingerprints on the document's front edges (46.3%) and corners (19.05%), while victims were more likely to leave fingerprints on the back edges (38.46%) and central areas (23.08%). This study provides valuable insights for forensic examiners dealing with fingerprints on fraudulent documents, thereby enhancing the ability to identify potential suspects.

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Fingerprint; Fraud; Paper

B153 The Development and Validation of a New GC/MS Decision-Point Method for Marijuana and Hemp Differentiation Using Hydrogen as Carrier Gas

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Learning Objectives: After attending this presentation, attendees will understand the process undertaken to develop, optimize, standardize, and validate a new Gas Chromatography/Mass Spectrometry (GC/MS) method for the analysis of cannabis materials. This new 1% decision-point method utilizes hydrogen as the carrier gas, a narrow-bore column, and a new Internal Standard (IS) compound.

Impact Statement: This presentation will impact the forensic science community by providing a deep dive into the processes used for evaluating and selecting the new IS, the preparation of a new positive control solution, and the performance characteristics evaluated during validation.

Abstract Text: During 2020, the Drug Enforcement Administration (DEA) laboratories started conversion of their GC/MS systems from using helium to hydrogen as a carrier gas in an effort to transition to a more sustainable and environmentally friendly alternative while also reducing maintenance costs and dependence on helium. In the years since, the move to using hydrogen along with parallel standardization efforts have resulted in the development and validation of various targeted and non-targeted qualitative analysis methods, including the more recent Global Uniform Analysis and Reporting of Drug-Related Substances (GUARDS) method, which combines the use of hydrogen with a narrow-bore (35%-phenyl-methylpolysiloxane) column for enhanced sensitivity. In order to further standardization efforts, the original DEA 1% decision-point method used for hemp and marijuana discrimination has now been adapted and validated to use the same GC/MS instrumental conditions as the GUARDS method. This adaptation allows for harmonization across DEA laboratories by allowing use of the same analytical instrumentation for cannabis testing and other (non-cannabis) routine screening analyses.

Past limitations encountered when using 4-androsten-3,17-dione and testosterone led to the search for alternative compounds and ultimately the selection of dextromethorphan as a new IS due to its comparable size and structure. Short- and long-term (18 weeks) experiments demonstrated not only similar instrument response as that recorded for previous IS compounds, but also stability of the prepared solutions under different storage conditions. This presentation will describe these evaluation tests and provide details about the new positive control solution used to easily visualize the abundance of the target analyte (delta-9-Tetrahydrocannabinol [THC]) and establish the THC:IS peak height ratio used as part of the DEA cannabis analytical scheme to discriminate between hemp and marijuana.

This report will also discuss the steps and performance characteristics evaluated during the validation of this new method at the DEA Special Testing and Research Laboratory, including selectivity, repeatability (short-term precision), and the linear response (around the critical decision-point region) of not only THC but also Cannabidiol (CBD). The validity (accuracy) of the decision-point method was also evaluated by testing cannabis materials with THC levels below, at, and above the 1% level (w/w). To demonstrate fitness for purpose, this presentation will also summarize results from the various method verification experiments performed and documented after transferring of the new method to additional DEA laboratories, a step required to demonstrate the ruggedness of the method prior to system-wide implementation and casework use.

Marijuana; Hemp; Hydrogen

B154 Evaluating the Performance of DEA's New GUARDS Method Via an Interlaboratory Study

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Learning Objectives: After attending this presentation, attendees will know about the Global Uniform Analysis and Reporting of Drug-Related Substances (GUARDS) method recently implemented throughout the Drug Enforcement Administration (DEA) laboratories, United States Customs and Board Protection (CBP), and other state/local laboratories. This non-targeted Gas Chromatography/Mass Spectrometry (GC/MS) method was established to better standardize drug analysis and reporting across the seized-drug community and to enhance the reliability and accuracy of the information being reported through the National Forensic Laboratory Information System (NFLIS).

Impact Statement: This presentation will impact the forensic science community by informing the audience of the advantages and disadvantages of the GUARDS method and by presenting and discussing data collected during a system-wide Interlaboratory Study (ILS).

Abstract Text: In response to the continual emergence of novel psychoactive substances and the ongoing opioid epidemic, GUARDS was designed to offer a GC/MS method that allows for standardized analysis of both commonly encountered drugs as well as emergent compounds. The ILS presented here involved the analysis of three sets of solutions containing a total of 14 compounds at different concentration levels. Fentanyl was evaluated at two different concentrations to mimic levels routinely encountered in lab submissions. An internal standard (octacosane; C_{28}), added at the 1% concentration level, was also included. Solutions were sent out to DEA field laboratories and analysts were instructed to analyze the solutions using the GUARDS method. Each ILS solution was divided into ten different autosampler vials (30 vials total) and each vial was analyzed once. If available, analysts were to transfer the vials to a different instrument and repeat the analyses. All raw data files were transferred to the DEA Special Testing and Research Laboratory for processing. In total, the ILS involved 21 different instruments across nine laboratories.

This presentation will summarize the ILS results and the final figures of merits determined for the GUARDS method. These will include assessment of Retention Time (RT) data collected for each compound tested. After compiling data across 21 instruments, results demonstrate high within- and between-instrument RT repeatability, with calculated standard deviations ranging from 0.053 to 0.172 minutes (0.72% - 3.7% RSD) and early eluting compounds showing higher variability.

Additionally, the precision and accuracy of the experimentally determined Relative Levels (RL = compound peak area/C_{28} peak area) were investigated in order to assess if the values across compounds, instruments, and laboratories were: (1) reproducible, and (2) could provide an estimated purity range. Data from three replicate injections (injections 1, 5, and 10) from each compound were evaluated in accordance to American Society for Testing and Materials (ASTM) E691 and results demonstrated good within-instrument precision.[1] However, consistency statistics highlighted various outliers and trends across the 21 instruments evaluated. The high instrument-to-instrument variability was demonstrated to be the most significant factor influencing the method's overall reproducibility standard deviation.

The accuracy associated with RL was evaluated using all 210 injections collected for each compound (10 injections across 21 instruments). Correction Factors (CF) were calculated by comparing each compound's concentration (as originally prepared) against the RL measured for each individual injection. The final 210 RLs and 210 CFs produced for each compound were statistically evaluated using box plots, histograms, and normality tests. Overall, results show that a significant portion of the RL measured underestimate the compounds' concentration in solution; CFs varied from compound to compound and from instrument to instrument; dimethyl sulfone and noscapine were significant outliers; and distributions were found not to be normal. Compilation of the 25th and 75th percentile CFs for all compounds resulted in two "global" correction factors (1.439 and 1.992) that could hypothetically be applied to generate an Inter-Quartile Purity Range (IQPR) for any compound tested using the GUARDS method under standardized sample preparation conditions.

Applying the "global" correction factors, a total of 210 IQPR were generated for each compound investigated. When evaluating those purity ranges against the compound concentrations (as prepared), it was found that, on average, only about 40% of the IQPR generated captured the true purity of the compound. In other words, the probability of producing an estimated purity range that does not capture the true purity of the substance being tested is about 60% (on average). These results are not surprising and highlight the natural variability of responses across GC/MS systems, even when using standardized acquisition conditions.

Reference:

1. ASTM E691-23, 2023, *Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method*" ASTM International, West Conshohocken, PA 2023, DOI: 10.1520/E0691-23, www.astm.org.

Seized Drugs; GC/MS; Interlaboratory Study

B155 Exploring Natural Aging and Depletion Processes of Latent Fingermarks With 2D and 3D Imaging

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Learning Objectives: After attending this poster presentation, attendees will have a better understanding of the complex Three-Dimensional (3D) structure of fingermarks and how different temporal mechanisms of degradation affect their topography as examined by 2D and 3D imaging.

Impact Statement: This presentation will impact the forensic science community by serving as an observation and comparison of the microscopic 2D and 3D topographical changes that simultaneously may occur during the (1) natural aging of latent fingermarks and (2) chronological order of sequential depositions (depletions). This preliminary study will have future implications for crime scene investigations as it provides useful data toward determining the chronological order of fingermark depositions.

Abstract Text: Latent fingermark age estimation remains a complex and unresolved challenge in forensic science.[1-3] Fingermarks are dynamic matrices that undergo time-dependent changes of topography, a process referred to as fingermark aging. These can be measured with 2D and 3D "chronomorphometrics," such as loss of color contrast between ridges and furrows and loss of ridge height and volume over time.[3-5] Physical, biological, and environmental factors each play a role in the severity and rate of degradation patterns of fingermarks. Early methods to estimate fingermark age focused on analyzing changes in chemical composition, while recent studies have shifted toward non-destructive, faster, and portable visual quantitative measurements. Currently, there are no standardized optical approaches to determine not only the natural age of latent fingermarks, but also the chronological order of depletions post-deposition. Investigating how these two temporal mechanisms affect the topography of ridges over time is important as they may play a role in fingermark aging patterns, simultaneously. Furthermore, establishing these patterns has the potential to identify both the suspect and the time at which a crime was committed.

This study aims to assess and record fingermark physical changes occurring over time on a glass substrate in complete darkness at four time points, days 0, 5, 13, and 27, utilizing a 2D imaging method with black magnetic powder and with a 3D imaging technique, an optical profilometer. With approval from the Institutional Review Board, triplicate sebaceous-rich fingermarks of the index finger and thumb of the dominant hands of four healthy male individuals were collected in sets of 15 depletions for each time point. A total of 1,440 fingermarks were deposited on glass microscope slides with medium pressure and examined under controlled environmental and lighting conditions. The 2D examinations provided morphometrical data: Color Contrast (CC), color-coded maps of ridge clarity (BlueGreen, BG), and fingermark visual Quality Scores (QS). The 3D approach collected microscopic measurements on the x, y, and z axes, and provided ridge height (S_a), and volume (Volume up, V_{up}) data.[6,7] It was hypothesized that 2D and 3D topographical changes occur in the two temporal degradation mechanisms, in which 3D measurements will be more sensitive in the detection of time-dependent changes.

Exploratory analysis included data cleaning, normalization of data, and regression analysis on Microsoft Excel. Further examinations included Grubb's tests for the detection of outliers, Boxplots for data distribution, and univariate and multivariate Analysis of Variance (ANOVA) analyses for each metric with IBM SPSS Statistics 25. Additionally, Principal Component Analysis (PCA) was utilized to reduce the dataset dimensionality and find correlations between variables.

Preliminary regression data for the depletion mechanism revealed the S_a and V_{up} metrics follow a power model, in which a significant initial decrease in ridge height and ridge volume, respectively, was observed for each time point. The CC metric, which represents the ratio of the average color (Mean Intensity [MI]) and the spread of color intensities (Intensity Amplitude [IA]), follows a positive linear model, in which the MI/IA ratio increased over 27 days. The BG metric, a color-coded measure of areas of high (blue and green) and low (red and yellow) ridge clarity, data revealed a linear decrease over time. Preliminary regression data for the natural aging mechanism revealed both the V_{up} and CC metrics follow a second-order polynomial model, both with an initial steady increase in ridge volume and MI/IA ratio, respectively. A positive linear trend was noted for S_a, while a negative linear trend was observed for the BG metric. This preliminary cross-analysis reveals there are differences in which the topographical changes of fingermarks occur over time when comparing the two temporal mechanisms.

References:

1. Girod, A., Ramotowski, R., Lambrechts, S., Misrielal, P., Aalders, M., & Weyermann, C. (2016). Fingermark age determinations: Legal considerations, review of the literature and practical propositions. *Forensic Science International*, 262, 212-226. https://doi.org/https://doi.org/10.1016/j.forsciint.2016.03.021.
2. De Alcaraz-Fossoul, J., Roberts, K., & Barrot, C. (2015). The paradigm of fingerprint age determination. *jacobs journal of forensic science*.
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7. De Alcaraz-Fossoul, J., Mancenido, M., Soignard, E., & Silverman, N. (2019). Application of 3D Imaging Technology to Latent Fingermark Aging Studies. *J Forensic Sci*, 64(2), 570-576. https://doi.org/10.1111/1556-4029.13891.

Latent Prints; Age Estimation; Imaging

B156 Utilizing Low Energy Ionization to Differentiate and Identify Fentanyl and Nitazene Analogs

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Learning Objectives: Many fentanyl and nitazene analogs generate similar or non-characteristic spectra when ionized at the conventional 70eV. This presentation will illustrate the ability to generate a molecular ion to provide confirmational and structural information by using isotopic fidelity and High-Resolution Mass Spectrometry (HRMS). Attendees will learn how low energy ionization works and how it can be applied to generate molecular ions that may not be produced under standard 70eV electron ionization. In addition, attendees will learn how to generate and interpret isotopic fidelity based on their masses and abundance ratios.

Impact Statement: Utilizing low energy ionization techniques could reduce or eliminate the difficulty of identifying compounds with similar EI spectra resulting in an enhanced level of confidence during the identification process and improve spectral certainty based on isotopic ratios and ion fragment stability in a low energy environment.

Abstract Text: Historically abused opiates such as heroin, are being "cut" with and/or replaced with many New Synthetic Opioids (NSO), such as the fentanyls and non-fentanyl-derived synthetic opioids (i.e., nitazenes).[1] Keeping up with the ever-evolving synthetic illicit drugs, precursors, and their metabolites is increasingly difficult and the need for retrospective data mining is invaluable. This research describes the workflow for employing a high-resolution accurate mass Gas Chromatograph/Mass Spectrometry (GC/MS), in combination with low energy ionization, to generate differentiating structural information on synthetic fentanyl analogs, including 4-ANPP, a scheduled precursor along with nitazene analogs from seized street drug case samples. This work compiles high resolution mass spectrometry low energy ionization spectra, the formation of an analyte's molecular ion, and the molecular ion's elemental isotopic ratios to assist in the identification of isobaric spectra generated under conventional nominal mass GC/MS 70eV ionization energy. Low energy ionization spectra were produced at 10, 12, 15, and 17eV ionization energies to assist in the formation of a molecular ion enabling the measurement of the isotopic abundances based on the elemental composition of the molecular ion. Comparisons of the exact masses and ratios (calculated) to the accurate masses and ratios (measured experimentally) are illustrated in this work. Low energy spectral patterns of known of nitazene and fentanyl analog standards were compared to seized drug case samples and used to identify nitazene analogs and other controlled substances based on the creation of their respective accurate mass molecular ion and isotopic ratios and patterns.

The data demonstrates the power and ability of low energy ionization and high-resolution MS to produce molecular ions of many illicit drugs that would normally be difficult to identify due to their similar or isobaric spectra when generated under the conventional 70eV ionization energies. This comprehensive analytical technique would be useful in identifying and differentiating NSOs that continually flood the illicit drug market, contributing to the increasing caseloads for seized drug analysis across the country.

Reference:

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Mass Spectrometry; Low Energy Ionization; Novel Psychoactive Substances

B157 The Development and Validation of a Combined Selected Ion Monitoring-Scan GC/MS Method for Nitazene Analogs

Alleigh N. Couch, BS, Sam Houston State University, Huntsville, TX; Emma Hardwick, BA, Sam Houston State University, Huntsville, TX; J. Tyler Davidson, PhD, Sam Houston State University, Huntsville, TX; Claire Phelps, MSFS, Sam Houston State University, Inver Grove Heights, MN*

Learning Objectives: After attending this presentation, attendees will have learned about a validated Selected Ion Monitoring (SIM) -scan Gas Chromatography/Mass Spectrometry (GC/MS) method for the identification of 20 representative nitazene analogs. The audience will learn about the concept of SIM data acquisition, including the use of retention time windows and ion ratios to increase method specificity. Finally, the audience will learn about the benefits of a combined SIM-scan acquisition method to identify low concentration nitazene analogs, even in the presence of commonly encountered controlled substances, adulterants, and diluents.

Impact Statement: This presentation will impact the forensic science community by providing a validated SIM-scan acquisition GC/MS method to identify 20 representative nitazene analogs. The validated method enables the detection of low concentration nitazene analogs using SIM acquisition, as well as the identification of other controlled substances and cutting agents using mass spectral library searching and scan acquisition. The developed SIM-scan GC/MS method provides a potential solution for seized drug laboratories to address the increasing presence of nitazene analogs in seized drug casework, which could easily be missed by routine scan data acquisition given the potency of nitazene analogs.

Abstract Text: The third wave of the opioid epidemic is characterized by overdoses due to synthetic opioids, such as fentanyl and fentanyl analogs. However, changing legislation in the United States and China has led to the emergence of a new class of synthetic opioids, known as 2-benzylbenzimidazoles, or nitazene analogs. These compounds are very potent and have grown in popularity within the United States since 2019. Given the potency of nitazene analogs and their increasing prevalence in seized drug casework, it is imperative that seized drug laboratories have sufficient methods to identify nitazene analogs. The central hypothesis of this research is that a combined SIM-scan acquisition GC/MS method will enable the necessary sensitivity and selectivity to identify nitazene analogs, even in the presence of higher-concentration controlled substances and cutting agents. The developed method was also validated in terms of the Limit Of Detection (LOD), carryover, selectivity, repeatability, reproducibility, and processed sample stability.

An Agilent GC/MS was used to develop the combined SIM-scan acquisition method. The 20 representative nitazene analogs analyzed in this study were: isotodesnitazene, 3'-methoxy metodesnitazene, protodesnitazene, 5-methyl etodesnitazene, nitazene, menitazene, 5-aminoisotonitazene, N-desethyl etonitazene, propylnitazene, N-desethyl isotonitazene, 4'-hydroxy nitazene, N-pyrrolidino metonitazene, ethyleneoxynitazene, iso-butonitazene, N-pyrrolidino isotonitazene, N-pyrrolidino 4'-hydroxy nitazene, N-piperidinyl etonitazene, N-piperidinyl 4'-hydroxy nitazene, N-piperidinyl protonitazene, and metodesnitazene. A mixture of the 20 nitazene analogs and a PCP internal standard was prepared at 50ppm in methanol for the Quality Control (QC) mixture used for the validation studies.

The LOD was determined based on the lowest concentration that provided a Signal-to-Noise (S/N) ratio of at least 3:1, a retention time within 1% of the QC, and ion ratios within ±20% of the QC. Carryover was monitored by analyzing each nitazene analog individually at 100ppm and assessing the subsequent methanol blanks. Selectivity was assessed by monitoring the ability to identify each nitazene analog in the presence of common interferences. The interference compounds included cocaine, caffeine, methamphetamine, xylazine, diphenhydramine, quinine, levamisole, phenacetin, alprazolam, diazepam, bromazolam, clonazepam, fentanyl, tramadol, and heroin. Interferences were prepared at various concentrations ranging from 25ppm to 250ppm. Repeatability was determined using ten injections within one day and reproducibility was determined using one injection per day for ten days. Processed sample stability was assessed in methanol at room temperature for up to four days.

All 20 targeted nitazene analogs had an LOD between 5ppm and 10ppm due to the enhanced sensitivity provided through SIM acquisition. No carryover was observed for any of the nitazene analogs. The 20 nitazene analogs were differentiated from each other and the common interference compounds that were included in this study. The retention times demonstrated acceptable repeatability and reproducibility, although there were inconsistent relative peak areas for several of the nitazene analogs. The processed sample stability study revealed that the majority of nitazene analogs in this study were stable in methanol at room temperature for up to 48 hours. The validated method was then applied to analyze 35 blind simulant samples. The ground truth nitazene analog composition was identified in 34 out of 35 simulant samples. The lone incorrectly reported sample was due to ion ratios outside of tolerance, likely because of a concentration below the limit of detection. Finally, two blind authentic samples were analyzed using the validated method. The identified controlled substances were consistent with those reported by the laboratory that provided the authentic samples. Thus, the validated SIM-scan GC/MS method is suitable for seized drug casework and should help provide an additional resource for seized drug analysts to combat the ongoing opioid epidemic.

Novel Synthetic Opioids; Analogs; Chromatography

B158 The Differentiation of Isobaric Methyl-Substituted Fentanyl Analogs Using Direct Analysis in Real-Time Mass Spectrometry (DART-MS) and All-Ion Fragmentation (AIF)

Christany Liggins, BS, Sam Houston State University, Huntsville, TX; Alleigh N. Couch, BS, Sam Houston State University, Huntsville, TX; J. Tyler Davidson, PhD, Sam Houston State University, Huntsville, TX*

Learning Objectives: After attending this presentation, attendees will have learned about the differentiation of isobaric methyl-substituted fentanyl analogs using DART-MS and AIF. The audience will learn about the fundamentals of AIF, characteristic differences in the AIF data of isobaric methyl-substituted fentanyl analogs, and how the location of the methyl substitution impacts the formation of characteristic fragment ions under AIF conditions.

Impact Statement: This presentation will impact the forensic science community by demonstrating the effectiveness of AIF combined with DART-MS for the differentiation of isobaric methyl-substituted fentanyl analogs. Without the incorporation of AIF, the DART mass spectra of the isobaric methyl-substituted fentanyl analogs are indistinguishable. The application of AIF with soft ionization sources such as DART provides a potential solution for the differentiation of isobaric analogs that are becoming increasingly present in seized drug casework.

Abstract Text: As the number of seized drug casework submissions continues to rise, forensic laboratories need more rapid screening techniques to address their growing backlogs. One potential solution is the incorporation of rapid seized drug screening using DART-MS, which is an ambient ionization technique that enables the analysis of samples with only minimal sample preparation. Unfortunately, because DART is a soft ionization technique that produces predominantly protonated/deprotonated molecules, DART-MS cannot readily distinguish between isomers. Tandem Mass Spectrometry (MS/MS) can be used to generate fragment ions through Collision-Induced Dissociation (CID) that may help differentiate isomers; however, targeted MS/MS analysis requires knowledge about potential compounds of interest. In comparison, AIF enables CID activation of all precursor ions present without prior knowledge regarding the sample composition. Given the abundance of fentanyl analogs in seized drugs casework, the reliable identification of fentanyl analogs is critical for forensic laboratories. However, the differentiation of isobaric methyl-substituted fentanyl analogs is quite challenging, especially when present in mixtures. The central hypothesis of this research is that the combination of AIF with DART-MS will enable the differentiation of isobaric methyl-substituted fentanyl analogs, even when present in mixtures, based on differences in fragmentation derived from the location of the methyl substitution to the core fentanyl structure.

A DART JumpShot ionization source coupled to an Agilent 6530 quadrupole Time-Of-Flight (qTOF) mass spectrometer was used to analyze a series of isobaric methyl-substituted fentanyl analogs. Samples were introduced to the DART ionization source by depositing 5µL of sample onto a QuickStrip, which was allowed to dry before analysis. All analyses were completed in positive ionization mode using helium as the source gas heated to 350°C. The isobaric methyl-substituted fentanyl analogs included 4-methyl fentanyl, 4'-methyl fentanyl, butyryl fentanyl, isobutyryl fentanyl, ortho-methyl fentanyl, trans-3-methyl fentanyl, α-methyl fentanyl, and β-methyl fentanyl, which only differ in the location of the methyl substitution to the core fentanyl structure. All standards were prepared at a concentration of 10ppm in methanol. Two-component mixtures of methyl-substituted fentanyl analogs were also prepared at a total concentration of 20ppm in methanol. AIF was used to obtain low (i.e., 0 eV), medium (i.e., 30 eV), and high (i.e., 60 eV) activation energy spectra.

Except for butyryl fentanyl and isobutyryl fentanyl, all methyl-substituted fentanyl analogs produced either a unique combination of fragment ions or distinct differences in ion abundance ratios that enabled successful differentiation. For example, ortho-methyl fentanyl produced fragment ions at m/z 230 and m/z 188 without the presence of a fragment ion at m/z 281 that enabled differentiation from amide-substituted fentanyl analogs, such as butyryl fentanyl and isobutyryl fentanyl. Trans-3-methyl fentanyl and 4-methyl fentanyl produced a fragment ion at m/z 202 without the presence of fragment ions at m/z 119 and m/z 91 that are present for analogs such as α-methyl fentanyl, β-methyl fentanyl, and 4'-methyl fentanyl. The presence of a fragment ion at m/z 230 enabled the differentiation of trans-3-methyl fentanyl and 4-methyl fentanyl. Likewise, α-methyl fentanyl can be differentiated from β-methyl fentanyl and 4'-methyl fentanyl based on the presence of a characteristic fragment ion at m/z 233. Finally, β-methyl fentanyl and 4'-methyl fentanyl can be differentiated based on the abundance of the fragment ions at m/z 119 and m/z 91, with β-methyl fentanyl favoring the formation of the tropylium ion at m/z 91. Unfortunately, due to the location of the methyl substitution on the amide moiety for butyryl fentanyl and isobutyryl fentanyl, these compounds remained indistinguishable even under AIF conditions. However, this study demonstrates the potential benefits of combining AIF with DART-MS for differentiating isomeric species, such as isobaric methyl-substituted fentanyl analogs.

Fentanyl; Analogs; Direct Analysis in Real Time (DART)

B159 Thermal Desorption-Direct Analysis in Real-Time Mass Spectrometry (TD-DART-MS) of Seized Drug Mixtures

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Learning Objectives: After attending this presentation, attendees will have learned about a TD-DART-MS screening method that provides a temperature-based separation of seized drug mixture components before DART-MS analysis. The audience will learn about the benefits of the developed TD-DART-MS method, including reduced competitive ionization and the ability to acquire DART-MS spectra for individual components of seized drug mixtures, reducing the challenges associated with mixture interpretation.

Impact Statement: This presentation will impact the forensic science community by providing an alternative rapid screening method for seized drug mixtures using TD-DART-MS. The developed TD-DART-MS method enables the separation of mixture components, reducing concerns with competitive ionization and mixture interpretation. The demonstration of similar performance using either helium or nitrogen as the source gas supports using more affordable nitrogen as an alternative source gas for the developed TD-DART-MS method.

Abstract Text: Seized drug mixtures are analyzed daily in forensic laboratories across the United States. Presumptive testing, such as color tests, is typically used to rapidly gain information about an unknown substance without extensive sample preparation or lengthy analyses. However, color tests struggle with the presence of multiple components in seized drug mixtures, which can lead to unpredictable or inconclusive results. Recently, DART-MS has gained popularity as an alternative seized drug screening method. DART-MS enables the rapid analysis of seized drug mixtures with little-to-no sample preparation; however, the presence of multiple components can lead to issues with sensitivity due to competitive ionization and it requires complex mixture interpretation. The central hypothesis of this research is that incorporating thermal desorption before DART-MS analysis will provide a temperature-based separation to improve analyte detection and the ease of identification due to reduced competitive ionization and the production of single-component mass spectra.

An Agilent 6530 quadrupole Time-Of-Flight (qTOF) mass spectrometer was coupled with an IonSense DART JumpShot ionization source and a BioChromato ionRocket thermal desorption unit for analysis of the seized drug mixtures. The thermal desorption temperature program included a 0.8-minute hold at 40 °C followed by a 30°C/minute ramp rate to 200°C with an additional 0.5 minute hold for a total analysis time of 6.6 minutes. All TD-DART-MS data was collected in positive ionization mode with a scan range of m/z 50-450. The analyses were completed using helium and nitrogen as the source gas to assess the impact on the resulting analysis. The seized drug mixtures analyzed in this study included controlled substances such as methamphetamine, cocaine, heroin, fentanyl, and oxycodone, and cutting agents such as dimethyl sulfone, aniline, procaine, caffeine, and xylazine. Pure samples and mixtures were prepared in methanol at concentrations ranging from 5ppm to 50ppm to assess the sensitivity of the developed TD-DART-MS method.

The analysis of each substance individually enabled the development of a temperature program to maximize the temperature-based separation of the individual components before mixture preparation. As expected, components with similar vapor pressures could not be readily separated. However, components with more drastic differences in vapor pressure can be readily separated before DART-MS analysis. For example, aniline/fentanyl and methamphetamine/fentanyl can be easily separated, whereas mixtures such as dimethyl sulfone/methamphetamine, cocaine/caffeine, and fentanyl/oxycodone cannot be readily separated. Several mixtures exhibited separation between several components, but not all components within the mixture. For example, xylazine can be readily separated from heroin/fentanyl and fentanyl can be readily separated from procaine/cocaine, but the remaining components cannot be readily separated.

The limit of detection for separated mixture components was slightly lower than when the components were unable to be separated, likely due to competitive ionization. Likewise, the separated components could be identified without complex mixture interpretation typically required for DART-MS analysis of seized drug mixtures. Even with the presence of competitive ionization in the non-separated mixtures, the limit of detection was still on the order of 5ppm for all mixture samples. Finally, similar TD-DART-MS results were acquired when using either helium or nitrogen as the source gas, which supports using the more affordable nitrogen compared to traditional helium as the source gas. The helium source gas provided slightly more sensitivity; however, sufficient performance was still achievable with the more affordable nitrogen source gas. The developed TD-DART-MS method improves upon existing DART-MS analysis by reducing concerns with competitive ionization and mixture interpretation, especially for components with disparate vapor pressures.

Rapid Drug Screening; Drug Analysis; Direct Analysis in Real Time (DART)

B160 An Evaluation of a Transportable Linear Ion Trap Mass Spectrometer for Rapid Seized Drug Screening

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Learning Objectives: After attending this presentation, attendees will have learned about the advantages and limitations of rapid seized drug screening using a transportable linear ion trap mass spectrometer. The audience will learn about the capabilities of direct tandem mass spectrometry analysis, including the development of an internal library and associated alarm system for the automated identification of individual compounds within commonly encountered seized drug mixtures.

Impact Statement: This presentation will impact the forensic science community by evaluating the use of a transportable linear ion trap mass spectrometer for rapid seized drug screening of commonly encountered seized drug mixtures. Assessing the performance of this instrumentation to enable automated seized drug identification will inform the community about the potential utilization of transportable linear ion trap mass spectrometers as an alternative rapid seized drug screening method.

Abstract Text: The increasing number of seized drug casework submissions is creating a significant problem for seized drug analysts. Not only are analysts having to examine more items of evidence per submission, but the backlogs of seized drug casework are continuing to grow. The implementation of field-portable or transportable instrumentation is one potential solution that could ease the current casework burden; however, extensive testing is required before these instruments can be reliably applied within the field. Ideally, law enforcement or forensic practitioners will be able to rapidly and accurately screen seized drug evidence on-site to reduce the number of casework submissions and improve the allocation of resources within forensic laboratories. The central hypothesis of this research is that the direct tandem mass spectrometry capabilities of a transportable linear ion trap mass spectrometer will enable rapid and reliable screening of seized drug mixtures, even by non-trained personnel, using an automated alarm system for compound identification.

In this study, a BaySpec Continuity transportable mass spectrometer coupled with a Thermal Desorption-Atmospheric Pressure Chemical Ionization (TD-APCI) source was used for the analysis of seized drug mixtures. The following controlled substances and cutting agents were analyzed to represent compounds commonly found within seized drug mixtures: cocaine, methamphetamine, heroin, fentanyl, caffeine, acetaminophen, pseudoephedrine, phenacetin, phentermine, and levamisole. Both full scan and product ion spectra were collected for all individual compounds. The parameters required for Collision-Induced Dissociation (CID) activation and characteristic product ions for each compound of interest were imported into the internal library. A library-based alarm system was implemented to enable the automated identification of unknown compounds by even non-trained personnel. Any precursor ion and corresponding product ion abundance above the optimized library alarm threshold resulted in compound identification and a corresponding system alarm. The following two- and three-component mixtures were analyzed to demonstrate the ability to identify each component within the mixture using the developed alarm system: (1) heroin and fentanyl, (2) cocaine and levamisole, (3) methamphetamine and pseudoephedrine, (4) methamphetamine and phentermine, (5) methamphetamine, caffeine, and acetaminophen, and (6) cocaine, caffeine, and phenacetin. Validation studies consisting of selectivity, reproducibility, repeatability, and Limit Of Detection (LOD) were performed to assess instrumental performance. Five blind simulant mixture samples and ten authentic adjudicated casework samples were analyzed to establish the efficacy of the developed approach for real-world casework samples.

The results indicate that the controlled substances and cutting agents analyzed in this study were reliably identified as both pure samples and mixtures. Implementation of a library-based alarm system enabled the rapid identification of each component in the analyzed seized drug mixtures. In addition, even isobaric species such as methamphetamine and phentermine were differentiated based on distinguishable Tandem Mass Spectrometry (MS/MS) fragmentation behavior. The LOD was established on a per-compound basis, with most compounds detected at a concentration of 15ppm or less. The correct controlled substance was identified in 100% of two- and three-component mixtures, blind simulant mixtures, and authentic adjudicate casework samples. One potential limitation of the library-based alarm system is the requirement for the compound to be in the internal library, highlighting the need for continued expansion of the current internal library. However, this limitation did not impede correct compound identification for the authentic adjudicated casework samples used in this study. The developed library-based alarm system implemented with a transportable linear ion trap mass spectrometer coupled to TD-APCI sample introduction enabled individual compound identification in seized drug mixtures, providing a potential solution for rapid on-site seized drug screening, even by non-trained personnel.

Rapid Drug Screening; Drug Analysis; Tandem Mass Spectrometry

B161 The Differentiation of CBD and Δ9-THC Isomers Using Copper Ion Complexation and Electrospray Ionization Tandem Mass Spectrometry

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Learning Objectives: After attending this presentation, attendees will have learned about the differentiation of Cannabidiol (CBD) and Δ9-Tetrahydrocannabinol (Δ9-THC) isomers using copper ion (Cu+) complexation and Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS). The audience will learn about subtle differences in the preferential binding affinity of Cu+ to CBD and Δ9-THC isomers and how these differences enable the formation of unique product ions or product ions ratios enabling cannabinoid differentiation.

Impact Statement: This presentation will impact the forensic science community by providing the first application of copper ion complexation for CBD and Δ9-THC isomer differentiation. The characterization of Cu-cannabinoid adducts for each isomer reveals the presence of unique MS/MS product ions or unique product ion ratios enabling their differentiation using a rapid direct mass spectrometry approach.

Abstract Text: After the enactment of the 2018 Farm Bill, marijuana was redefined as *Cannabis sativa* L. or any derivative thereof that contains greater than 0.3% Δ9-THC, whereas hemp contains no more than 0.3% Δ9-THC on a dry weight basis. The Controlled Substances Act classifies marijuana as a Schedule I controlled substance, prohibiting marijuana at the federal level. Therefore, the ability to differentiate between legal hemp and controlled marijuana is critical within the seized drug community. CBD and Δ9-THC are the main cannabinoids present in hemp and marijuana, respectively. However, there are additional cannabinoids present within cannabis, many of which have identical chemical formulas yet slightly differ in their structural arrangements, resulting in nearly identical product ion spectra under ESI-MS/MS analysis. Likewise, there are concerns with thermal degradation and interconversion between cannabinoid isomers with gas chromatography/mass spectrometry analysis. The central hypothesis of this research is that the application of copper ion complexation and ESI-MS/MS will enable the differentiation of CBD and Δ9-THC isomers based on the formation of unique MS/MS product ions due to subtle differences in the binding affinity between cannabinoid isomers.

An Agilent 6530 quadrupole Time-Of-Flight (qTOF) mass spectrometer coupled to direct injection ESI was used to analyze Cu adducts of the targeted cannabinoids. The following compounds were analyzed in this study: Δ9-THC, CBD, Δ8-tetrahydrocannabinol (Δ8-THC), exo-tetrahydrocannabinol (exo-THC), Δ6a, 10a-tetrahydrocannabinol (Δ6a,10a-THC), cannabicitran (CBT), cannabichromene (CBC), tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), cannabigerol (CBG), and cannabinol (CBN). These cannabinoids included Δ9-THC isomers, as well as other common cannabinoid interferences found in cannabis. Product ion spectra were collected for the precursor ions of the Cu adducts formed as [Cu(cannabinoid)*n*]+ adducts (*n* = 1, 2, or 3) observed at *m/z* 377, *m/z* 691, and *m/z* 1003, respectively. Product ion spectra were collected using a 4 Da isolation width and collision energies of 15-55eV. Spectral comparisons were utilized to characterize the Cu-cannabinoid adducts and to identify unique MS/MS product ions or unique product ion ratios that enable differentiation between CBD and Δ9-THC isomers. The efficacy and robustness of the developed approach were assessed by analyzing known mixtures of Δ9-THC isomers and methanolic extracts of cannabis plant material to identify the impact of matrix effects and potential overlapping product ions from isobaric cannabinoids.

The results indicate that CBD and Δ9-THC isomers can be differentiated using copper ion complexation and direct injection ESI-MS/MS. The differentiation of isomers is based on differences in the binding affinity between the Cu+ and alkene functionalities within the cannabinoids; therefore, small differences in structure between each isomer enable subtle differences in the formation of MS/MS product ions. Three variations of the Cu adducts were formed, including [Cu(cannabinoid)*n*]+ adducts (*n* = 1, 2, or 3), and when fragmented under optimal activation energies, unique product ions were observed enabling isomer differentiation. For example, when fragmenting the precursor ion at *m/z* 691 under 45eV activation energies, the presence of the product ion at *m/z* 313 and the absence of the product ion at *m/z* 254 enables the differentiation of Δ9-THC from CBD. Likewise, the presence of a product ion at *m/z* 245 indicates the presence of Δ8-THC rather than exo-THC. Known cannabinoid mixtures and authentic cannabis plant material extracts were analyzed and Δ9-THC and CBD isomers were able to be identified, even in the presence of common cannabinoid interferences. The results of this study highlight the potential for the combination of copper ion complexation and ESI-MS/MS as an alternative method for the forensic science community to differentiate CBD and Δ9-THC isomers, which is necessary for the differentiation of hemp and marijuana.

Marijuana; Hemp; Tandem Mass Spectrometry

B162 Enhancing Latent Print Development With Lanconide Powder

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Learning Objectives: From this poster presentation, attendees will learn: (1) the importance of white fingerprinting powders, (2) safety concerns with the current lanconide powder, (3) the method for altering lanconide to make it more effective as well as the results of those compositions, and (4) possible chemical properties that explain why TiO2 inhibits print development.

Impact Statement: While more complex technologies for latent print development are being created, powders are always a valuable tool to keep in the toolbox of crime scene techs and forensic investigators. Continuing to improve on old techniques, specifically powders, is incredibly valuable in the field of forensics. White fingerprinting powders are drastically under-researched, so the study described in this poster presents enhanced latent print development using lanconide, which could be applied to crime scene and trace evidence scenarios.

Abstract Text: Latent fingerprints found on crime scenes cannot be seen with the naked eye and require development techniques for visualization and analysis.[1] Because fingerprints are composed of oily sebaceous secretions, powders used in dusting adhere to the fingerprint oils.[2] The fine powder particles help visualize the print increasing contrast of the ridges and minutiae. The most common fingerprint powder is a black powder made of carbon black or powdered acacia. The composition of black powder is far more researched, but the limitation of this powder comes when the latent prints are found on dark surfaces.[3] Since black powders would not provide the necessary contrast for photo documentation in this situation, a white-colored powder must be used. However, there are very few literature compositions of white powders developed.

The current study then focuses on altering the composition of lanconide, a white powder composed of zinc sulfide (ZnS), zinc oxide (ZnO), barium sulfate (BaSO4), titanium (IV) oxide (TiO2), bismuth oxychloride (BiOCl), and calcium carbonate (CaCO3). The purpose was to improve the standard composition for lanconide powders to enhance latent print development. By removing one of the inorganic salts per trial, seven total powder compositions were tested to see if specific compounds had inhibitory or enhancing effects in the powder. The resulting prints and minutiae were analyzed for clarity to determine which powder provides the best contrast for print photography and analysis. A grading scale for analyzing prints was developed to standardize the process and quantify the data. While all powders developed prints, the highest quality resulted from removing TiO2 or BaSO4. More research needs to be done to confirm why these results occurred, but qualities such as acidity, hygroscopicity, solubility, and density were explored. The current hypothesis is that non-hygroscopic compounds (such as TiO2 or BaSO4) inhibit print development. This study posits that removing TiO2 from the powder recipe produces a more effective and safe lanconide powder.

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Fingerprint; Latent Prints; Trace Analysis

B163 A Modified and Optimized Duquenois Test for Classical and Designer Cannabis Products

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Learning Objectives: After attending this presentation, attendees will better understand the routinely used Duquenois-Levine test for cannabis products. They will have the opportunity to hear about modifications made to this presumptive color test to make it safer, simpler, and quicker. The removal of chlorinated solvents in this test makes it much safer for scientists and others, such as police officers, to utilize it in-field. This modified test can screen various popular designer formulations, such as gummies, oils, and plant material, and it has the potential to screen others as well for the presence of cannabinoids such as delta-9 THC, delta-8 THC, CBD, HHC, and other similar compounds.

Impact Statement: The results of this project can have long-lasting effects on forensic practitioners and law enforcement, as the removal of harmful solvents makes the work significantly safer. The modifications made on Duquenois-Levine, which has been the gold standard of cannabis color tests for over 50 years, not only increase the stability of the test result but also make it quicker, simpler, and a completely different color from the classical blue/purple extracted into the organic layer. This research also utilizes squeeze droplet Ultraviolet/Visible (UV/Vis) spectroscopy and is semi-quantitative.

Abstract Text: The classical Duquenois-Levine test is the primary presumptive color test used for cannabis products. This has been the preferred test for cannabis for over 50 years, and little changes have been made in that time. This test involves various harmful chemicals, including chloroform, a suspected human carcinogen. The substitution or elimination of this solvent would make the test significantly safer and was one of our major research goals. Another goal was improving results interpretation and exploring the possibility of differentiating color results for different cannabinoids and matrices using droplet UV/Vis spectroscopy and Red/Green/Blue (RGB) values. However, this was deemed not plausible using the classical or modified Duquenois-Levine as this test primarily confirms or denies that a sample contains cannabinoids.

A modified Duquenois reagent was prepared using the same reagent ratios as in the classical Duquenois reagent but replacing vanillin with 4-Hydroxy-3-Methoxycinnamaldehyde (HMC). Combining this three-component reagent (acetaldehyde and HMC in 95% ethanol) with hydrochloric acid and a cannabinoid-containing sample produced a deep green color. In addition to chloroform, cyclohexane, heptane, and hexane were all tested as solvents for this test result, and it was found that the addition of an organic solvent (Levine's modification) did not have a significant effect on the results. Using the modified Duquenois reagent, the chloroform step in Duquenois-Levine could be eliminated without altering the end color. It was discovered that the ethanol solvent used in the preparation of the chromophore was adequate at turning out the positive colors for both vanillin-based classical Duqunois tests and modified tests that were based on HMC.

Small amounts of each reagent (~0.3mL) were added to spot plates containing small quantities of each sample (10–20mg for plant material, around 100mg of gummy, and 100µL of oils). Photographs of the color results were collected with a smart phone camera, and UV/Vis spectra were collected for each sample using an NP80 Nanophotomer. 0.3µL aliquots of the colored matrix in the spot plates were transferred directly onto the instrument and analyzed using a squeeze droplet design that minimizes the sample's contact with the environment during analysis to prevent evaporation. A λ_{max} of between 655 and 657nm was observed for cannabinoid-containing gummies, 662 and 663nm for marijuana and hemp plant material (respectively), and between 652 and 658nm for oils.

Using HMC as a replacement for vanillin in the Duquenois reagent, the chemistry of the reaction was successfully altered to produce a different color (green) so that it was easier to identify a positive test result, and the absorption band separated more clearly from the other bands in the UV/Vis spectrum. The elimination of chloroform in the test also makes it safer and simpler. The resultant chromophore solution appears to be more stable and less volatile compared to classical Duquenois-Levine test solutions.

UV/Vis Spectroscopy; Duquenois Test; Color Test

B164 Discriminating Between the Tandem Mass Spectra of Isomeric Synthetic Cannabinoids Using the Expert Algorithm for Substance Identification (EASI)

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Learning Objectives: In this presentation, attendees will learn how the abundances of fragment ions in replicate tandem mass spectra display correlations that can be used to discriminate between spectrally similar synthetic cannabinoids.

Impact Statement: This presentation will provide the forensic community with a robust algorithm capable of identifying substances from their tandem mass spectra, even when collected under different activation conditions. EASI aims to minimize false identifications and improve forensic analysts' confidence in their identifications and testimony.

Abstract Text: The identification of isomers is one of the greatest challenges currently facing the seized drug community. Whether through traditional techniques, such as Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS), or emerging techniques, such as Liquid Chromatography/Electrospray Ionization/Tandem Mass Spectrometry (LC/ESI/MS/MS), seized drug analysts are faced with the difficult task of correctly identifying compounds that may contain numerous structural isomers. For example, the synthetic cannabinoid class of novel psychoactive substances contains multiple controlled substances, such as AB-PINACA, that have several known isomeric species that must be differentiated for an analyst to reach the correct conclusion. Previously, we have demonstrated the application of EASI to the differentiation of isomeric fentanyl analogs, synthetic cathinones, and cannabinoids using GC/EI/MS instrumentation. This study expands our work with EASI to LC/ESI/MS/MS instrumentation. Our central hypothesis is that relative ion abundances of replicate tandem mass spectra are not independently variable but highly correlated and anticorrelated. We use the measured correlations (confirmed by Pearson correlation matrices) between abundances in replicate spectra in a series of general linear models to make accurate predictions of ion abundances within the spectra. This hypothesis is supported by unimolecular fragmentation theories like Rice–Ramsperger–Kassel–Marcus (RRKM).

More than 60 replicate spectra were collected for each synthetic cannabinoid analyzed in this study. These chosen synthetic cannabinoids were isobaric or spectrally similar to AB-PINACA and included AEP-PINACA and ADB-BUTINACA. Tandem mass spectra were collected on an Agilent 6530 Q-TOF mass spectrometer at four collision energies. The replicate spectra were randomized and distributed so that 80% of the data was in a training set, and the remaining 20% was in the test sets for General Linear Modeling (GLM). Since the fragment abundances vary at different collision energies, the 30 most abundant fragments across all collision energies were selected as variables for the linear models and entered in a stepwise addition method. 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, including the weighted dot-product and the mean absolute residual. Each metric was then used as a binary classifier to generate Receiver Operating Characteristic (ROC) curves and to determine the true 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 four times smaller than those from the consensus-based approach. Using all the modeled fragment abundances as input variables for classification, the consensus-based approach discriminated between selected compounds with an average of ~80% accuracy. However, EASI distinguished between the analogs with an average of >90% accuracy. Abundance predictions were more accurate for both EASI and the consensus approach when the training set and query spectra were kept at a fixed collision energy. Demonstrating an extension of EASI to tandem mass spectra generated using multiple collision energies with LC/ESI/MS/MS instrumentation further highlights the utility of EASI for compound identification regardless of the instrumental configuration.

Novel Psychoactive Substances; Tandem Mass Spectrometry; Modeling

B165 An Analysis of *Mitragyna Hirsuta* and *Mitragyna Diversifolia* Being Offered as Legal Alternatives to Kratom

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Learning Objectives: While not controlled at the federal level, kratom is regulated or controlled in 13 states and is considered a drug and chemical of concern by the Drug Enforcement Administration (DEA). This poster provides attendees with understanding of the current state of the kratom, *Mitragyna speciosa*, alternatives being sold in the United States. The primary focus is on the sale and analysis of two *mitragyna (M.)* species, *M. hirsuta* and *M. diversifolia* which do not contain mitragynine, the psychoactive substance used to identify kratom. *M. hirsuta* and *M. diversifolia* are sold online and in head shops as a legal alternative to kratom as neither contains the psychoactive alkaloid.

Impact Statement: This presentation aims to add to analysts' and law enforcement's understanding of the different species of mitragynine being sold as legal alternatives to kratom. The knowledge gained from this presentation can also be applied to future regulations regarding kratom.

Abstract Text: *Mitragyna (M.) speciosa* is a tree native to Southeast Asia, where its leaves have traditionally been consumed for medicinal purposes through smoking or ingestion in teas, capsules, or with food. The plant is typically reported as having both stimulant effects in low doses and sedative effects when taken in high dosage. The effects are caused by the active alkaloids, mitragynine and hydroxymitragynine or 7-hydroxymitragynine.[1] Different strains of *M. speciosa* have characteristic levels of the active alkaloids.[2]

In 2016, the DEA published a notice of intent to list mitragynine and 7-hydroxymitragynine as a Schedule 1 substance under the Controlled Substances Act's emergency scheduling provisions. Following public backlash from kratom organizations and lobbyists, the DEA withdrew its expedited scheduling and, eventually, the attempt at scheduling entirely. It remains a DEA drug or chemical of concern.[3] Although there are no federal regulations regarding kratom, the possession and sale of kratom has been regulated in 13 states, with complete bans on the active alkaloid put in place in six states, including Alabama.[1] Since the scheduling of kratom in Alabama, head shops and gas stations have begun selling other mitragyna species, including *M. hirsuta* and *M. diversifolia* ,which do not contain the mitragynine alkaloid but are known to provide similar effects as *M. speciosa*.[4]

Samples of *M. hirsuta*, and *M. diversifolia* in capsule form were purchased from a head shop in Birmingham, AL. Products labeled as "Kratom," including Red Malay and Red Bali strains, were purchased from gas stations in Alabama and Georgia. Approximately 0.15g of each capsule's contents were dissolved in 7.5mL of methanol to create a 20mg/mL solution of the product. Nine hundred µL of the sample solution was added to a gas chromatography vial, along with 100µL of 1mg/mL dibucaine internal standard. The samples were run on an Agilent 8890 GC System 5977B Gas Chromatography/Mass Selective Detector (GC/MSD) with an external standard of mitragynine. All labeled kratom samples were positive for mitragynine, as expected for *M. speciosa*. The *M. hirsuta* and *M. diversifolia* samples were also positive for mitragynine, despite previous studies documenting these species lack the alkaloid. Due to the presence of mitragynine in the sample, it is possible that companies may be selling *M. speciosa* as the uncontrolled *M. hirsuta and M. diversifolia* in order to circumvent regulation.

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Kratom; Mitragyna hirsuta; Mitragyna diversifolia

B166 The Development of a Virtual Liquid Chromatography Method Development Tool

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Learning Objectives: By attending this presentation, attendees will understand the background and development process of a virtual Liquid Chromatography (LC) method development tool. The validation of this tool will be explained in depth to emphasize the accuracy of the modeler when compared to experimental, in-lab results.

Impact Statement: This presentation will impact the forensic science community by exploring the use of a novel technology being used to improve the analytical method development process.

Abstract Text: The development and optimization of LC separations can be time consuming and costly, often requiring several steps, including literature research, column selection, method scouting, method development, and method optimization. In an effort to eliminate these steps, an instrument-free, software modeling tool that gives users the ability to select compounds from a database and instantly model a separation on different column stationary phases was developed. Optimization of the model can be performed while maintaining critical pair separations by adjusting for instrument/system effects (e.g., dwell volume and extra column volume), mobile phase preferences, number of gradient steps, and more. The initial database consists of a Drugs of Abuse (DoA) library containing approximately 250 compounds with plans to continually expand the utility.

To build the chromatogram modeler, a DoA library containing approximately 250 compounds was created. Data was collected using a standard High-Performance Liquid Chromatography (HPLC) system coupled to a triple quadruple mass spectrometer (LC/MS/MS). Retention times were first collected using a fast/slow gradient, 30°C/60°C column temperature points, and ACN/MeOH mobile phases on a single column dimension (50 x 2.1mm, 2.7µm). Some additional data points outside of these runs were also collected for the development of a semi-empirical correction factor that was used to improve modeling accuracy. To assess the accuracy of the modeler, experiments comparing compound retention time values between wet-lab and modeled data were conducted. After the initial DoA library was built, the modeler was evaluated over four increasingly more complex stages of verification. In the final, most complex stage, new compounds not previously part of the initial DoA library were added and then compared by testing two different column dimensions, two different columns lengths, two different mobile phases, two different stationary phases, three different gradients programs, and three different temperatures against modeled retention time values. Because the semi-empirical correction factor was developed using only the original library compounds, this stage assessed the viability of adding future compounds to existing libraries.

During software development, the acceptance criteria for retention time agreement between wet-lab and modeled values was set at +/- 15 seconds. This range was chosen because it represents a typical Multiple Reaction Monitoring (MRM) window. In the most complex portion of the verification, 704 retention time data points were collected in total for the 25 compounds used in the evaluation. Only 13 data points exceeded the +/- 15 second window, with no compounds missing acceptance criteria by more than five seconds, giving an overall pass rate of 98.2%.

An online chromatographic modeling tool was successfully developed that allows users to select columns and compounds for separation. A modeled chromatogram and instrument-ready conditions are automatically generated and can be further optimized by users.

Method Development; Liquid Chromatography; Drugs of Abuse

B167 The Drug Enforcement Administration's Basic Forensic Chemist Training Program Becomes the First Forensic Training Program Accredited by the Federal Law Enforcement Training Accreditation Board

Jessica M. Rasmussen, MS, United States Drug Enforcement Administration, Stafford, VA*

Learning Objectives: This poster will enable attendees to understand the requirements, process, and importance of becoming an accredited forensic science entry-level training program. It will outline the structure of Drug Enforcement Administration's (DEA's) Basic Forensic Chemist Training Program (BFCTP) as a model to better equip attendees to create or re-organize their respective training programs should accreditation be desired.

Impact Statement: This poster will impact the forensic science community by highlighting the importance of maintaining a comprehensive, structured training program. As the forensic science community faces increased scrutiny in the criminal justice system, it is of the utmost importance that the public maintains a high level of confidence in the competency, proficiency, and professionalism of forensic examiners. Achieving and maintaining accreditation demonstrates a training program's effectiveness in producing forensic examiners able to meet these standards and expectations of the criminal justice system.

Abstract Text: On May 9, 2024, the DEA BFCTP became the first forensic training program to be accredited by the Federal Law Enforcement Training Accreditation (FLETA) Board.[1] The centralized program is located at the DEA Training Academy in Quantico, VA. It is a rigorous 18-week program that trains incoming Basic Forensic Chemists (BFCs) on DEA policy and procedures; methods and techniques used in seized drug analysis; and quality management, law, ethics, and courtroom testimony. Additionally, elements of professionalism, such as demeanor, integrity, and judgement are evaluated.

The accreditation process includes applying and preparing for the assessment, an onsite inspection, and an interview panel at the Federal Law Enforcement Training Center (FLETC) in Glynco, GA. This process confirms that the program meets several standards and ensures it has sufficient organization and oversight for accreditation. Specifically, the program must maintain records on curriculum content, such as lesson plans, training materials, practical and written evaluations, and respective answer keys. Ethics training as it relates to the forensic science profession is also required. For each class, the roster, the inclusive course dates, the dates each lesson was taught, and the instructors that taught each lesson must be recorded.[2]

The DEA BFCTP uses a federal agency training software to store most of the documentation regarding training dates, instructors, and completed practical assessment rubrics. Formal lesson plans and test questions are maintained by the DEA Training Academy's Policy and Learning Development Section. Each lesson includes one or a combination of PowerPoint presentations, written classroom exercises, hands-on laboratory exercises, verbal assessments, laboratory practical skills assessments, and written exams.

In addition to program content, the following information must be maintained on each trainee: course prerequisites met by each trainee (degree requirements, medical clearances, etc.), evaluation and examination scores, waivers or reasonable accommodations granted, and transcripts documenting the successful completion of the course.[2] The training software used by the BFCTP collects and retains much of this information for each BFC, including their educational background and work experience and progress through the course.

Finally, the program must adhere to its organizational policies, such as equipment maintenance, student misconduct, and security of controlled substances, hazardous substances, practical lesson samples, classrooms, and training laboratories.[2] The BFCTP is inspected on a routine basis by the DEA's Office of Forensic Sciences to ensure these organizational requirements are being met.

The FLETA accreditation validates the high quality, standards, and professionalism of the BFCTP, ensuring that DEA forensic chemists are fully prepared to analyze complex seized drug evidence. It further establishes credibility to the program, its curriculum, and ultimately, its graduates as competent, professional forensic chemists.

References:

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Training; Accreditation; Drug Analysis

B168 The Development and Testing of a Novel Sorbent Material for Headspace Analysis With Application to Illicit Drugs

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Learning Objectives: The audience will learn about a novel method of Volatile Organic Compound (VOC) collection for use in drug analysis and the distinct VOCs present in illicit drugs such as xylazine and nitazenes.

Impact Statement: The results will show the novel sorbent material's ability to be used as a headspace sampling technique in comparison to Solid-Phase Microextraction (SPME) fibers for illicit drug sampling. This technique offers a cost-effective and more rugged approach to headspace sampling methods in the field.

Abstract Text: SPME is an extraction technique used with Gas Chromatography/Mass Spectroscopy (GC/MS) that uses a polymer-coated fused silica rod to extract compounds from the liquid or gaseous phases. This technique is highly versatile, with uses in many different fields such as environmental, pharmaceutical, toxicology, and forensics. For this research, VOCs from pharmaceutical-grade and seized drug samples are identified to better understand the headspace profile of illicit drugs for use in development of canine training aids. SPME fibers are available with different polymer chemistries and thus varying fiber affinities for application to many targets, matrices, and applications. The most common polymer for SPME fibers is Polydimethylsiloxane (PDMS). The polymer coating acts to adsorb VOCs in the vapor profile of a sample, prior to desorption and analysis. The main drawback to SPME is the fragility of the fiber and cost, making sampling in the field challenging.

As an alternative to the SPME fiber, a novel headspace sampling material that is both rugged and inexpensive was designed using a functionalized glass surface for use in seized drug detection. The glass surface was functionalized with various amino silanes to improve vapor collection. Silanes are often used in functionalizing GC columns and SPME fibers; amino silanes have been previously used for small molecule/ volatile capture and drug delivery.[1] Amino silanes have an advantage on PDMS as a chemical modifier; since it includes a polar amine group, it allows for a greater ability to adsorb polar compounds allowing for broad selectivity. These functionalized glass surfaces were tested for their efficacy using a case study of various illicit drug samples such as nitazenes and xylazine and compared to the headspace analysis using SPME fibers. This sampling method is comparable to the SPME fiber for collection of VOCs in the headspace of illicit drug samples and more cost effective. During analysis of the samples, several target VOCs were found, and the most abundant targets were focused for each drug. The targets were found with similar abundances in the SPME fiber as well as the functionalized glass surface, showing a comparable technique to SPME and were more cost effective.

Reference:

1. Moon, J. H.; Shin, J. W.; Kim, S. Y.; Park, J. W. Formation of Uniform Aminosilane Thin Layers: An Imine Formation to Measure Relative Surface Density of the Amine Group. *Langmuir* 1996, 12 (20), 4621–4624. https://doi.org/10.1021/la9604339.

Seized Drugs; Absorption; Solid Phase Microextraction

B169 The Optimization of Fast Blue B Test for Cannabis Products

Ryan A. Smith, BS, Hofstra University, Swansea, MA; Ling Huang, PhD, Hofstra University, Hempstead, NY; Marie E. Lemay*, Hofstra University, Jamestown, New York*

Learning Objectives: After attending this presentation, attendees will understand the capabilities and shortcomings of the current Fast Blue B Test for cannabis products, and how to optimize the test by avoiding hazardous solvents and differentiate THC, CBD, and HHC using droplet Ultraviolet/Visible (UV/Vis) spectroscopy across multiple sample matrices such as plant material, oil, and gummy, with only 0.3-µL test solution. In addition to the optimization of the Fast Blue B Test, this presentation will demonstrate the capabilities of the Nanophotometer NP80's squeeze droplet design and the benefits of this setup over conventional UV/Vis spectrometers and other types of droplet spectrometers.

Impact Statement: The results of this experiment can have long-lasting benefits for law enforcement, harm reduction, and consumer safety as a quick and improved presumptive test can be utilized to differentiate cannabinoids in different designer formulations without the use of hazardous solvents. This research will also help to improve presumptive drug tests.

Abstract Text: The Fast Blue B test is a common colorimetric test used for the presumptive screening of cannabis products, which is commonly found in the form of a KN-S Nik Kit. It is composed of a color agent, a base, and the hazardous solvent trichloroethylene. This test would yield a dark red color change as a positive for the THC-containing samples and an orange color for CBD. The current test has its limitations as it was originally designed solely for a sample matrix which is plant material, but there has since been an emergence of designer cannabinoids such as HHC, THC-O, and THC-P found in various matrices such as oils and gummies.

In our investigation, the color change that is observed after cannabinoids react with Fast Blue B agent was quantified as an absorbance maximum wavelength using the Nanophotometer NP80 and UV/Vis spectroscopy as its squeeze droplet design prevented the evaporation of a small sample liquid droplet with more reliability than other droplet spectrometers. This allowed the analysis of the cannabinoid samples to only require 0.3µL, using a greener solvent than the chlorinated solvent used in the current Nik Kits.

To begin the test, approximately 0.05 grams of the Fast Blue B salt was dissolved in 10mL of solvent (either dimethylsulfoxide, DMSO, or deionized water which were found to be the best solvents). From here 100µL of the newly produced FBB reagent was mixed with 100µL 0.1 M NaOH and the cannabinoid-containing sample in a well plate and the color change was photographed and recorded. These cannabinoid samples ranged from marijuana leaf and hemp cigarettes to CBD oils to cannabinoid-containing gummies. Using only 0.3µL of each test solution from each well, it was discovered that THC consistently had an absorbance peak at 490nm, CBD consistently had an absorbance peak of 505nm, and HHC consistently had an absorbance of 495nm. These results provide evidence that the cannabinoid present in the samples of various forms can be differentiated using this optimized Fast Blue B test and UV/Vis spectroscopy.

To conclude, the end goal of being able to differentiate between cannabinoids across various sample matrices through their absorbance maximums using UVVis spectroscopy was achieved, with the added benefit of the test being quick to use and portable due to the Nanophotometer NP80's small size. In addition to the goal of the experiment being achieved, great strides in the field of public health have been made, as trichloroethylene has been shown to be unnecessary for the test to proceed, making the test safer to perform. Our improvements have also benefited law enforcement, as this presumptive test is minimally destructive since it required only 0.3µL of a sample to reliably produce results.

Cannabinoids; Color Tests; UV/Vis Spectroscopy

B170 The Detection and Quantitation of Xylazine, Medetomidine, and Detomidine as Adulterants to Heroin or Illicitly Manufactured Fentanyl Using NanoLC With Various Mass Spectrometers

Mehdi Moini, PhD, George Mason University, Manassas, VA; Brian Eckenrode, PhD, George Mason University, Aldie, VA*

Learning Objectives: After attending this presentation, attendees will understand the dangerous role of xylazine, medetomidine, and detomidine as adulterants to heroin or illicitly manufactured fentanyl to produce a mixture which in the United States is known as FAAX, "tranq," or "tranq dope," on the individuals using it, and the importance of the quantitative analysis of this mixture with nano Liquid Chromatography in conjunction with Mass Spectrometry (nLC/MS).[1]

Impact Statement: This presentation will impact the forensic science community by discussing the key advantages of nLC/MS in the quantitative analysis of xylazine, its derivatives as adulterants to heroin or illicitly manufactured fentanyl, and alert the forensic community concerning the increased prevalence of illicit drug overdose deaths involving xylazine.[2]

Abstract Text: In November 2022, the United States Food and Drug Administration (FDA) issued an alert concerning the increased prevalence ofxylazine and medetomidine in illicit drug overdose deaths involving xylazine.[3] As such, quantifying these compounds using LC/MS has become an important task. NLC/MS, with its high sensitivity, high separation efficiency, economy of sample size, solvent and gas consumption, and virtually no liquid waste production has become the instrument of choice in sample-limited applications such as proteomics. However, the application of nLC/MS to the forensic analysis of illicit drugs remains elusive. This report will present a case study in the analysis of illicit drugs containing xylazine and its derivatives as a practical application of nLC/MS to seized drugs. It is recommended that analysts become familiar with the principles of nLC/MS in seized drugs containing xylazine, medetomidine, and detomidine.

In this study, an nLC was used in conjunction with three mass spectrometers: (1) a Linear Trap Quadrupole (LTQ), (2) an orbitrap, and (3) a triple-quadrupole. These systems were used for the analysis of complex illicit drugs that included xylazine, heroin, fentanyl, and their derivatives. Analytical figures of merit, including detection limit and dynamic range, were studied and compared. Under the conditions used here, baseline resolved peaks were obtained for almost all compounds. Preliminary results indicated detection limits in the pg/uL range under positive ionization mode. Experiments were also conducted under negative ionization mode; however, the detection sensitivity for most compounds was lower than positive mode. Overall, Oribtrap, with its high resolution and high mass accuracy, provided the best performance since there were many overlapping peaks with the same nominal mass but different accurate mass, and a high resolution, high mass accuracy mass spectrometer was needed to identify the drug of interest with better quantitation.

Phytocannabinoids show a strong memory effect on our C18 column. Once a sample with a concentration above one ng/uL was used, removing the background peaks related to these compounds was difficult. Because of this, we could not find a thorough detection limit since our background was about ½ of the lowest (50 picograms on column injection) levels. Also, we noticed that xylazine ionization efficiency was much lower than most other compounds, such as phytocannabinoids. It is important to note that these comparisons were performed under full-scan mode for all instruments. The results obtained under full-scan mode will be compared with those from multiple ion monitoring modes.

References:

1. Jocelyn V. Abonamah, Brian A. Eckenrode, Mehdi Moini, On-site detection of fentanyl and its derivatives by field portable nano-liquid chromatography-electron lonization-mass spectrometry (nLC-EI-MS). *Forensic Chemistry*, 2019, 100180.
2. Ball NS, Knable BM, Relich TA, Smathers AN, Gionfriddo MR, Nemecek BD, Montepara CA, Guarascio AJ, Covvey JR, Zimmerman DE. Xylazine poisoning: a systematic review. *Clin Toxicol (Phila)*. 2022; 60: 892-901.
3. Drug Enforcement Administration. Public Safety Alert. *DEA Reports Widespread Threat of Fentanyl Mixed with Xylazine, Public Safety Alert*. 2022. Available from: https://www.dea.gov/alert/dea-reports-widespread-threat-fentanyl-mixed-xylazine#:~:text=The%20DEA%20Laboratory%20System%20is,suffering%20a%20fatal%20drug%20poisoning [Last accessed 13th February 2024].

Xylazine; NanoLC/MS; Mass Spectrometry

B171 The Development of an LC/MS/MS Method for the Identification of Psilocybin and Psilocin From Seized Drugs

Alexia Cassman, BS, Sam Houston State University, Huntsville, TX; Kristen J. Head, MS, Harris County Institute of Forensic Sciences Houston, TX*

Learning Objectives: After attending this presentation, attendees will have learned about the benefits of incorporating Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) instrumentation into seized drug analysis for the detection of challenging substances such as psilocybin and psilocin. The audience will learn about the necessary method optimization required to chromatographically separate and identify psilocybin and psilocin using Multiple Reaction Monitoring (MRM).

Impact Statement: This presentation will impact the forensic science community by serving as a model for the development of LC/MS/MS methods for the analysis of difficult seized drug casework samples. Unlike traditional GC/MS methods, LC/MS/MS methods can accommodate non-volatile, thermally unstable, and otherwise hard-to-detect analytes using GC/MS instrumentation. Informing the seized drug community about the capabilities of LC/MS/MS instrumentation and providing an example of a developed method for the identification of psilocybin and psilocin will assist with the adoption of LC/MS/MS instrumentation for the analysis of seized drug evidence.

Abstract Text: The analysis of suspected mushroom samples or samples suspected of containing psilocybin (i.e., edibles) requires derivatization before analysis using traditional GC/MS instrumentation due to the elevated temperature of the inlet and oven. In comparison, LC/MS/MS instrumentation eliminates the need to derivatize thermally unstable compounds, making this technique more suitable for suspected mushrooms or edibles suspected of containing psilocybin. In addition, LC/MS/MS instrumentation is more compatible with edible matrices. The central hypothesis of this study is that an LC/MS/MS method can be developed to separate and identify psilocybin and psilocin for application to seized drug casework samples including suspected mushrooms or edible samples.

An Agilent 1200 LC coupled to a Sciex QTRAP ABI 3200 mass spectrometer using Electrospray Ionization (ESI) and MRM was utilized for the analysis of psilocybin and psilocin. An Ascentis Express biphenyl column and an Ascentis Express biphenyl guard column were chosen for analysis due to the high polarity of both analytes. Two MRM transitions were optimized for each analyte as follows: psilocybin m/z 285.4/240.1 and m/z 285.4/58.1, and psilocin m/z 205.2/58.1, m/z 205.2/160.1. The optimized collision energy for psilocybin was determined to be 23V and psilocybin was 25V. A gradient elution was used with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as the mobile phases as previously demonstrated by Goff et al.[1] The gradient starts at 6% B from 0-1 minutes and is increased to 25% B from 1 minute to 3 minutes, increased to 95% from 3.0 to 3.1 minutes, held at 95% until 3.2 minutes. From 3.2 to 3.3 minutes, B is decreased to 6% B and held until 4.5 minutes, when the method runs to completion.

Using the developed method, psilocybin and psilocin had retention times of 1.08min and 1.73min, respectively. The optimized MRM transitions led to consistent signal for samples analyzed as low as 25ng/mL of psilocybin and 2.5ng/mL of psilocin. Performance characteristics of the method were assessed consistent with the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations.[2] Baseline separation, acceptable chromatographic peak shape, signal-to-noise ratio, and ion ratios were assessed across analyses of each analyte and their corresponding internal standard to statistically assess significant differences in retention time and ion ratios.

The selectivity of this method was tested by using ten compounds related to casework samples or compounds similar in structure to psilocybin and psilocin. Three replicates of each compound were diluted to 100ng/mL and analyzed. The only potential interference observed was 4-acetoxy-DMT, which had the same retention time as psilocin, but did not produce any signal for psilocybin. Matrix effects were tested by running 20 samples of blank matrices mimicking edible matrices. These samples were extracted using HCIFS Drug Chemistry protocol and then sampled for the LC/MS/MS method to confirm the absence of analytical signal for psilocybin and psilocin. Selectivity was also tested by running five replicates of each analyte and internal standard ten times total to confirm no signal was observed from any of the other analytes or internal standard. The LOD was determined through creation of five calibrators for each analyte ranging from 50ng/mL to 1ng/mL. Each calibrator was analyzed three times across three days and monitored for acceptance criteria. The determined Limit Of Detection (LOD) for psilocybin was 25ng/mL. The LOD of psilocin was determined to be 2.5ng/mL. Ion suppression was studied by running the same ten compounds as mentioned above in mixture with psilocybin and psilocin, with their corresponding internal standards, at their LOD.

In conclusion, a method for the separation and detection of psilocin and psilocybin has been developed and produced qualitative results.

References:

1. Roman Goff, Morgan Smith, Sabrina Islam, Sue Sisley, Jonathan Ferguson, Scott Kuzdzal, Sunil Badal, Arun Babu Kumar, Uma Sreenivasan, Kevin A. Schug. "Determination of psilocybin and psilocin content in multiple psilocybe cubensis mushroom strains using liquid chromatography – tandem mass spectrometry." *Analytica Chimica Acta*, vol. 1288, Feb. 2024, p. 342161, https://doi.org/10.1016/j.aca.2023.342161.
2. *Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations.* SWGDRUG Approved Recommendations, www.swgdrug.org/approved.htm. Accessed 23 July 2024.

Liquid Chromatography; Method Development; Seized Drugs

B172 DSI-GC/MS Analysis of Designer Cannabis Gummies

Hannah LaVena, Hofstra University, Barrington, RI; Kevin Bisceglia, PhD, Hofstra University, Hempstead, NY; Ling Huang, PhD, Hofstra University, Hempstead, NY*

Learning Objectives: After attending this presentation, participants will better understand the operational principles of Direct Sample Introduction (DSI) Gas Chromatograph/Mass Spectrometry (GC/MS) and how it can be applied to the analysis of designer cannabis gummies.

Impact Statement: This presentation will impact the forensic science community by demonstrating the utility of DSI-GC/MS as an effective and efficient tool to differentiate, identify, and quantify cannabinoids and their isomers with minimal sample preparation, and it can identify inactive components within the sample matrix, allowing for the linkage of samples to specific manufacturers or distributors.

Abstract Text: After attending this presentation, participants will better understand the operational principles of DIS-GC/MS and how it can be applied to the analysis of designer cannabis gummies.

This presentation will impact the forensic science community by demonstrating the utility of DIS-GC/MS as an effective and efficient tool to differentiate, identify, and quantify cannabinoids and their isomers with minimal sample preparation, and it can identify inactive components within the sample matrix, allowing for the linkage of samples to specific manufacturers or distributors.

DIS-GC/MS has the ability to separate and detect target analytes in the presence of a complex, viscous, and often "dirty" sample matrix. It also allows for a smaller sample size to be analyzed and the elucidation of the sample matrix composition. It employs a Programmable-Temperature Vaporization (PTV) injector in combination with a ChromatoProbe accessory, in which a solid or liquid sample in a disposable glass vial can be heated to create vapor or aerosol prior to GC separation and MS detection.

Designer cannabis gummies can be found in various forms and configurations (e.g., hard, soft, cube-shaped, or bear-shaped). Additionally, they can be deliberately enhanced by distributors by combining currently available strains in order to generate a product that is both higher in quality and potency, containing a vast array of cannabinoids such as delta-8 THC, CBD, THC-A, and HHC. These products are then sold through private sellers, online retailers, and delivery services, often maintaining a low profile to avoid attention from law enforcement.

In our investigation, gummies were cut into smaller pieces (8 to 16), dissolved in solvent, and treated with manual agitation prior to analysis. Each GC run was completed in approximately 15 minutes, and the resulting mass spectra contained m/z signatures characteristic to cannabis-containing compounds.

Once identified, each cannabis-containing compound was quantified utilizing a CBD external standard calibration curve, resulting in a CBD range of 2.9%-76.8%. With library searches, additional components such as food dyes and sucrose were also detected. Additionally, the small sample volume (around 1–2µL) allows for repetitive sample analysis and additional testing.

The complexities and irregularities contained within these products pose serious threats to consumers. As there is only partial regulation by states within the cannabis industry and no systemic federal regulation on the contents of these designer products, users do not always know exactly what they are consuming.

After consumption, the delayed onset of the active cannabinoids also allows the potential for inadvertent overconsumption and extreme side effects, including psychotic episodes, impaired motor ability, and paranoia. Children and adolescents were reportedly the victims of these designer cannabis products with poor quality control and unknown pharmacology.

The DIS-GC/MS method allows for the rapid analysis and identification of cannabinoids in designer products with minimal sample preparation and identification in real time of designer cannabinoids and other components. Cannabinoids not listed as ingredients were identified, indicating this method can be used for quality assurance and the identification of contaminants in samples, thus informing consumers of the actual components of these popular designer gummies.

Further improvement of the scope and accuracy of designer gummy analysis can result from the evaluation of sugar, oil, and flavor matrix components, taking into account the unique and custom formulations across products as well as their manufacturers and suppliers. DIS-GC/MS is presented as an analytical technique that uses temperature programming, rapid analysis times, and smaller sample sizes as an adjunct to the investigation of seized designer cannabis products.

This report will present a thorough analysis of designer cannabis gummies utilizing DIS-GC/MS.

Cannabinoids; Gas Chromatography/Mass Spectrometry (GC/MS); Direct Sample Introduction (DSI)

B173 GC/VIR Technology in Seized Drug Analysis

Sherri L. Tupik, BS, Drug Enforcement Administration/Department of Justice, Dulles, VA*

Learning Objectives: The poster will show the power of Gas Chromatography/Vapor Phase Infrared Spectroscopy (GC/VIR) in identification and differentiation of nitazene compounds in seized drug analyses.

Impact Statement: The use of GC/VIR as an alternative technology in seized drug analysis combats challenges in identification of chemically and structurally similar substances when using traditional instrumental techniques such as Gas Chromatography/Mass Spectrometry (GC/MS).

Abstract Text: The ongoing emergence of Novel Psychoactive Substances (NPS), synthetic opioids, nitazenes, xylazine, and analog compounds creates challenges in identification of chemically and structurally similar substances using traditional instrumental techniques such as GC/MS. Often in forensic samples, analogs or isomers are present and possess similar chemical and physical properties. Multi-component mixtures are also a challenge. GC retention times can overlap, be difficult to differentiate, and are limited by the separation capabilities of the instrumentation. GC/MS fragmentation patterns can be very similar.

This has led to the introduction and use of alternative technologies in seized drug analysis. GC/VIR is a separatory and confirmatory technology capable of distinguishing the smallest of chemical structure subtleties in a compound enabling identification of drug substances within specific chemical classifications. GC/VIR is capable of chromatographically separating a mixture of compounds and obtaining singular infrared spectra as each compound elutes from a capillary column. GC/VIR can distinguish between different ring and side chain regioisomers, substituted aromatics, functional groups, and analogs. Confirmatory VIR spectroscopy can distinguish differences in spectra based on the arrangement of substituents in a molecule and provides identification with exclusion of all other isomers. GC/VIR use in forensic laboratories eliminates the need to purchase costly reference materials of all possible isomers to use for identification. There is ease in sample preparation, small amount of sample needed, and ability to resolve compounds of interest away from complex matrices. Using the data analysis software, spectra can be overlaid or stacked assisting in identification of specific analytes in structurally similar compounds.

The increased presence of synthetic opioids, specifically the nitazene compounds, in seized drug evidence has enabled GC/VIR to be a valuable tool in identification and analysis of such compounds. This poster shows the power of GC/VIR in identifying and differentiating nitazene compounds.

Chromatography; Spectroscopy; Forensic Science

B174 Inconvenient Truths in the Friction Ridge Discipline

Alice White, BS, Evolve Forensics, Partlow, VA*

WITHDRAWN

B175 The Development and Optimization of a Method for the Quantitation of Seized Drugs Using Rapid Gas Chromatography/Mass Spectrometry

Marissa Ho, BS, The George Washington University, Washington, DC; Briana A. Capistran, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Edward Sisco, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Ira S. Lurie, PhD, The George Washington University, Washington, DC*

Learning Objectives: After attending this presentation, attendees will be able to understand a newly developed method for the quantitation of seized drugs using rapid Gas Chromatography/Mass Spectrometry (GC/MS). In addition, attendees will also be informed on the use of Selected Ion Monitoring (SIM) with rapid GC/MS technology.

Impact Statement: The presentation will have an impact on the forensic science community by providing insight into the use of rapid GC/MS for quantitation of seized drugs. The method presented will demonstrate how rapid GC/MS can be used to reduce overall analysis times for forensic drug samples and ultimately help in decreasing the backlog of seized drug samples.

Abstract Text: With the increasing prevalence of seized drug mixtures in the United States in addition to the growing backlog of forensic seized drug samples, leveraging highly sensitive and selective screening techniques can be a useful complement to traditional confirmatory techniques. Rapid GC/MS has previously been demonstrated as a successful screening technique for seized drugs, providing chromatographic separation in under two minutes with limits of detection comparable to traditional GC/MS methods (e.g., low µg/mL range). Previous work focused on validating an analytical method for representative drug classes, such as opiates/opioids and synthetic cathinones.[1] From these results, rapid GC/MS has been shown to be a viable preliminary screening method to current GC/MS methods in situations where fast analysis is required.

Another recent concern in the forensic drug landscape is the need for quantitation of seized drugs. The implementation of the 2018 Farm Bill, which requires quantitation of Tetrahydrocannabinol (THC) in cannabis, has set the precedent for the imminent possibility of required quantitation of other seized drugs. In anticipation of potential legislation, it is important to begin researching new methods for quantitation now. Using rapid GC/MS for initial quantitation would provide forensic analysts with an understanding of the amount of a drug present during the preliminary screening stage, therefore allowing them to better determine which confirmatory method to use for further analysis. This project aims to explore the quantitative capabilities of rapid GC/MS for seized drugs.

In this study, a quantitative rapid GC/MS method was developed for a 14-compound test mixture consisting of seized drugs, adulterants, and diluents. The method was optimized for maximum separation, applying deconvolution to identify coeluting compounds. The use of SIM for rapid GC/MS analysis was studied for the first time in comparison with full scan MS data in order to determine which data collection method resulted in the most repeatable peak areas. Investigations into a suitable internal standard for quantitation as well as a determination of the best method for incorporation of the internal standard were also completed. Three methods for incorporation were tested: incorporation into the calibration curve solution (Method 1), external addition to the calibration curve solution (Method 2), and reconstitution of the calibration curve solution in solvent spiked with the internal standard (Method 3). The method was validated by determining the concentrations of compounds with known amounts using calibration curves. Lastly, mock and adjudicated case samples were analyzed to determine the real-world applicability of the method in a practical environment.

The developed method had a final run time of 1.63 minutes (97.8 seconds) and resulted in maximum separation for all compounds in the mixture. SIM was utilized to improve specificity and eliminate background noise, resulting in an overall decrease in variation for normalized peak areas. Ethylparaben was selected as the internal standard due to its symmetrical, narrow peak shape and early elution time. Preliminary results demonstrate the highest degree of calibration curve linearity using Method 1 for the incorporation of the internal standard, which resulted in coefficients of determination (R2) for single analytes as high as 0.9998. Initial quantitation results indicate acceptable performance of the calibration curve and internal standard incorporation method. For example, a known 20µg/mL heroin solution was determined to contain 14µg/mL ± 1 µg/mL heroin using the corresponding calibration curve, which is adequate for obtaining a good estimate of purity to satisfy sample analysis requirements. Overall, this method demonstrates a rapid workflow allowing for semi-quantitation of seized drugs and can potentially be utilized in forensic laboratories in order to reduce the backlog, decrease analysis times for seized drug samples, and provide fast feedback to law enforcement.

Reference:

1. Bloom MB; Sisco E; Lurie IS. Development and validation of a rapid GC-MS method for seized drug screening. *Forensic Chem*. 2023;33:100479. https://doi.org/10.1016/j.forc.2023.100479.

Quantitation; Seized Drugs; Rapid Gas Chromatography/Mass Spectrometry

B177 A Needle in a Haystack: Are Those "Rare" Minutiae in Palms Really as Uncommon as You Think?

Heidi Eldridge, PhD, The George Washington University, Washington, DC*

WITHDRAWN

B178 The Proliferation of Nitazenes in the United States Drug Market: Examining Powders and Counterfeit Tablets

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Learning Objectives: After attending this presentation, attendees will be able to describe the range of benzimadazoles ("nitazenes"), potent novel synthetics, in contraband powders and counterfeit pills.

Impact Statement: This presentation will impact the forensic science community by raising awareness of the presence of highly potent Novel Synthetic Opioids (NSOs) in the United States drug supply, allowing forensic chemists to identify these in their casework, especially counterfeit pills.

Abstract Text: A class of benzimidazole opioid agonists, the "nitazenes", are non-fentanyl synthetic opioids that were discovered by a Swiss drug company in the 1950s. They were known to activate the mu opioid receptor, producing opioid-like effects, but were never approved for use due to their high potency and low therapeutic index.

Nitazenes were identified in the United States drug supply both on their own as novel psychoactive substances and combined with illicitly manufactured fentanyl. These combinations are especially dangerous to People Who Use Drugs (PWUD) and have increased overdose rates throughout the United States. In 2021, the Drug Enforcement Administration enacted emergency scheduling to control a subset of the known nitazene compounds; however, other analogs have emerged on the illicit market. This makes testing difficult because new variations manufactured in clandestine labs continue to enter the drug supply. In addition to analytical challenges associated with identifying the drugs when they first appear, these drugs pose a public health challenge as the effects of nitazenes in combination with other drugs can be extremely detrimental to PWUD. Comprehensive testing of seized materials is necessary for laboratories to be able to track the emergence of nitazenes and analogs, as this knowledge will enhance harm reduction strategies and ultimately impact the treatment and outcomes of acutely intoxicated patients.

Illicit powders (n=3) and pills (n=60), collected during November and December 2023 were received at the Center for Forensic Science Research and Education (CFSRE) in April 2024. Both tablet types were collected on the same date. Powder Types 2 and 3 were collected on the same date, and Powder Type 1 was collected on a different date. Both types of tablets were round, green tablets monogrammed "OC 80" meant to resemble pharmaceutical 80mg oxycodone tablets but were different in appearance to pharmaceutical "OC 80" tablets. The tablets were assigned to two separate groupings based on differing characteristics. All powders and 30 of the tablets underwent comprehensive drug testing. The samples were analyzed using a workflow that included microscopic imaging of tablets using the MiScope Megapixel MP3, color measurement, and qualitative analysis of drugs, adulterants, precursors and reaction byproducts using Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). Quantitative estimates were made by measuring relative peak areas to most intense peak (relative parts). Nitazenes were identified in all samples tested (n=33). The main component in the tablets was metonitazene. Tablet Type 2 had three nitazenes present in addition to ketamine related compounds, and synthetic cathinones. The three powders had different nitazenes as the largest peak as outlined in Table 1.

Table 1. Drugs Identified in powder and tablet exhibits.

Material	Date of Seizure	Drugs Identified (Relative Parts Calculation, Main Component = 1p)
Tablet Type 1 (n=15)	11/2023	N-desethyl Metonitazene (trace) Metonitazene (1 p)
Tablet Type 2 (n=15)	11/2023	Phenyl-2-propananone (P2P) (trace) Amphetamine (trace) O-PCE (trace) 2-fluoro-2-oxo-PCE (0.56 p) Pentylone (0.02 p) N,N-Dimethylpentylone (0.26 p) N-desethyl Metonitazene (0.04 p) Metonitazene (1 p) N-pyrrolidino Protonitazene (trace)
Powder Type 1 (n=1)	12/2023	Protonitazene (1 p)
Powder Type 2 (n=1	12/2023	N-pyrrolidino Metonitazene (1 p)
Powder Type 3 (n=1)	12/2023	N-pyrrolidino Protonitazene (1 p)

The continued emergence of nitazenes in the illicit drug supply is concerning due to their relatively high potency. Studies evaluating the potency of nitazenes suggest that metonitazene, N-desethyl-etonitazene, and protonitazene are 1.5-10X more potent relative to fentanyl.[1] Furthermore, in Tablet Type 2, the combination of several NPS (stimulants, hallucinogens, and opioids) presents additional concerns about the potential for adverse polydrug toxicity for PWUD. These cases also reinforce the need for crime laboratories to have updated scopes that include new and emerging substances.

Reference:

1. Vandeputte, M. M.; Van Uytfanghe, K.; Layle, N. K.; St. Germaine, D. M.; Iula, D. M.; Stove, C. P. Synthesis, Chemical Characterization, and μ-Opioid Receptor Activity Assessment of the Emerging Group of "Nitazene" 2-Benzylbenzimidazole Synthetic Opioids. *ACS Chem. Neurosci*. 2021, 12 (7), 1241–1251. https://doi.org/10.1021/acschemneuro.1c00064.

Nitazenes; Drug Chemistry; Surveillance

B179 A Discovery of False Positive Illicit Drug Identification With Portable Surface-Enhanced Raman Spectroscopy (SERS)

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Learning Objectives: The goal of this presentation is to share the findings of a reproducible false-positive identification of cocaine from a mixture of fentanyl and acetaminophen when using a portable Raman spectrometer equipped with a 785nm laser paired with a SERS field test kit.

Impact Statement: This presentation will impact the forensic science community by sharing the first reproducible false positive identifications of an illicit drug using portable SERS.

Abstract Text: For several decades, the standard procedure for testing suspected controlled substances in the field involved the use of color-based or colorimetric presumptive tests. Although color tests are quite sensitive with low limits of detection, they have several recorded limitations, including a lack of specificity which means they suffer from false positives. A recent publication by the Quattrone Institute reported:

"Each year approximately 773,000 drug-related arrests involve the use of presumptive tests. Although the true error rate of these tests remains unknown, estimates based on the imperfect data that are available suggest that around 30,000 arrests each year involve people who do not possess illegal substances but who are nonetheless falsely implicated by color-based presumptive tests."[1]

The availability of robust portable spectrometers has great promise for addressing some of the limitations of field color tests. The majority of instrumental methods used in the forensic analysis of illicit drugs in the laboratory have field portable versions, which includes the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) and the American Society for Testing and Materials (ASTM) identified confirmatory methods of Raman spectroscopy, infrared spectroscopy, and mass spectrometry.[2,3] However, portable spectrometers may have meaningful differences in their performance characteristics when compared to their benchtop counterparts, which must be evaluated and understood prior to deployment of these instruments in the forensic field.

The major advantages of Raman spectroscopy for the identification of illicit drugs are its rapid, non-destructive, non-contact (e.g., through glass and plastic containers) analysis, which generates a reviewable record (i.e., a spectrum) with very high discriminating capabilities. When paired with SERS, a technique which enhances Raman scattering through molecular interactions with rough metal surfaces or nanostructures, there can be a meaningful improvement in the limits of detection for illicit drugs to include trace-level detection for analytes of interest like fentanyl, heroin and cocaine.

During the course of evaluating a commercially available hand-held narcotics analyzer (a Raman spectrometer equipped with a 785-nm laser) in SERS mode for the detection and identification of low dose fentanyl mixtures, a false positive for cocaine was identified when the fentanyl was dispersed in an acetaminophen matrix at low concentrations. Other matrices evaluated in the same manner did not generate this false identification. The SERS kit uses a gold nanoparticle-infused substrate in a polycarbonate holder and a solvent for dissolving a sample of interest. It is critical for police officers and other first responders who are using this commercially available technique for the field identification of suspected controlled substances to be aware of this reproducible false positive identification of cocaine and its potentially hazardous repercussions. This resulting false positive for cocaine and absence of identification for fentanyl will inevitably complicate subsequent laboratory testing, has implications for safety, and may have important legal ramifications

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Raman Spectroscopy; SERS; Illicit Drugs

B180 Illicit Drug Analysis in the Field With a Portable Instrument "Toolkit"

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Learning Objectives: After attending this presentation, attendees will learn the capabilities and limitations of a portable instrument "toolkit" for the field detection and identification of illicit drugs.

Impact Statement: This presentation will impact the forensic science community by directly comparing seven different portable technologies for the detection and identification of seized illicit drugs and providing recommendations for a "toolkit" approach for their successful implementation in the field.

Abstract Text: Portable instruments offer numerous advantages for combatting the illicit drug market in the field. Portable spectrometers have detection, identification, and analysis capabilities that provide valuable investigative and adjudicative information about a sample that can be used on its own or to inform further evidence selection, preservation, and collection. The real-time data provided by portable instruments, which have the ability to identify both bulk and trace components of a mixture, can be of particular value to first responders given the modern dynamic illicit drug landscape that contains highly dangerous and New Psychoactive Substances (NPS), including fentanyl-related substances. Still, given the diversity of commercially available portable instruments for illicit drug identification, there remains questions regarding which portable technologies are best and what are the best practices for their implementation in the field.

This research evaluated seven different field portable methods for the analysis of 38 adjudicated seized illicit drug samples. The 38 samples represented a range of illicit drug products, including, but not limited to, cocaine, heroin, methamphetamine, MDMA, PCP, and NPSs. The technologies included in this study included: (1) field color tests, (2) a portable Fourier Transform Infrared (FTIR) spectrometer, (3) a hand-held Raman spectrometer equipped with a 1,064nm laser, (4) a hand-held Near Infrared (NIR) spectrometer; (5) a portable Gas Chromatograph/Mass Spectrometer (GC/MS) equipped with a quadrupole mass analyzer, (6) a portable GC/MS equipped with an ion-trap mass analyzer, and (7) a portable High Pressure Mass Spectrometer (HPMS). Samples were also analyzed using traditional laboratory benchtop GC/MS and FTIR instrumentation for comparison. In agreement with the work by Lanzarotta et al., this research concluded that a toolbox or "toolkit" approach is best when working with field portable instrumentation.[1] Lanzarotta et al. proposed using a three-instrument toolkit for detection and identification of the Active Pharmaceutical Ingredients (APIs) in illicit medications and foreign unapproved pharmaceutical products. With their three-instrument toolkit, consisting of a portable FTIR spectrometer, a hand-held Raman spectrometer equipped with a 1,064nm laser, and a portable Direct Analysis in Real-Time Mass Spectrometer (DART-MS), 81 of 82 (92.0 %) of the APIs were detected by at least one technique. The current research demonstrated the reliable detection and identification of both bulk and trace components of seized illicit drugs when at least two field deployable devices are employed. Since multiple instruments can be used in combination to achieve these results, a "toolkit" approach is recommended, which provides flexibility for use by agencies with a range of resources and is consistent with recommendations by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) and *ASTM E2329: Standard Practice for the Identification of Seized Drugs*.[2,3]

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Drug Analysis; Scene Investigation; Seized Drugs

B181 Optimizing Extraction Methods for Cannabinoid Quantitation in Cannabis Gummies: A Comparative Study Using LC-PDA

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Learning Objectives: Attendees will gain insight into the challenges and solutions associated with extracting and quantifying cannabinoids, specifically delta-9-Tetrahydrocannabinol (Δ9-THC) and Cannabidiol (CBD), in cannabis gummies.

Impact Statement: This presentation impacts the forensic community by providing a thorough evaluation of diverse extraction methods for cannabis gummy products for cannabinoid quantification. By optimized protocols compatible with Liquid Chromatography-Photodiode Array (LC-PDA), this study supports forensic laboratories in adapting to the evolving legal requirements and improving their analytical capabilities, thereby enhancing the reliability of forensic evidence related to hemp and cannabis products.

Abstract Text: The Agriculture Improvement Act of 2018 (Farm Bill) legalized hemp plants containing 0.3% or less Δ9-THC as hemp, removing them from the list of controlled substances. The change of legal landscape necessitates that forensic laboratories shift from just qualitative to quantitative cannabinoid analysis. Various methods have been proposed for this purpose, but the diverse range of sample matrices in gummy products complicates the extraction of the regulated substance Δ9-THC. Gas Chromatography/Mass Spectrometry (GC/MS) and LC-PDA are widely used techniques for cannabis analysis. However, the extraction process for GC/MS is typically lengthy and involves multiple steps to prevent matrix-related issues like syringe clogging or column damage. LC-PDA, on the other hand, may be more compatible with simpler extraction protocols, which are preferable in forensic laboratories.

This study offers a comprehensive comparison of various extraction methods and the factors influencing cannabinoid extraction for the efficient quantitation of Δ9-THC and CBD in gummies using liquid chromatography coupled with a LC-PDA. More than ten extraction methods were evaluated, incorporating various solvent combinations such as two types of basic water (NaOH or NH4OH), acetonitrile, basic acetonitrile, methanol, basic methanol, and hexane. Additionally, enzymatic methods (lipase and amylase) and the use of QuEChERS and lipid filtration were also compared. The study also examined the effects of incubation temperature and duration on extraction efficiency. A simple combination of water and basic acetonitrile provided optimal recovery. Although lipid filter and QuEChERS might slightly increase extraction efficiency, the effects were not significant. The incubation temperature and duration also impacted Δ9-THC and CBD quantitation.

Gummies, being very sticky and hard to grind mechanically, often require cryo-milling, which is effective but limits sample throughput and is not accessible to most crime labs. Sampling a single gummy may result in biased results due to potential variations in cannabinoid content, especially in homemade THC gummies. Therefore, the sampling procedure was assessed to address sample homogenization issues. Overall, this study provides insights into optimizing extraction protocols for accurate cannabinoid quantitation in edible gummy products, meeting the needs of forensic laboratories, and ensuring compliance with regulatory standards.

Cannabinoids; Gummies; Extraction

B182 The Presumptive Identification of Psilocybin and Psilocyn in Mushrooms and Food Products Using Matrix-Assisted Ionization Vacuum (MAIV) and Time-of-Flight/Mass Spectrometry (TOF/MS)

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Learning Objectives: The information in this presentation discusses the challenges of analyzing psilocybin and psilocyn in mushrooms and food products. Attendees will learn how MAIV can be used with TOF/MS to screen for both compounds under conditions that will not initiate conversion.

Impact Statement: This presentation will impact the forensic science community by providing a fast and efficient instrumental screening technique for both psilocybin and psilocyn in mushroom material as well as edible products.

Abstract Text: Psilocybin is readily dephosphorylated to psilocyn under various conditions, such as heating and hydrolysis in solution.[1,2] Thus, the identification of psilocybin can be difficult and time-consuming. Further complicating this is the complex matrix of edible products available today that contain one or both hallucinogenic compounds. Psilocybin identification may be accomplished by Liquid Chromatography/Mass spectrometry (LC/MS) or Gas Chromatography/Mass Spectrometry (GC/MS) with derivatization. If these methods are not available, determining whether psilocybin was present before analysis or if it was converted to psilocyn during analysis is difficult.

The Virginia Department of Forensic Science (VA-DFS) uses Direct Analysis in Real Time-Time of Flight Mass Spectrometry (DART-TOF/MS) for presumptive screening of general unknown samples and for confirmation of pharmaceutical preparations.[3-5] The DART ionization source is paired with a TOF/MS to measure each ion's exact mass.[4] Function switching mode on the DART-TOF utilizes an in-source collision induced dissociation (CID) to switch the orifice 1 voltages between 20, 30, 60, and 90V.[6,7] Under these conditions psilocybin is converted into psilocyn. This study focuses on the use of 3-nitrobenzonitrile (3-NBN) crystals instead of the DART ionization source to facilitate matrix assisted ionization vacuum (MAIV).[7-9] This matrix ionizes the sample while keeping the psilocybin intact.

Samples are prepared by a solvent extraction and combined with a 3-NBN solution. This is dried with air to create a crystallized 3-NBN:sample matrix. The DART is turned off to remove the instrumental ionization source. The crystallized 3-NBN:sample matrix is scraped from the test tube using a spatula which is then placed in front of orifice 1 of the TOF-MS. Various orifice 1 temperatures and 3-NBN solutions were evaluated for psilocybin and psilocyn ionization. In addition, the occurrence of dephosphorylation was evaluated. The ideal conditions were found to be 60˚C and a 3-NBN solution made with DI water:ACN with 0.1% formic acid, respectively. The developed method showed 77.8% rate of ionization for psilocybin and 2.8% rate of psilocybin to psilocyn conversion.

The 3-NBN is the ideal matrix to facilitate MAIV due to its low thermal energy requirement to produce ions from atmospheric pressure.[7] Further testing is required to fully optimize and streamline the Matrix-Assisted Ionization Vacuum-Time of Flight (MAIV-TOF)for psilocybin and psilocyn screening and to understand how 3-NBN is facilitating the ionization at low temperatures. This method has the potential to be applied to other compounds relevant to drug chemistry, especially those with thermal instability. The implementation of this screening method will provide verification of psilocybin and/or psilocyn's presence in food products prior to dephosphorylation during routine analysis.

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Mass Spectrometry; Drug Analysis; Hallucinogens

B183 A Green Method for Seized Drug Analysis Using a Portable Liquid Chromatograph

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Learning Objectives: After attending this presentation, attendees will understand how seized drug analysis may be performed using a portable Liquid Chromatograph (LC) employing dual capillaries with dual wavelength detection, and how it can provide a greener and relatively low-cost method for seized drug analysis.

Impact Statement: The use of a portable LC, as presented in this presentation, will impact the forensic science community by providing a green and relatively low-cost option for seized drug analysis, all the while likely adhering to the guidelines outlined by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG).

Abstract Text: Like other chromatographic methods, the portable LC described in this presentation is amenable to mixtures in contrast to other portable instrumentation, such as Raman and Fourier transform infrared spectroscopy. The portable LC can either be kept in a laboratory setting or brought into the field. Unlike traditional, full-sized chromatographs, this relatively inexpensive, small-footprint LC only weighs 17.6 pounds, uses a fraction of the materials and solvents normally used, and generates considerably less waste. These features would be of interest to developing countries and jurisdictions with limited resources and present for these entities a possible low-cost alternative to gas chromatography Mass Spectrometry (MS) and LC/MS instrumentation. This study employed a 3µm, 0.15 × 100mm capillary column in series with a 3µm, 0.15 × 50mm biphenyl capillary column with dual on-column Ultraviolet Light Emitting Diode (UV LED) detection at either 235nm and 275nm or 255nm and 275nm using acetonitrile as the organic modifier and trifluoracetic acid as mobile phase additive. For representative compounds from different drug classes, including stimulants, depressants, opioids, opiates, hallucinogens, anabolic steroids, cannabinoids, synthetic cannabinoids, synthetic cathinones and phenethylamine analogs, UV LED detection at 255nm and 275nm was found to be more universal and was utilized for further investigation. Analytes of interest were identified based on relative retention times for both capillaries and UV absorbance ratios obtained from dual UV LED detection.1

The proposed methodology could satisfy SWGDRUG requirements which, in the absence of a category A method of analysis, allow for two category B methods and a category C method of analysis.[2] The use of dual capillaries with different stationary phases (C8 and phenyl) would exploit different chemical or physical properties of the analytes and thus qualify as two category B techniques. In addition, dual wavelength detection, which imparts selectivity through general or class information, would appear to qualify as a category C technique.

The LC instrumentation presented for seized drug analysis is applicable to both field and laboratory use, being lightweight, portable, green, and economical. The dual capillary on-column UV detection could satisfy SWGDRUG requirements, which would be particularly useful for developing countries.

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Seized Drugs; Liquid Chromatography; Green Chemistry

B184 Drug Detection in Complex Materials Using Confocal Raman Microspectroscopy

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Learning Objectives: Attendees will review current difficulties associated with seized drug analysis, which includes analysis of counterfeit drugs. The application of Confocal Raman Microspectroscopy (CRM) in the forensic field is examined to address the current difficulties experienced with illicit drug detection.

Impact Statement: Law enforcement currently relies upon scientific techniques for illicit drug analysis, which have some challenges that hinder the detection of some drugs. This presentation will look at the use of CRM as a possible type of analytical technique that is not presently used widely in forensic analysis, which includes the detection of illicit drugs.

Abstract Text: Counterfeit drugs and food items are a global problem, and the World Health Organization estimates that roughly 10% of medical products in low- and middle-income countries are substandard or falsified.[1] Often, counterfeit drugs contain unknown substances that are difficult to detect without destroying the sample. Moreover, in 2023, over 80 million fentanyl-laced fake pills were seized in the United States, according to the Drug Enforcement Administration, making the analysis of counterfeit drugs an urgent topic.[2]

Here, we report on the use of CRM to identify drugs such as counterfeit pills. CRM is a powerful analytical tool that provides information about the molecular composition of various samples. CRM works by giving a microscopic image where scientists can pinpoint specific areas to measure the molecular structure of compounds. This is a useful tool to microscopically examine mixtures and chemically analyze the different components. It offers non-destructive analysis at the molecular scale that can be automated, making it valuable for counterfeit pills or other mixtures received for forensic analysis because it maintains the integrity of the evidence for future testing if needed. CRM also reduces the scientist's interaction with hazardous samples, such as fentanyl.

Our research, conducted in collaboration with the Ada County Forensic Lab, leverages CRM to address the detection challenges posed by complex matrices in counterfeit pills and food items. Our research improves the methodologies for detecting different mixtures of illicit drugs that are difficult to analyze using standard techniques. One of the most common methods in seized drug detection is sample extraction followed by analysis using Gas Chromatography/Mass Spectroscopy (GC/MS). Various drugs and matrices have shown to be challenging to detect the illicit substance using GC/MS, which leads to possible incorrect reporting of no controlled substance detected. By integrating CRM into routine forensic workflows, we enhance the capabilities of law enforcement agencies and forensic scientists. Our findings underscore the importance of innovative technologies in forensic science, contributing to more effective drug enforcement through a chemical analysis that has not been typically used in forensics.

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Drug Analysis; Raman Microscopy; Raman Spectroscopy

B185 The Identification of Isobutane Hash OIL (IBHO), an Uncommon Cannabis Product Seized for the First Time by the Brazilian Federal Police

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WITHDRAWN

B186 Back to the Future: Applying Decades-Old Probabilistic Models to Today's DNA Results When Activities Are of Interest

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Learning Objectives: After attending this presentation, attendees will understand that there is a robust, foundational, probabilistic framework for the evaluation of biological results given activity level propositions.

Impact Statement: This presentation will impact the forensic science community by explaining this probabilistic model for assigning the value of evidence to biological findings given each of the three different categories of activity level proposition pairs (i.e., no alternative activity, different alternative activity with the same person of interest, same alternative activity with an unknown person) and typical case scenarios encountered by DNA analysts.

Abstract Text: Evaluating scientific results given activity level propositions is nothing new. A robust probabilistic framework exists for this evaluation, with the original model published in the *Journal of the Royal Statistical Society* over 40 years ago.[1] This model introduced the concept of material being transferred during an alleged activity and the concept of material being present by chance as part of background noise, both of which are model parameters required for a probabilistic evaluation of the results given activity level propositions. This model provides a foundational basis for the evaluation of findings given activity level propositions, regardless of the forensic science discipline these findings fall into.

We have taken this foundational basis and adapted it to the evaluation of the DNA typing results seen today to create a forensic biology specific framework. This model has been validated for the presence of DNA showing the same alleles as the person of interest's profile, alleles different from the person of interest's profile, and the absence of DNA. In addition, it includes the case's specific circumstances, such as cohabitation of a defendant and complainant, which impact the evaluation of the results. The translation of this framework into Bayesian networks for each of the three categories of proposition pairs (i.e., no alternative activity, different alternative activity with the same person of interest, same alternative activity with an unknown person) and specific case circumstances is straightforward and provides us with the fundamental Bayesian network structures for each type of proposition pair and case scenario. The underlying mathematical equations for the value of evidence highlight which parameters are the most crucial for establishing the order of magnitude of the value of evidence.

This presentation provides valuable insight into a way forward toward a robust, structured, fundamental, and overarching model for the evaluation of DNA results given activity level propositions.

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Activity Level; Likelihood Ratio; Bayesian Network

B187 The Construction of a Microhaplotype Panel for Complex Mixture Deconvolution Using Next-Gen Sequencing

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Learning Objectives: By attending this presentation, attendees will learn about a novel microhaplotype panel in development specifically for analysis of complex mixtures. Attendees will learn about locus selection, sequencing platform agnostic assay development, and overall assay sensitivity to minor contributor detection.

Impact Statement: This presentation will impact the forensic science community by demonstrating that a microhaplotype panel, specifically constructed for the analysis of complex mixtures, can complement Short Tandem Repeats (STRs) when inconclusive results are obtained. Overall, this presentation will further demonstrate the usefulness of implementing current sequencing technologies to improve DNA typing results.

Abstract Text: Microhaplotypes (MHs) are short DNA regions (< 300 nucleotides) containing two or more Single Nucleotide Polymorphisms (SNPs) with multiple allelic combinations.[1] Current sequencing technologies reveal that MHs offer significant advantages over STRs in analyzing complex and imbalanced mixtures. Key benefits include the absence of stutter artifacts and uniform allele size, which makes preferential amplification of smaller alleles a non-issue.[2] Moreover, loci with a high effective number of alleles (Ae) also provide substantial ancestry information (In), much more so than conventional forensic STRs, potentially allowing biogeographical ancestry inference even for minor contributors to a DNA mixture.[3]

Large genome sequencing projects, including, but not limited to, 1000 Genomes, Genomes Asia, and gnomAD, have provided databases of whole genome sequence information. These resources have allowed researchers to explore SNP variation across populations using advancements in bioinformatic tools and identify numerous MH loci with an Ae comparable to, or exceeding, forensic STRs.[4] The comprehensive repository of MH loci, MicrohapDB, includes over 3,000 loci detailing marker locations, Ae, In, and specific allele-defining SNPs5. Aiming to develop a sequencing-platform agnostic multiplex MH assay for complex mixtures, 43 MHs were selected for primer design with the Ampliseq system. Using MicrohapDB, loci were sorted based on their Ae focusing on markers with lengths less than 270 base pairs and an Ae > 5. This narrowed-down pool was further assessed based on the following set of parameters: ranges and standard deviation of Ae values from the different populations, presence and frequency of indels, the occurrence of homopolymers longer than five nucleotides, and the BLAST search results for each marker. Initially, approximately 250 markers were identified, some of which had been included in a panel by Bode Technology and in the Kidd 24-locus panel. Markers that demonstrated a high Ae value, but were too long, were evaluated for length reduction and Ae values were re-calculated. Any marker that showed a significant reduction in allelic diversity was further eliminated.

Ten individuals from diverse ancestries were chosen from an Institutional Review Board (IRB) -approved GW collection. Various mixtures, ranging from two to five contributors with different ratios, were sequenced on a Gene Studio S5. Sequence alignment was conducted with the Torrent Suite Server by TMAP to produce mapped bam files. The bam files were then processed with the JAVA-based application mh.jar, available from Thermo Fisher Scientific, which generates allele calls based on relative coverage. Although primer concentration can be optimized, the preliminary data shows a read coverage ratio of sister alleles, averaged across loci and samples, of 77%, with random match probabilities less than one in 10^50 for single-source samples. The sensitivity to detecting minor contributors is equal or greater to that of STRs, detecting 58% and 43% of unique minor contributor alleles in a 1:40 and 1:60 mixture, respectively. The multiplex is also being tested on the MiSeq sequencing platform and next steps include: (1) generating allele frequencies both empirically by testing ~250 GW samples from individuals across the major United States populations and *in silico* by utilizing data from large sequencing projects such as 1000 Genomes and gnomAD, (2) optimizing inter-locus balance by adjusting primer concentrations, and (3) adapting probabilistic genotyping tools to incorporate MH data for mixture deconvolution and analysis.

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Biomarkers; Next Generation Sequencing; Low DNA

B188 DNA Evidence Analysis Using the Same Number of Contributors for Prosecution and Defense Will Often Frame a Non-Contributing Suspect

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Learning Objectives: Prosecution and defense are each entitled to choose whatever hypothesis they prefer. In particular, a prosecution DNA mixture analyst cannot assume that the best choice of number of contributors for the prosecution will also be fair for the defense.

Impact Statement: I hope the audience will come to a better understanding that "number of contributors" in mixture analysis is not a fixed number like "ground truth," but rather is dependent on context and hypothesis.

Abstract Text: If there is a low-level contributor who shares some alleles with a non-contributing suspect S, there is a lively chance to frame the suspect. ("Frame" here means reporting that the evidence is against S when a correct understanding of the data would be in S's favor.) The error consists in failing to realize that the prosecutor's choice of number of mixture contributors must not be foisted on the defense.

Suppose DNA mixture evidence for a particular case is analyzed using a leading edge, fully continuous, a.k.a. "probabilistic," model program. For this case, we have an electropherogram that includes some low-level signals, signals not much above a standard rfu detection threshold. It is not particularly unusual for about half of suspect's genotype to overlap with alleles of the epg's.

Then we have two possibilities for the unseen suspect alleles. It may be that the suspect is a contributor and the remaining but missing suspect alleles dropped out (whether visible below threshold or completely absent). That's the prosecution's hypothesis, Hp. Or it may be that the suspect is a non-contributor, maybe wrongly arrested, and the alleles that are tempting evidence against suspect are purely coincidence—defense's hypothesis Hd.

Then it is common that prosecution and defense should not agree on number of contributors to assume for analysis. Instead, it is likely that the phantom contribution that S comprises under Hp, disappears as a contributor under Hd leaving a contributor count one fewer. (The discussion of discrepant numbers of contributors by [Taylor et al] is similar.)[1] The simple way to assign the appropriate choice of number of contributors for each of Hp and Hd is to accept, for each, the number which maximizes the likelihood, independently, under each H. For further simplicity just to explain the idea, let's suppose that the mixture consists of the alleles/peaks that cover half of the suspect's genotype, plus one more peak, and there is not much peak height variation. Then under Hp we need at least two contributors—the suspect, plus an "unknown" second contributor (and a lot of allelic stacking) to cover the extra allele. But under Hd it is easy to imagine that one contributor is enough. Further, the likelihood of one random genotype covering that contributor is obviously much more than the likelihood of two contributors covering the same set of peaks and staying within bounds as well. Hence, it is quite intuitive that, when about half of the suspect's genotype is observed in a mixture, the appropriate number of contributors to assume for likelihood ratio calculation is one fewer contributor for Hd. Moreover, it is common that wrongly applying the prosecution's larger number of contributors to the defense's calculation results in evidence against suspect S, while correctly allowing each of Hp and Hd its own preference results in evaporating away any evidence against suspect S.

There are additional analysis improvements for a future presentation. One is, given the assumption that S did *not* contribute, to the extent that we can find a legitimate way to take advantage of sub-threshold data, expect not merely neutral but positive support of the innocence of suspect S.

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Forensic DNA; Mixture Analysis; Justice

B189 The Development of Universal STRmix Parameters for Legacy Kits

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Learning Objectives: Attendees will learn about community-sourced Probabilistic Genotyping (PG) parameters for legacy Short Tandem Repeat (STR) kits. The purpose of these parameters is to allow for the application of modern PG software, specifically for laboratories that have adopted STRmix, to old cases that require interpretation due to ongoing investigation, Forensic Investigative Genetic Genealogy (FIGG) results, or other post-conviction activities.

Impact Statement: This presentation will impact the forensic DNA community by presenting parameters that will allow legacy samples to be interpreted using updated methods. This can have an impact on the outcome of on-going court activities by allowing for modern methods to be applied to old cases. The use of a fully continuous likelihood ratio can take advantage of all the information in a sample and may provide exculpatory weights of evidence that are not possible to obtain using Combined Probability of Inclusion (CPI) and Random Match Probability (RMP) methods.

Abstract Text: PG is quickly approaching ubiquity in the United States forensic DNA community. The most commonly used PG software in the United States is STRmix. As of the beginning of 2021, over 50% of certified laboratories were using this software with another 30% moving toward implementation. Each laboratory does their own independent validation prior to use to generate required parameters from known single source samples, which then get applied to unknown case work samples. In casework, the stochastic modelling parameters and evidence profiles generate weightings for allowable genotypes.

There is a disconnect from current best practices involving PG software when data using legacy STR kits is evaluated. Old cases are revisited from time to time, such as an unsolved case that has recently generated a suspect, or post-conviction activities as part of an ongoing appellate process. When this happens, laboratories essentially roll the clock back to the status of mixture interpretation and statistical analysis to that of the time the analysis was performed. This means that modern PG analysis is not used for STRmix laboratories. Laboratories are limited to the use of CPI or RMP, which have limited utility at best, and may be considered to having been inappropriately calculated by some critics at worst. One of the most striking differences between the CPI and RMP calculations compared to STRmix is that the legacy methods cannot return a numerical weight in favor of the defense.

This concept of only using what was available at the time of the original analysis contrasts with many other areas of science, such as medicine and engineering. Newer tools, such as cancer-fighting drugs and powerful mathematical software, are used on cases that may pre-date the current need for additional attention by decades. This is generally considered beneficial, whether for someone diagnosed with cancer in their 20s whose illness has returned in retirement or for stress analysis of a crumbling bridge or building constructed long ago.

A recent paper has been published that shows STRmix is remarkably tolerant of a wide range of stochastic parameter (stutter ratios and variance parameters).[1] In this study, eight laboratories provided validated stutter data and variance parameters along with 20 mixtures of 2- to 4-contributors. This included both GlobalFiler and Investigator 24plex laboratories, and both 28- and 29-cycle PCR amplifications for each kit. The results of this paper suggest that STRmix should perform well using community-derived universal parameters for older kits, such as Profiler/Cofiler, Identifiler, PowerPlex 16, and others.

This presentation will present a grassroots, community-driven set of parameters suitable for use with legacy STR kits for STRmix. One of the challenges of creating parameters for these older kits is that any one individual laboratory may not have a sufficient set of samples to generate the needed allele specific stutter models or the 100 samples of varying quality to generate their own variance parameters. However, as these are to be considered universal parameters, collecting a few samples from many labs can both generate the samples needed and ensure that the parameters developed consider a wide range of laboratory data. These parameters will be publicly available and shared without constraint. It is expected that internal testing will need to be conducted by any laboratory that may be interested in using these parameters on legacy kits.

Reference:

1. S. Boodoosingh, H. Kelly, J.M. Curran, T. Kalafut, An inter-laboratory comparison of probabilistic genotyping parameters and evaluation of performance on DNA mixtures from different laboratories, *Forensic Sci. Int. Genet.* 71 (2024) 103046. https://doi.org/10.1016/j.fsigen.2024.103046.

Probabilistic Genotyping; Interpretation; Legacy Data

B190 Touch/Trace DNA in Activity-Level Propositions: Anatomy of a DNA Transfer

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Learning Objectives: In this presentation, we will describe a quantitative evaluation of DNA transfer at a crime scene supported by our extensive experimental touch DNA data. Attendees will learn about the vagaries of primary vs. secondary DNA transfer events, DNA recovery, and how they impact activity-level questions at a crime scene.

Impact Statement: The impact to the forensic science community is rooted in the empirical evaluation of the fundamental events of DNA transfer and recovery and the metrics we can use to describe them. This expression of transfer events could contribute to a Bayesian evaluation of a crime scene, adding value to the forensic scientists' interpretation to assist the trier of fact.

Abstract Text: Consider this scenario: your DNA is found on a spent bullet casing at a death scene. The prosecution presents this as evidence that you deposited your DNA as you loaded the gun and committed the crime. Your defense, however, argues that you shook hands with the actual perpetrator earlier in the day, and they transferred your DNA to the casing as they loaded the gun and did the shooting. Each of the scenarios is possible; your DNA could be transferred along both pathways. The critical question becomes not "whose DNA is it," but, rather "how did the DNA get there?"

Evaluations of the evidence given the donor's activities inform such activity-level propositions; such assessments of alternate scenarios can provide tremendous value when they are backed by empirical data.[1] The forensic scientist is uniquely qualified to interpret data to inform activity-level questions and present them to the trier-of-fact. However, there is a dearth of empirical data available in the literature.

The goal of this project was to generate empirical data in support of activity-level evidence interpretation. We asked the question: Can we tell a difference between primary and secondary transfers using DNA recovery values? To address the question, we used a well-established protocol—the domesticated fingerprint, a ground truth sample containing a known quantity of DNA.[2] We mocked three crime scenes, tracking DNA transfer through a primary versus a secondary pathway. Using the domesticated fingerprint and its transfer device, the domesticated hand, we transferred DNA across pathways that involved three commonly occurring surfaces in criminal casework—a subway pole, a drug baggie, and a knife. Samples were collected from each surface and from the domesticated hand. Quantifying the DNA recovered from each surface allowed us to track DNA across the pathway and compare the results of primary and secondary transfers.

By repeating each pathway 20 times independently, we were able to generate mean recovery values, measure the standard deviation, and run an analysis of variance. We found that the difference in recovery after a primary vs. secondary transfer pathway is statistically significant difference for all three crime scenes. Therefore, we suggest that assessments of alternate scenarios provide tremendous value when they are backed by empirical data and may aid the trier-of-fact in a determination of which scenario is most probable.

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DNA; Activity Level; Touch/Trace

B191 Evaluating the Consistency of a DNA Mixture Interpretation Protocol

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Learning Objectives: After this presentation, attendees will understand the requirements of a DNA mixture interpretation protocol verification as defined by Standard 4.4 of American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 020, Standard for Validation Studies of DNA Mixtures and Development and Verification of a Laboratory's Mixture Interpretation Protocol. Attendees will learn how to design and administer a mixture interpretation protocol verification that demonstrates the consistency and reliability of the laboratory's procedures for interpretation of DNA mixtures.

Impact Statement: This presentation will encourage other forensic DNA testing laboratories to evaluate the effectiveness of their procedures for DNA mixture interpretation by performing a protocol verification. The protocol verification discussed in the presentation will be offered as a template for other laboratories interested in strengthening the quality of their DNA interpretation procedures by measuring consistency and reliability in a controlled study. The results from the most challenging samples will demonstrate for the forensic DNA testing community that protocol verification can illuminate gaps in an existing or new DNA interpretation procedure, thereby offering opportunities for improvement.

Abstract Text: To comply with ANSI/ASB Standard 020, Standard for Validation Studies of DNA Mixtures and Development and Verification of a Laboratory's Mixture Interpretation Protocol, a laboratory must perform a mixture interpretation protocol verification to demonstrate consistency and reliability in its conclusions.[1] In May 2023, the Harris County Institute of Forensic Sciences revised the laboratory's DNA interpretation procedure and later that same year endeavored to evaluate the performance of the procedure and its application by performing a protocol verification planned to coincide with the training program for a newly implemented capillary electrophoresis platform.

A wide range of DNA samples was prepared from previously extracted saliva samples of nine known donors. The breadth of samples, as required by Standard 020, was intended to reflect the range of DNA mixtures routinely encountered in casework, with consideration given to Number Of Contributor (NOC) range, mixture ratios, DNA template amounts, and donor combinations. DNA mixtures with more than one acceptable interpretation were intentionally included in the study to challenge the participants with genuinely ambiguous data. In total, 21 DNA samples were prepared and administered in a blind format to 19 qualified DNA Analysts and two trainees. Participants in the study were provided with three references for comparisons against the 21 test samples. Comparison results were expressed as likelihood ratios or visual exclusions. A total of 441 NOC interpretations were made in the protocol verification, and 436 of 441 (98.9%) were deemed to be within the predefined acceptable range of variation for each sample. Four of the five interpretation errors were attributed to a misreading of peak height imbalance in the stochastic range for the Polymerase Chain Reaction (PCR) typing kit and detection platform. Considering ground truth NOC, 73 of 441 NOC interpretations (16.6%) did not agree with the true NOC by addition or subtraction of one contributor. Fifty-seven interpretations were N-1 relative to the true NOC, while 17 interpretations were N+1 relative to the true NOC. Conclusions omitting one true donor were considered acceptable in select samples where alleles from trace-level contributors failed to amplify or were masked by higher-template contributors. Insertion of a phantom contributor was deemed acceptable in select samples if multiple peak height imbalances or spuriously elevated stutter gave justification for an additional contributor.

Participants evaluated a total of 63 comparisons in the study. Fifty-eight of 63 (92.1%) comparisons provided likelihood ratio support or visual exclusions concordant with the ground truth. Among comparisons discordant with ground truth, four provided false exclusionary support and one false support for inclusion. In each case, the relevant samples were amplified at suboptimal DNA template less than 0.5ng. The incidence of false exclusionary results was driven by the incorporation of trace-level contributors in several test samples. The laboratory's own internal validation and published studies predict this risk.[2-4] The one result incorrectly supporting inclusion of a non-contributor was an instance of coincidental allele sharing. The magnitude of the average likelihood ratio (=9), however, appropriately weights the false inclusion within the "limited support" category of the Scientific Working Group on DNA Analysis Methods (SWGDAM) -recommended verbal scale.[5]

An unexpected outcome of the protocol verification allowed the laboratory to identify an opportunity to improve the interpretation of four-person mixtures. Three such DNA mixtures were included in the study, and each provoked inconsistent decisions on suitability for comparisons. Most participants deemed these DNA mixtures unsuitable for comparisons. The minority who decided that these complex DNA mixtures were suitable for comparisons obtained likelihood ratios that mostly supported ground truth. Based on these findings, the laboratory intends to supplement its internal validation with more complex mixtures and update the DNA interpretation protocol to promote consistency with this sample type.

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Protocol Verification; Mixture Analysis; Probabilistic Genotyping

B192 A Perspective on More Than 30 Years of Forensic DNA Research and Technology Development

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Learning Objectives: After attending this presentation, attendees will understand the history of the development of methodology for DNA typing including Capillary Electrophoresis (CE), mini Short Tandem Repeats (miniSTRs), DNA extraction, Polymerase Chain Reaction (PCR) inhibition, body fluid analysis, and Rapid DNA. Projections for future technology such as body fluid detection and microbial forensics will also be discussed.

Impact Statement: This presentation will assist the forensic science community in understanding the background research involved in the development of current methodology for forensic DNA typing. The presentation will discuss history as well as project future technology as well as technology transfer from research to validation to implementation.

Abstract Text: This presentation will discuss the various approaches taken by the McCord research group in developing methods for genetic typing and body fluid analysis from 1989 to the present, a span of 35 years. Among the items covered will be the initial development of CE and STRs for use in forensic DNA typing. Here, the author will discuss the transition from gel electrophoresis to CE and key factors such as the optimization of soluble polymer buffers. Experiments to determine the interesting effects of electrokinetic injection on DNA separations in which slight changes in conductivity affect signal intensity and resolution will also be discussed. Other topics will include the application of CE-based analysis for mixture determination and how signal-to-noise differences and variations in PCR efficiency can affect overall results.[1,2]

A second portion of the presentation will discuss experimental studies of PCR inhibition and DNA degradation. This includes early work on the application of real-time PCR for the detection of PCR inhibition and the development of miniSTRs. Here experimental data demonstrated the multiple ways inhibitors can alter peak intensity and peak dropout.[3] Portions of this work led to interesting experiments involving the development of miniSTRS and microfluidic systems for DNA typing.[4] This work demonstrated the improvement in recovery and inhibition resistance when CE and PCR are used with smaller amplicons. An interesting side result was that smaller amplicons also permitted the development of ultra-high-speed PCR, ultimately resulting in devices capable of multiplex PCR amplifications as fast as seven minutes with 80-second genotypes.[5]

The presentation will end with a discussion of non-traditional applications for forensic DNA analysis, including epigenetic body fluid detection with sensitivity and specificity that is fully compatible with STR typing.[6] DNA methylation can also be used for phenotyping, and we have examined its use for age determination and smoking status.[7] More recently, our laboratory has been examining microbial DNA. Here we show how microbial transfer can be used to detect sexual assault and also to examine contamination of agricultural produce.[8,9] The discussion will end with the author's comments on technology transfer and the potential topics for future research in forensic analysis.

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Forensic DNA; Body Fluid Analysis; Microbial Forensics

B193 From Planning to Practice: Navigating the Implementation of Rapid DNA Technology and Tracking Success Metrics

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Learning Objectives: Attendees will: (1) gain detailed knowledge of the four key stages of implementing Rapid DNA technology, from pre-purchasing concerns to pre-implementation considerations; (2) understand the details and resources available to cover the tasks outlined in each of the key stages; and (3) learn how to utilize the implementation checklist and metric tracker to ensure a thorough and successful integration of Rapid DNA technology within their agencies.

Impact Statement: This presentation will provide a comprehensive roadmap for law enforcement agencies considering or in the process of integrating Rapid DNA technology. Attendees will gain critical awareness into the implementation process from procurement to practical application and will be equipped with two valuable tools: an Interactive Implementation Checklist and a Metric Success Tracker. These resources will enable agencies to effectively manage and develop their Rapid DNA programs, ensuring robust and successful integration.

Abstract Text: In this presentation, attendees will gain insights into a comprehensive approach that has been utilized by law enforcement agencies currently integrating Rapid DNA technology. Experiences from 18 months of supporting multiple Byrne Discretionary Grants Program grantees adopting Rapid technology, the presenter will expand upon several critical considerations for implementation—from procurement to practical application of the Rapid DNA technology.

Rapid DNA technology is gaining prominence among law enforcement agencies across the United States. Agencies may choose to implement it for various reasons, including disaster victim identification, booking stations, and crime scene analysis. While current regulations restrict entry of forensic samples into the Federal Bureau of Investigation's (FBI's) Combined DNA Index System (CODIS), some jurisdictions have developed or utilize local databases for these types of samples. Additionally, the Scientific Working Group on DNA Analysis Methods (SWGDAM) is developing new standards for forensic sample data generated from a Rapid DNA instrument to be uploaded into CODIS, which are anticipated to be in place by mid-2025. Agencies must carefully consider the steps required to implement Rapid DNA technology, and this presentation will provide a roadmap for planning accordingly.

Specifically, the implementation process is separated into four key stages: Prior to Instrument Procurement, Prior to Receiving Instrumentation, After Receiving Instrumentation, and Prior to Implementation. Pre-procurement will cover key considerations such as establishing partnerships and consultants and determining fit-for-purpose given agency needs. Pre-receiving will cover key considerations such as security, staffing, and training. Post-receiving will cover strategies for Quality Assurance/Quality Control, sample types, and validation. Last, Pre-Implementation will cover approaches associated with sample assessment and acceptance, comparisons, reporting, and metrics tracking.

As part of the presentation, attendees will be given a preview and have access to two downloadable tools at no cost that have been met with a positive response:

1. Interactive Implementation Checklist: This comprehensive checklist covers all stages and aspects of the implementation process. The tool offers interactive features with key information and associated resources that will allow for a thorough and robust implementation process.
2. Metric Success Tracker: An Excel-based metrics tracker that agencies can customize to monitor the success of rapid DNA technology after implementation. Tracking of critical information will allow agencies to effectively manage or develop their program.

Attendees can bring both tools back to their agency for practical application and sharing.

Rapid DNA; Forensic Investigation; Forensic DNA

B194 A Sensitivity Evaluation of the Applied Biosystems RapidHIT ID System v2.0 and RapidINTEL Plus Sample Cartridge

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Learning Objectives: This presentation provides a better understanding of the sensitivity and performance of the newly released RapidHIT ID system v2.0 and RapidINTEL Plus sample cartridge via experiment and analysis

Impact Statement: By providing better understanding of the performance and sensitivity limitation of RapidHIT ID system v2.0 and RapidINTEL Plus sample cartridge, this presentation demonstrates the potential implementation of this system in casework, thus eliminating the lengthy process in obtaining Short Tandem Repeat (STR) profiles and alleviating the backlog issue in many laboratories.

Abstract Text: The RapidHIT ID is a fully automated system that is designed to perform cell lysis, DNA isolation, Polymerase Chain Reaction (PCR) amplification, electrophoretic separation, and data analysis from saliva or blood sample swabs.[1-4] The RapidHIT ID systems require less than one minute of hands-on preparation and 90 minutes of processing time.[1,2,5-8] Currently, the most recent RapidHIT ID firmware released by the manufacturer is the RapidHIT ID System v2.0, which works in conjunction with the new RapidINTEL Plus Sample Cartridge, the RapidLINK Software v2.0, and utilizes GeneMapper ID-XR Software for profile analysis.[5-8] Samples processed with this cartridge and the accompanying upgraded software are automatically analyzed utilizing validated locus-specific stochastic thresholds, which are much lower than those previously recommended.[5] Additionally, the RapidHIT ID System v2.0 has a minimum peak height ratio of 0.2 or 0.4 (depending on the locus), whereas the previous version used the minimum peak height ratio threshold of 0.4 across all autosomal markers. Furthermore, the RapidINTEL Plus sample cartridge is equipped with small and large quantification markers (QTS and QTL), which allows the estimation of the DNA quantity of the template DNA analyzed. Given the firmware, software, and cartridge updates associated with the recently released RapidHIT ID System v2.0, the new, more specific analytical settings, and the desire to use this platform for more complex evidence-type samples, it is critical that the sensitivity and other operational limitations be carefully evaluated. In this study, the range of detection and performance of the updated instrument and software were experimentally evaluated using buccal swab samples from four different donors with total DNA inputs of 5ng, 7ng, 10ng, 20ng, and 50ng. Each swab was processed using the RapidHIT ID and analyzed with the GeneMapper ID-XR Software, following the general protocol with lysis volume of 500uL. The resulting STR profiles were compared to the corresponding reference STR profiles to confirm accuracy and concordance. Each experimental concentration tested was compared to the values generated from the on-board quantification markers and the sensitivity of the platform determined.

Across the five tested DNA input groups, all STR profiles observed were fully concordant with the profile from the traditionally tested reference buccal swabs. Interestingly, the QTL quantification values indicated that only ~1.15% of the total input of each sample was metered for amplification. Accordingly, the average percentage of expected alleles detected for the DNA inputs of 5ng, 7ng, 10ng, 20ng, and 50ng were 59.41%, 72.78%, 86.11%, 98.17%, 100.00%, respectively. From all sample sets, the average peak heights of all DNA input groups were below the internally validated optimal peak height range used by the RapidLINK Software v2.0. However, the average intralocus heterozygous peak height ratio of the STR profiles across all testing inputs ranged from 0.67 to 0.79, which is above the minimum peak height ratio of 0.2 or 0.4 (marker-specific) recommended by the manufacturer.5

Overall, the results show that the updated system provides several improvements to the rapid DNA process, including valuable quantification data. However, with such a small volume of the DNA extract moved into amplification chamber of the cartridge, it will be important for examiners to carefully consider the specific nature of evidence items being tested. For very challenged DNA samples or for those expected to yield low template DNA, it may be beneficial to increase the amount of substrate loaded into the cartridge or use the specialized protocol that has a lower lysis volume (100ul). Future studies may include experimental evaluation of the sensitivity of the lower volume specialized protocol of RapidHIT ID System v2.0 when coupled with RapidINTEL Plus Sample Cartridge.

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Rapid DNA; STR Profiles; Backlogs

B195 An Assessment of the RapidHIT ID System V2.0 for Use on Evidentiary Samples and Its Suitability for Implementation Into Law Enforcement

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Learning Objectives: After attending this presentation, attendees will have a better understanding of how the Applied Biosystems RapidHIT ID System v2.0 is evolving Rapid DNA technology to be more suitable for law enforcement in terms of processing evidentiary samples and will understand the capabilities of this system compared to standard laboratory processing. Specifically, attendees will learn about the new RapidINTEL Plus chemistry and how to determine the correct protocol option based on sample type.

Impact Statement: This presentation will impact the forensic science community by providing insight on the potential benefits and limitations of the RapidHIT ID System v2.0 for processing crime scene samples and how implementation of this new system is facilitating the advancement of Rapid DNA technology to ensure that it is reliable, responsible, and appropriate for obtaining evidentiary DNA profiles to upload to DNA databases.

Abstract Text: The rise of Rapid DNA technology in the field of forensic science has been fueled by the desire for faster processing times of biological samples, the expansion of DNA typing to include non-traditional operators, and the ability to process samples from remote locations. As a fully automated "sample in-answer out" system that obtains a DNA profile in under 90 minutes, Rapid DNA has shown to be a viable option for obtaining arrestee profiles at booking stations and producing investigative leads. Currently, the only sample type approved for enrollment and/or search in the Combined DNA Index System (CODIS) following Rapid DNA processing is reference samples since crime scene evidence can vary in quality and quantity of DNA.[1] However, there has been significant interest from law enforcement to extend the capabilities of this technology to approve Rapid DNA analysis on evidentiary samples for database upload in addition to reference-type samples. In response, manufacturers have made efforts to improve the ability of Rapid DNA instruments to process more challenging samples. This study assessed the performance of the Applied Biosystems RapidHIT ID (RHID) System v2.0 and the RapidLINK Software v2.0 with a focus on the new RapidINTEL Plus Sample Cartridge for evidentiary samples. This cartridge provides a specialized protocol to increase sensitivity with the goal of improving first-pass success rates for a range of crime scene samples. Additionally, it includes internal quality control and quantification markers to assist with identification of low quantity, degraded, and inhibited samples.[2] These markers allow sample processing with the RapidINTEL Plus cartridge to be in line with the required enhancements needed for Rapid DNA crime scene evidence processing as outlined by the Federal Bureau of Investigation's (FBI's) Rapid DNA Technology Advancement Task Group.[1]

Various studies were conducted to assess the performance of the RHID System v2.0 with the RapidINTEL Plus cartridge and evaluate the first-pass success rate for a variety of evidentiary-type samples in comparison to standard laboratory processing. The sensitivity of the RapidINTEL Plus cartridge was determined by comparison against processing with the original RapidHIT ID ACE GlobalFiler Express (GFE) cartridge as well as standard laboratory processing.

Overall, the RHID System v2.0 with the RapidINTEL Plus and ACE GFE cartridges produced accurate, reproducible, precise, and sensitive results that were free of contamination. The RapidINTEL Plus specialized protocol provided the highest level of sensitivity as shown by obtaining a searchable DNA profile from even a 1:1,000 blood dilution sample. In most cases, evidentiary items from blood or mouth contact samples run using the specialized protocol obtained full DNA profiles comparable to standard processing. High-quality bone samples also obtained full profiles with the RapidINTEL Plus sample cartridge. Touch samples, specifically handled items and fingerprints, did not consistently obtain searchable DNA profiles due to low-level DNA or presence of a mixture that is not suitable for Rapid DNA analysis. The quantity and quality markers in the RapidINTEL Plus cartridge accurately depicted sample quantity, inhibition, and degradation.

These results indicate that the RHID System v2.0 is a suitable tool for processing most evidentiary sample types and obtaining database-ready DNA profiles. The RapidINTEL Plus cartridge advances Rapid DNA technology toward being eligible to analyze crime scene samples for search and/or upload to DNA databases. Additionally, the implementation of this system into law enforcement allows Rapid DNA to maximize the potential of crime scene samples by generating more reliable investigative leads.

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Rapid DNA; Crime Scene Samples; Law Enforcement

B196 An Analysis of Pre- and Post-Coital Microbial Transfer Using Deep Shotgun Metagenomic Studies

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Learning Objectives: After attending this presentation, attendees will understand how the genital microbiome can be observed pre- and post-sexual intercourse. They will also be informed on how to perform sample collection to increase bacterial DNA yields.

Impact Statement: This presentation will assist the forensic science community in understanding the potential of the genital microbiome as an aid in forensic investigations by describing a sample collection method and demonstrating bacterial transfer.

Abstract Text: The microbiome is becoming an emerging field of interest within forensic science with high potential for individualization; however, little is known about signature bacterial species within the genital area or their ability to transfer between individuals during sexual contact. There have been some forensic-focused studies suggesting that metagenomic analysis of human pubic hair could be a useful tool to trace sexual contact when traditional forensic methods are inconclusive.[1] As per Locard's Principle of Exchange, the genital microbiome may indicate proof of sexual contact between two individuals, each of male and female sex. Our goal was twofold: first, to examine the effects on vaginal, labial, and penile microbiome before and after sexual intercourse, and second, to determine what types of bacteria were transferred and to assess whether the specificity of recovered microbial genotypes is sufficient to trace individual bacterial origins.

In this study, we investigated microbial transfer dynamics in seven monogamous, heterosexual couples by collecting pre- and post-sexual intercourse samples from their genital areas, including penile, vaginal, and labial locations. The samples were extracted and the microbial DNA in the samples were then quantified using Real Time Polymerase Chain Reaction (qPCR), an intercalating dye, and universal bacterial primers. Utilizing shotgun metagenomic sequencing, we sequenced the microbial profiles of these samples. To determine the number of microbial species (richness and evenness of bacterial species distribution) associated with these biological specimens, alpha diversity analysis was carried out. Next, to measure the differences in the microbiome composition among different species studied, beta diversity was examined, and a principal coordinate analysis was performed to assess and visualize the compositional similarities of the microbial communities associated with these specimens. Subsequently, we used a couple-wise comparison among pre- and post-coital microbiomes to determine shared taxa and possible transfer of microbiota after sexual contact. Finally, when shared bacterial species were evident, we utilized the evolutionary analysis method using Single Nucleotide Polymorphism (SNP), which helps determine the genetic variations at a single base position in the DNA, thereby, offering a high-resolution assessment of the genetic similarity of two candidate bacterial strains in order determine a potential transfer of strain through sexual contact.

Our findings reveal significant transfer from the vaginal microbiome onto the penile microbiome, predominantly originating from the labial genitalia. For example, *Lactobacillus crispatus* abundance level increased by 70% in penile samples for two couples. Moreover, strain analysis unveiled distinct differentiation between the same species of bacteria across individuals, underscoring the potential for microbial forensics to distinguish individuals. This study contributes to our understanding of microbial transfer during sexual contact and highlights the forensic implications of the genital microbiome.

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Microbial; Sexual Crimes; Sequencing

B197 Exploring the World of "Touch DNA" and "Touch Microbiome": One-Shot DNA Collection and Extraction

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Learning Objectives: After attending this discussion, participants will have an improved understanding of the precise method for extracting human and microbial DNA from a fingermark and the information that the microbial signature can reveal in association with the donor of the fingermark.

Impact Statement: This presentation will impact the forensic science community by providing a new methodology to co-extract from a standardized fingermark surface extension to obtain successful DNA genotyping and microbial sequencing.

Abstract Text: Microorganisms are present on both the interior and exterior surfaces of the human body, outnumbering human cells at a 3:1 ratio.[1] Like human DNA shed from skin cells, microbes are also transferred to the surrounding environment.[2,3] Due to the robust structure of bacterial DNA, it withstands degradation more effectively than human DNA. Given the increasing role of microbiome analyses in forensics, and the presence of enough microbial DNA in a fingerprint to generate a reliable profile, this presentation examines the transfer and recovery of touch DNA and microbiomes using 4N6FLOQSwabs.[2]

At a crime scene, the simultaneous collection of fingermark ridge patterns and related evidence can be challenging. When a fingermark is compromised, investigators are often advised to prioritize DNA recovery over dactyloscopy. Fingermarks frequently contain low quantities of DNA, sometimes below the 0.002ng/µL threshold for obtaining a meaningful Short Tandem Repeat (STR) profile.[4] Despite this, forensic DNA analyses can still yield high-quality profiles.[5-7] Additionally, a person's microbial signature can reveal more about their lifestyle and health than human DNA alone. By analyzing both nuclear DNA (nDNA) and microbial DNA from the same swab, investigators can gain further insights, using established forensic genetics methods while maintaining the accuracy of human identification profiles.

A total of 40 participants provided fingermarks of their Dominant Hand's Index (DHI) on a standardized and sterilized glass surface. Prior to deposition, participants washed their hands and allowed natural "recharge" for one hour. Fingermarks were deposited using a cutout mold, applying 500g pressure for 60s. DNA was collected by swabbing the entire fingermark with dried flocked 4N6FLOQSwabs. NDNA extraction was done using the Qiagen QIAamp DNA Blood and Tissue kit.[8] DNA quantitation was conducted using the Human Quantifiler kit on a 7500 Real-Time PCR System. Human DNA was amplified by AmpFLSTR Identifiler Plus PCR Amplification Kit on a 9700 GeneAmp PCR System. The amplified samples were processed by capillary electrophoresis and STRs analyzed by GeneMapper ID 3.2 software. Using the same extracts, the V4 region of the 16s rRNA gene was amplified and sequenced using the Illumina MiSeq System. FASTq files were analyzed in QIIME2 (ver. 2024.5.0), were quality filtered using DADA2, and trimmed accordingly. Taxonomic assignments were made using silva-138-99. Data were further analyzed in R (version 4.2.3) with the "Phyloseq" packages.

Results showed that the co-extraction of touch DNA and touch microbiome is possible, and the use of standardized conditions for the deposition and collection of the fingermark significantly improves the success of the analysis when compared with previous studies conducted in less ideal and optimized conditions.[3] The microbial profiles obtained from the various fingers from the same donor are distinct one from another one but still more similar than between individuals in terms of bacterial composition, bacterial relative abundance, alpha and beta diversity. Specific taxa (ASVs) unique to specific donors were found in the deposited finger marks after excluding the environmental signature found on the glass slide, highlighting the potential use of touch microbiome as an additional tool to identify the donor of the trace found on touched objects on the crime scene.

On the basis of our preliminary results, it is possible to affirm that the obtention of human DNA and microbial DNA with standardized approaches may help forensic analysts in performing a more appropriate personal identification and simultaneously obtain information about habits of a suspect.

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Touch DNA; Touch Microbiome; Personal Identification

B198 Size-Selective Purification to Separate High and Low Molecular Weight DNA

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Learning Objectives: Attendees of this presentation will learn about a modification to the Qiagen Investigator Kit protocol for DNA extraction that can allow for the selective isolation of low and high molecular weight DNA.

Impact Statement: Forensic DNA samples can be mixtures with contributors differing in DNA quality. This simple modification to an already-known extraction method can be used to separate the degraded DNA from higher-quality DNA, which can benefit mixture deconvolution of casework samples.

Abstract Text: When working with DNA evidence containing multiple contributors, separating and identifying contributors with varying levels of DNA integrity can be challenging. Solid phase extraction methods utilize chaotropic salts and the affinity of DNA for silica in the presence of ethanol to isolate DNA from cell lysates. However, commercial forensic kits are not always optimized to purify highly degraded DNA, leading to potential loss during the initial post-lysis purification step.

We optimized a straightforward modification to the QIAmp Investigator Kit protocol to recover degraded DNA that might otherwise be lost. This allows for the separation and potential differentiation of contributors with different DNA qualities within the same sample. The Size Selective Purification (SSP) method involves adding extra ethanol to the first flow-through of the lysate through the silica-based column and then passing it through a second column. The additional ethanol enhances the binding of degraded DNA that was not captured by the first column to the second one. Both columns are then processed in parallel following the standard protocol. The amount of ethanol added to a lysate before spinning through the column affects binding efficiency and the size of the DNA fragments recovered.

Although there is a potential to co-elute other undesired or inhibitory substances, modern amplification methods are better equipped to overcome Polymerase Chain Reaction (PCR) inhibitors. We tested 12 pairs of high-quality DNA samples (extracted from whole blood) from different individuals, enzymatically fragmenting one sample in each pair. The paired samples were mixed and processed using both the regular protocol and the SSP method. Results demonstrated that the SSP method enabled the separation of fragmented samples from non-fragmented ones in a mixture, allowing for the recovery of approximately 20% more alleles from the degraded DNA samples, otherwise undetectable with the regular protocol. In some cases, the separation was so effective that it enabled us to conclusively attribute alleles to the degraded contributor, which were masked by the non-degraded individual and unidentifiable in the regular extraction.

The SSP method can enhance mixture deconvolution and could potentially be applied to DNA mixtures involving a source of degraded DNA and another of higher quality, such as ancient DNA with recent contamination or criminal evidence involving firearm handlers (degraded source) and victim blood (higher quality).

Low DNA; Forensic DNA; STRs

B199 Probabilistic Clustering of Single-Cell Investigative Evaluations

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Learning Objectives: After attending this presentation, attendees will learn how peak-height-based probabilistic clustering of single-cell data enhances forensic investigations when there is no suspect. This presentation will showcase the effects of accurate clustering, an essential step in single-cell interpretation. By comparing two probabilistic clustering methods—a Peak-Height-Based model and the previously published Model-Based Clustering (MBC)—we highlight the merits of faithful clustering when determining the posterior probability distribution of a genotype at a locus in non-suspect casework.

Impact Statement: This presentation will impact the forensic science community by demonstrating a significant improvement in clustering accuracy for single-cell Electropherograms (scEPGs), narrowing the estimated number of contributors explaining the evidence which, in turn, leads to a concentration in the credible set of genotypes that explain it. We will show that the concentration is persistent, even when the evidence is complex or the donor contributed as little as two cells.

Abstract Text: In forensic DNA analysis, one of the primary goals of the analyst is to generate data that will discriminate candidate hypotheses. Traditionally, these data are obtained through bulk treatments, where DNA of all contributors is extracted in a single vessel, resulting in DNA fragments that are equally distributed in solution. If the concentration of any one contributor is low, then allele drop-out can result. If there are many donors, allele overlap will result. With all contributors' DNA being sampled together, the result is that evidence (i.e., the likelihood ratio) approaches one as allele drop-out or the Number of Contributors (NoC) to a mixture increases. These issues mean that there is scope to improve the robustness of mixture interpretation.

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 (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 *m* single-cell, single-source contributor electropherograms (scEPGs). The interpretation of these highly resolved data, therefore, changes from one of determining the weights given multifarious genotype combinations to one that relies on clustering the scEPGs by similarity. With this modification, the NoC to the admixture is an output, no longer relying on human interventions. For non-suspect casework, a multi-hypothesis evaluation that estimates the probability of genotype *g* given the cluster can, therefore, provide significant gains in investigative genetics.

Previously, we used MBC to group scEPGs, based on a Gaussian mixture model.[1,2] Though fruitful, MBC resulted in some instances of over-clustering, where scEPGs from the same genotype were split into multiple clusters, which inflated the estimated NoC. We extend this previous work by introducing a model that uses conditional probabilities based on peak heights, allele positions, and allele frequencies to assess the universe of possible partitions, resulting in more accurate clustering.

To test the new clustering approach, we analyzed the same 630 admixtures used to test MBC. These mixtures contained 17 to 75 scEPGs from 2 to 5 contributors. We applied the Peak-Height-Based model, implemented in Evidentiary Evaluation of Single Cells (EESCIt), and compared its performance to MBC. We then use EESCIt to estimate the probability of the scEPGs in a cluster, *C*, given *g*, the given genotype within the set of all common genotypes according to background frequencies. In summary, EESCIt assigns 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 evaluate the performance of each clustering method, we determined the proportion of admixtures that gave correct and incorrect number of clusters (i.e., NoC). The Peak-Height-Based model outperformed MBC, eliminating over-clustering. Specifically, while MBC over-clustered 86 (13%) of the 630 admixtures and mis-clustered one sample (< 0.1%), the Peak-Height-Based model achieved perfect clustering, recovering the over-clustered samples from MBC.

We also report the proportion of loci for which only one genotype is in the credible set and that genotype is the true genotype. When using the Peak-Height-Based model, 90% of the time the credible contained one genotype and it was the correct one, compared to 84% for MBC. Furthermore, the Peak-Height model showed improved posterior distributions, resulting in a higher percentage of loci (94%) with only one or two candidate alleles in the credible set, compared to 89% for MBC. With the peak height model demonstrating improved clustering results, it is the investigative evaluation process that is improved, narrowing the number of investigative leads to one or two genotypes.

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Single-Cell Genetics; Investigative Genetics; Probabilistic Clustering

B200 The Comparison and Evaluation of Predictors on Single-Cell Weights of Evidence

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Learning Objectives: After attending this presentation, attendees will know what single-cell Likelihood Ratio (LR) features to assess and learn how to query the calibration of single-cell Weights of Evidence (WoE). They will hear about what procedures support the efficient construction and validation of single-cell assessments and will become aware of models that effectively predict single-cell WoE.

Impact Statement: This presentation will impact the forensic science community by demonstrating that at the boundary of one cell and as the information in a single-cell signature increases, LRs approach the inverse of the random match probability when H1 is true and approach 0 when H2 is true. We discuss the effects of extreme, though rare, stutter events that occur when amplifying single copies of DNA and proffer model adaptations describing ways in which these rare, impactful events can be addressed. These findings, therefore, not only show how single-cell forensics can be used in casework but demonstrate the value of single-cell data as a research tool able to illumine fundamental forensic problems.

Abstract Text: Recent developments in single-cell analysis have revolutionized basic research and have garnered the attention of the forensic domain. Though single-cell analysis is not new to forensics, the ways in which these data can be generated and interpreted are. Modern interpretation strategies report LRs that rely on a model of the world that is a simplification of it. It is, therefore, plausible that different reasonable models will assign impactfully different WoEs to some of these data. With one goal of research being to identify and understand sources of inconsistencies during early stages, we undertake a study that evaluates WoE at the limit of one single-cell Electropherogram (scEPG) across three architecturally distinct models.

To do this, we performance test three models on a set of 996 individual scEPGs and conduct one H1-true and 201 H2-true tests per scEPG. With these results, we confirm that scEPGs can well resolve between *source* hypotheses, regardless of what model was applied. We also observe that the WoE increases, on average, by 1 for every 1,000RFU of total intensity added until a plateau near the logarithm of the inverse of the random match probability is reached at ca. 22,000RFU.

By querying WoE calibration for each model, we determine if the evidence is over- or under-stated. We find that for WoE $\geq$ -1 hardly any calibration discrepancy is observed. There are rare instances, however, for which WoEs too strongly point in the negative direction, though H1 was true. This is the result of five scEPGs that not only exhibit extreme signal in stutter positions, but also carried little information in other loci. These findings show that all three models appropriately state WoEs for scEPGs when reporting positive WoE, and the two continuous model's WoE reasonably represent the findings when WoE < -1 for most loci. To further explore, we continue with paired analyses that evaluate the consistency in WoE, per scEPG, across models. Unlike unpaired analyses, this evaluation determines if similarly, well performing models return equivalent results for the same scEPG. With Pearson correlations of at least 0.98, and with 975 of 996 WoEs being within a range of 3, we conclude that the models, generally, perform equivalently. For the 21 scEPGs that did not return equivalent results and for the five scEPGs returning too strong negative WoE for true contributors, we proffer interpretive adaptions that extend beyond manually addressing the phenomena and maintain the locus independence assumption.

Single-Cell; Forensic DNA; Probabilistic Genotyping

B201 Low-Level DNA Recovery From Fired Cartridge Casings, Firearms, and Explosive Devices: Comparing Conventional Methods and Sequencing-Based DNA Typing

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Learning Objectives: After attending this presentation, attendees will gain insight into a modified purification procedure that can be utilized on low template DNA samples, to include Fired Cartridge Casings (FCCs), Firearms (FAs), and swabbing from material recovered from Improvised Explosive Devices (IEDs). Attendees will also learn about both conventional and novel approaches to processing low-level DNA type samples.

Impact Statement: This presentation will benefit the forensic science community by presenting a modified automated extraction method to aid in the recovery of DNA from evidence items known to contain low levels and quality of DNA, such as FCCs, FAs, and IEDs. Additionally, the results will showcase the effectiveness of both conventional and novel downstream processing approaches for handling low-level DNA sample types.

Abstract Text: Low-level DNA samples often pose challenges for forensic laboratories in their successful profile recovery. However, these evidence samples remain crucial in forensic cases due to their impact on public safety within communities. Between 2017 and 2021, the National Integrated Ballistic Information Network reported a 103% increase in cases, reaching a total of 843,719 casings.[1] This rise in FA-related crimes in the United States highlights the critical need for laboratories to efficiently process FCCs and FA swabs. Another sample type, IEDs, made from various materials, including explosive materials and shrapnel, have shown DNA recovery even after exposure to explosive charges and fingerprinting recovery techniques in previous studies.[2-4] However, DNA recovery can be challenging for all these sample types due to the presence of metal in materials, heat exposure, and the inherently low quality of DNA present.

To successfully obtain DNA profiles, it is crucial to focus on two key steps: efficient upstream collection and extraction followed by downstream DNA typing. Various collection techniques are employed in DNA recovery, including the traditional method of using a wet swab followed by a dry swab. Recent developments in novel methods, such as Massively Parallel Sequencing (MPS), can provide an additional tool for laboratories to process these low-level DNA sample types.

This study focuses on processing these samples utilizing a modified automated extraction procedure, followed by splitting the extract into two parts: one for conventional DNA methods using Capillary Electrophoresis (CE) and the other for MPS. The study involved the following sample types (extracts) from participants: 90 FCCs, 24 FA swabs, and 20 IED swabs. For the IED collection, galvanized steel pipe bombs were constructed and handled prior to the Render Safe Procedure (RDS). Wet and dry nylon swabs were collected at five locations from the IEDs (end cap, blown end cap, pipe-blown end, pipe middle, and the pipe closed-end). For the FA collection, the trigger and the handle were tested, and FCCs were collected after test firing rounds. All samples were processed using a customized extra-large volume method on QIAGEN's EZ2 Connect instrument. This automated DNA purification technique facilitates substantial lysate volumes from either two swabs or two FCCs (2,000µL) and allows for low elution volumes (20µL), enhancing DNA fragment recovery with ethanol. Quantification was conducted using the Quantiplex Pro RGQ Kit, with average quantification values and degradation indices (DI) calculated.

From the IEDs, the DNA yields from the combined swabs reached up to 1,376pg, with a maximum Degradation Index (DI) of 15.07. For the FCCs, DNA yields reached up to 568pg, with a maximum DI of 37.9. The FAs yielded DNA up to 2,750pg, with a maximum DI of 28.68.

Samples were then processed using the Investigator 24plex QS Kit on the 3500 Genetic Analyzer for CE and the ForenSeq MainstAY kit on the MiSeq FGX for MPS. Data analysis was performed using GeneMapper ID-X (CE) and Universal Analysis Software (UAS). Based on quantitation results, successful outcomes are anticipated from samples using both downstream kits. This will result in DNA profiles, along with their corresponding quantitation results, that can be used to identify the best approach for processing these sample types.

Overall, this study demonstrates the successful recovery of DNA from these challenging samples, offering laboratories a valuable automated tool for handling this challenging sample type. Additionally, a decision tree for downstream processing will be developed, guided by both the quality and quantitative results obtained. Future work will include additional donors, test explosive procedures, and various types of explosive materials to further validate these methods.

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Low DNA; MPS; Extraction

B202 Getting More From Less: Low-Level DNA Mixtures on Cartridges

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Learning Objectives: TrueAllele Casework technology was able to use more DNA information than manual interpretation from cartridge casings, especially low-level and mixture data, providing more informative results.

Impact Statement: Cartridges are common pieces of evidence at crime scenes, and DNA information from cartridges is hard to obtain. TrueAllele technology can find information from this challenging data while also providing match information quickly.

Abstract Text: Cartridge casings are the empty shells left behind after a gun was fired and they are typically made of different metals and have different calibers. Nearly 200,000 cartridge cases are recovered annually at United States crime scenes.[1] The crime scenes where cartridges can be collected can range from homicides, aggravated assaults, robberies, and gang-related crimes. It has been shown that cartridges that were fired degrade any DNA that was left and have significantly less DNA.[2] Items with less DNA make it harder for crime labs to interpret the data and for crime scene investigators to collect DNA.

The cartridge study was designed to determine how much single-source identification information can be recovered from firearm cartridges as well as the best collection method and most informative cartridge type. Our study examined DNA data from seven different casing materials for a total of 910 cartridge samples. The cartridge samples were then collected using five different DNA collection methods. Once the DNA was collected, both manual interpretation and a probabilistic genotyping software, TrueAllele Casework, were used to analyze the cartridge data.

Once the single source cartridge samples were created, the George Washington University lab manually interpreted the DNA data. The lab used a threshold and an allele counting method. The laboratory counted how many alleles matched the reference that was expected to be present in the sample. The laboratory found the reference sample was present in 205 of the cartridge samples.

A TrueAllele trained analyst set up single-source requests for the program to solve. Upon review of the data, it was determined that the cartridge data was low-level mixtures. There was a total of 202 samples that were low level. There was a total of 431 mixtures present, which made up 47% of the total samples.

The presence of additional contributors was an unexpected result, so a TrueAllele analyst did further processing considering multiple contributors, with the number of contributors that was observed in the data. The total number of contributors for these mixtures ranged from two to five.

TrueAllele measured more information by looking at the lower-level data and mixtures and found a previously unidentified contributor. The unknown profile was informative with an expected genotype statistic of 30.36 ban. The unknown contributor was also found in 138 of the samples, ranging across the seven different cartridge types. Manual interpretation did not consider this unknown person as the method focused on the reference's allele pair only, limiting their interpretation of the data.

Based on the results of the study, the known reference was found in 351 cartridge samples using TrueAllele, compared to 251 samples using manual interpretation. The unknown person was found in 138 samples using TrueAllele where manual interpretation ignored the unknown person.

The most informative collection methods based on high genotype statistics were wet:wet or wet:dry. The least informative collection methods were scraping and soak and sonicate. The most informative cartridge types based on high genotype and inclusionary statistics were aluminum and steel. The least informative cartridge type was 45 fired.

Overall, TrueAllele was able to use more DNA information than manual interpretation from cartridge casings, especially low-level and mixture data.

References:

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2. Prasad, Elisha, et al. "Touch DNA recovery from unfired and fired cartridges: Comparison of swabbing, tape lifting and soaking." *Forensic Science International*, vol. 330, Jan. 2022, p. 111101, https://doi.org/10.1016/j.forsciint.2021.111101.

Probabilistic Genotyping; Cartridge Casings; Mixture Analysis

B203 Integrated On-Chip PCR-Capture-Sizing-CE Microsystems for Ultrasensitive Forensic DNA Typing

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Learning Objectives: Conventional forensic Short Tandem Repeat (STR)) typing suffers from the challenges posed by: (1) mixture samples from several contributors, and (2) Low-Copy Number (LCN) DNA samples. Hence, a fully integrated microdevice for ultrasensitive forensic STR analysis has been developed that includes Polymerase Chain Reaction (PCR), post-PCR cleanup and inline injection, and Capillary Electrophoresis (CE).

Impact Statement: The high sensitivity of this on-chip forensic STR analysis can help solve challenges from LCN DNA samples and it has potential for single-cell forensic STR analysis, thus providing an ultimate solution for forensic DNA typing of mixture samples. Given high potability of this fully integrated microsystem, this portable forensic STR analysis microsystem with sample-in-answer-out capability is a significant advance in the development of rapid, sensitive, and reliable micro-total analysis systems for on-site human identification.

Abstract Text: Forensic scientists are always seeking techniques that can improve STR analysis for better throughput, cost, sensitivity, and reliability. Conventional Forensic STR typing suffers from the challenges posed by: (1) mixture samples from several contributors, and (2) LCN DNA samples. Microfabricated bioanalysis devices have the potential to address these challenges by providing lower reagent consumption, the ability to integrate multiple analytical steps on a single device, and facile scaling capability. Hence, a fully integrated microdevice for ultrasensitive forensic STR analysis has been developed that includes PCR, post-PCR cleanup and inline injection, and CE. After on-chip cell lysis, DNA was amplified in the PCR chamber with an integrated heater. The resulting biotin-labeled PCR products are electrophoretically injected through a streptavidin-modified capture gel where they are captured to form a concentrated and purified injection plug. The thermally released sample plug was injected into a 14-cm-long CE column for fragment separation and detection. We used DNA male sample 9948 as a model system to optimize DNA capture process and on-chip PCR-capture-sizing-CE. Since K562 cell line (instead of Jurkat cell) was reported to exhibit stable STR profiles over time, we applied the approach to test samples of K562 cells for multiplex STR typing. This system can produce full 9-plex STR profiles from oral swabs in about 3 hours. During the multiplex STR typing, this on-chip microsystem exhibited a limit of detection of close to single cells. The high sensitivity of this on-chip forensic STR analysis can help solve challenges from LCN DNA samples and it has potential for single-cell forensic STR analysis thus providing an ultimate solution for forensic DNA typing of mixture samples. Given high potability of this fully integrated microsystem, this portable forensic STR analysis microsystem with sample-in-answer-out capability is a significant advance in the development of rapid, sensitive, and reliable micro-total analysis systems for on-site human identification.

DNA; Criminalistics; Forensic Analysis

B204 So, You Want a Job in Forensic Science? Getting Hired as a Forensic Scientist From the Hiring Manager's Perspective

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Learning Objectives: Attendees will learn how to be effective in obtaining employment at a forensic science laboratory. Attendees will learn about errors, pitfalls, and things to avoid doing that will stifle an application to work in a desired lab or field.

Impact Statement: Hundreds of students and student affiliates attend the American Academy of Forensic Sciences (AAFS) meeting every year. There is a hiring session for labs to interact with potential employers. However, there has never been a presentation for students about what employers are looking for, how they go about the hiring process, and what things will lead you to not get a job with a publicly funded agency. This presentation will be a huge benefit for these students by helping them to avoid pitfalls they will encounter as they apply for full-time work. The presentation will also help colleges and universities that are preparing their students for these interviews and jobs. Further, the impact to other forensic hiring managers in tools that will be discussed to help them make better hires will lead to a better forensic science workforce.

Abstract Text: Forensic science service providers advertise openings for positions every year and hope to find qualified candidates to fill those positions. Often, candidates for these positions do not understand what is behind the hiring curtain at state and local agencies making these hiring decisions. Programs like the Forensic Science Education Programs Accreditation Commission (FEPAC) -accredited organizations and other colleges and universities try to prepare their students to qualify for and secure these positions. However, there are certain applicant knowledge and experience gaps that have been identified by forensic science service providers that are blind spots to the applicants and even educational institutions. Examples include preparation for technical- and situational-based interview questions, appropriate attire and demeanor for government employment interviews, working with government agency Human Resource (HR) representatives, providing accurate and complete documentation, and what interview panels are looking for in an excellent candidate. Often, very qualified candidates on paper are not successful in obtaining employment in a forensic lab because of background or polygraph issues, inappropriate social media postings, or poor references from former employers or internships. Something as simple as not knowing anything about the local area, the organization, or the organization website, mission, vision, values, size, forensic disciplines, etc. may completely derail an otherwise qualified applicant interview.

This presentation by current forensic science service provider hiring managers will focus on skills, tools, abilities, and knowledge that will help applicants obtain gainful employment in a forensic science laboratory. The presenters have extensive experience hiring new trainees and are currently writing minimum qualification documents, evaluating applicants, participating in candidate interviews, and offering positions at forensic science laboratories. Presenters have also attended many focus groups on this topic with national organizations interested in improving the hiring and onboarding processes. While this topic will be focused on individuals looking for their first job in forensics, some focus will be directed to those scientists looking to move from one laboratory to another, and what laboratory leaders and managers are looking for in experienced scientist applicants. Information will also be presented about new standards and state laws regarding minimum qualifications for positions that are changing the way employers are hiring. In addition, presenters will discuss tools that hiring managers could be using to evaluate and select candidates. These tools include artificial intelligence, cognitive tests, and deception detection testing. Employers are looking to hire and retain exceptional employees, and the vetting process for new employees is a critical part of the selection. Attendees will take away new ideas and thoughts about obtaining employment or hiring in a more effective way.

Job; Interview; Education

B205 The Role of the National Technology Validation Implementation Collaborative (NTVIC) in Forensic Technology Implementation

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Learning Objectives: Attendees will learn about the new National Technology Validation and Implementation Collaborative (NTVIC) and the mission, vision, and values of the organization. They will also learn about a new collaborative model to implement new and emerging technologies in a more expeditious and comprehensive way. Attendees will be provided with information about data publication and sharing. A major learning objective is how to leverage partnerships between researchers, instrument manufacturers and representatives, forensic science practitioners, and others to develop best practices and excellent work practices. The final learning objective is for the attendees to understand how collaboration will lead to established publish policy, procedures, validation plans, validation studies, and implementation plans for Forensic Investigative Genetic Genealogy (FIGG), Rapid DNA, 3D Firearms Analysis, and Single-Cell DNA instruments and technologies in public forensic laboratories.

Impact Statement: The NTVIC is a revolutionary organization that is changing the way that forensic science technology implementation and advancement is happening in the United States. Attendees will gain a new appreciation of the need to publish validation data in a collaborative way to aid the expedited implementation of technology. Attendees will understand how practitioners are leading the implementation of FIGG technology into publicly funded laboratories in the United States and understand more about the path for this implementation to happen in their laboratory. Attendees will also understand more about other emerging technologies and potential timelines for those to be implemented in laboratories. The goal is to help the forensic science community understand that the lift to implement new technology is easier when we are all pulling toward the same goal and leveraging the resources that we have to get to that goal.

Abstract Text: The NTVIC was established in 2022 with a vision to collaborate nationally on forensic science validation, method development, and implementation in the public sector.[1] The NTVIC has expanded to include the state public forensic laboratories in over 30 states (and growing). Efforts of the NTVIC to collaborate validations between public laboratories, instrument manufacturers, public and private researchers, and other industry experts has proven to increase the speed of implementation, quality, and scientific nature of these technologies. This presentation will discuss plans to establish and publish policy, procedures, validation plans, validation studies, and implementation plans for Forensic Investigative Genetic Genealogy (FIGG), Rapid DNA, 3D Firearms Analysis, and Single-Cell DNA instruments and technologies in public forensic laboratories. NTVIC publications will be discussed, plans for future growth will be outlined, and the vision of the organization to collaborate on forensic science validations will be presented. The NTVIC strategic vision of open, transparent, and scientific publication of validation data will advance forensic science by ensuring that new technologies implemented in forensic science laboratories have been scientifically evaluated, peer reviewed, and transparently published for use in legal settings.

Specifically, this presentation will discuss the implementation of FIGG technology in the United States by the NTVIC setting forth robust policy and procedure guidelines. These NTVIC publications include model state legislation and recommended best policies and procedures for responsible use of the technology and the investigative technique.[2-4] The process of collaboration employed by NTVIC to achieve consensus recommendations along with the adjudication process the NTVIC applies to vet and respond to public criticisms and comments will be presented. In addition, efforts to develop model FIGG training programs for DNA staff, genealogy researchers, and law enforcement investigators will be discussed. Further, NTVIC publications regarding validation of various FIGG technologies and validation plans for Whole Genome Sequencing in public laboratories in the United States will be summarized. Finally, other considerations, such as cost benefit analysis evaluation for implementation strategies, will demonstrate the value represented by the NTVIC collaborative approach.

References:

1. Establishment of the National Technology Validation and Implementation Collaborative (NTVIC) and Forensic Investigative Genetic Genealogy Technology Validation Working Group (FIGG-TVWG). Matthew J. Gamette and Ray A. Wickenheiser.
2. National Technology Validation and Implementation Collaborative (NTVIC) policies and procedures for Forensic Investigative Genetic Genealogy (FIGG). Ray A. Wickenheiser, Jennifer Naugle, Brian Hoey, Rylene Nowlin, Swathi A. Kumar, Alana Minton, Lance Allen, Claire Glyn.
3. National Technology Validation and Implementation Collaborative (NTVIC): Guidelines for establishing Forensic Investigative Genetic Genealogy (FIGG) programs. Ray A. Wickenheiser, Jennifer Naugle, Brian Hoey, Rylene Nowlin, Swathi A. Kumar, Mark A. Kubinski, Claire Glynn, Raymond Valerio, Lance Allen, Stephanie Stoiloff, Jennifer Kochanski, Christi Guerrini, Anne Marie Schubert.
4. NTVIC Recommended General MOU for FIGG Participants, July 2024.

Validation; Technology Transition; Quality Assurance

B206 A National Survey and Interviews on Training in Forensic Science Service Laboratories in the United States Evaluative Report

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Learning Objectives: By attending this presentation, attendees will gain insights into the content and emphasis of forensic DNA training onboarding programs at public and private Forensic Science Service Providers (FSSPs) in the United States through survey responses and development of a standardized curriculum for forensic DNA training and education.

Impact Statement: This presentation will impact the forensic science community by providing an opportunity to learn the results of a national survey and interviews conducted in 2024 on the content and emphasis of DNA training programs at public and private FSSPs in the United States funded by the National Institute of Standards and Technology (NIST) through a cooperative agreement to Towson University.[1] New hires in forensic laboratories undergo training that may last from six months to two years. The time commitment (six month minimum) is dictated by the Federal Bureau of Investigation (FBI) Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories for a DNA analyst who "conducts and/or directs the analysis of forensic samples, interprets data and reaches conclusions."[2] Additionally, there are consensus-based standards for lab testing and reporting, and new hires need to be aware of these standards and how they impact their work. Standards are a means to strengthen forensic science and promote scientifically supported methods and analysis approaches.

Abstract Text: To gather information and opinions from forensic science practitioner leaders about training activities, consent and 20-question multiple-response survey and paired semi-structured set of interview questions were created, and Institutional Review Board (IRB) approval was obtained. The survey and interview requests were sent to forensic lab directors, assistant directors, and/or DNA technical leaders across the United States to public contacts or those obtained through social media. Thirty-two survey responses were collected, and 12 interviews were conducted. The qualitative interviews were developed to learn about the characteristics or values that are sought in new analysts, the focus and timeline of the training program, training materials used, concepts new hires learn, how learning is assessed, the passing threshold score, ideas, needs or plans for improvement, and near-future modification plans. The top three focuses of the training are lab techniques (extraction, quantitation, amplification, and capillary electrophoresis), Short Tandem Repeat (STR) typing, and mixture analysis. The top items respondents would like to spend more time offering training on are troubleshooting, validation, and root cause analysis. Ethics violations and failure to pass the competency tests and mock trial, even after remediation, are the top reasons new hires fail training. Interviewees reported that they valued critical thinking, decision-making, ethics, willingness to admit mistakes, and strong pipetting skills and shared that they use a variety of materials, including books, recorded presentations, slides, manuals, and Standard Operating Procedures (SOPs) for training. Labs employ different training program models, including modular, start-to-finish, individual trainee, and cohort. Training is individualized and remediation on components is offered, if necessary. Interviewees communicated that they would like more off-the-shelf training materials and commercial options of training samples and mock cases.

Insights from this study were used to guide the preparation of a forensic DNA training and education curriculum that aligns to American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 1153 that is being uploaded to a public website and accessible by the forensic community and beyond at no cost. The study provides insight into processes and pathways that can be implemented to more rapidly train new hires so they can contribute to casework processing and reducing or eliminating backloads. Additionally, this project can serve as a model for other disciplines seeking to understand training content and emphasis in their specialties through mapping training documents to standards, creating surveys and interviews to understand training goals and priorities, and standardizing training curriculum to published standards.[1-3]

References:

1. This presentation is made possible through the following financial assistance award #70NANB23H276 awarded to Towson University from the U.S. Department of Commerce, National Institute of Standards and Technology (NIST).
2. FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS)*, September 1, 2011.
3. *ANSI/ASB Standard 115, Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection*. 2020. 1st Ed. https://www.aafs.org/sites/default/files/media/documents/115_Std_e1.pdf.

Training; Standards; Survey

B207 Planning for and Executing a Disaster Response: The Rapid DNA Mass Disaster Emergency Response and Preparedness Toolkit

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Learning Objectives: Attendees will learn the following from this presentation:

- A background on how the Rapid DNA Mass Disaster Emergency Response and Preparedness Toolkit was developed, including key collaborations with agencies who have previously deployed to mass disaster events through virtual questionnaires and discussions and completion of virtual mock exercise scenarios.
- An overview of all 30+ materials contained within the Rapid DNA Mass Disaster Emergency Response and Preparedness Toolkit as well all additional resources that have been collated into an easy-access repository to support agency's emergency response preparedness.
- An explanation of the intended usage of materials contained within the Rapid DNA Mass Disaster Emergency Response and Preparedness Toolkit, including demonstrations of how to access the toolkit; download materials; use the interactive features; and adapt them to fit their agency's needs and existing frameworks, policies, and standard operating procedures.
- How to get in contact with the team that developed the Rapid DNA Mass Disaster Emergency Response and Preparedness Toolkit with any questions, suggestions for improvement, or development of additional materials to integrate within the toolkit for further benefit to the forensic community.

Impact Statement: Mass disaster events by nature are unpredictable events that are unique in nature and are coupled with their own particular challenges. When an agency receives notification that a mass disaster event has occurred and response is needed, time is of the essence. This presentation of the Rapid DNA Mass Disaster Emergency Response and Preparedness Toolkit will provide valuable no-cost, adaptable resources to support immediate deployment response to mass disaster events of any kind (e.g., building collapses, wildfires, aviation accidents). The entirety of the forensic community from law enforcement, DNA laboratory personnel, crime scene investigators, medical examiner/coroner personnel, and other first responders and emergency response personnel will benefit from having access to these resources. Within the forensic community, due to the nature of the work and ever-prevalent resource barriers, many agencies do not have the time, funding, personnel, or other resources to devote to building a robust emergency response plan. These resources will equip agencies with a foundation of over 30 materials to alleviate the resources required for developing an emergency response plan that can be further adapted to meet any agency's specific needs and fit within their existing frameworks, policies, and standard operating procedures. When it comes to mass disaster events, every second counts, and this toolkit aims to alleviate the burden faced by the agency when that unpredictable and unfortunate response call is received.

Abstract Text: The National Institute of Justice (NIJ's) Forensic Technology Center of Excellence (FTCOE) is dedicated to elevating the status of forensic science through advancing technology, sharing knowledge and addressing challenges encountered within the field. Through engagement with early adopters, the FTCOE develops no-cost, evidence-based resources that support technology adoption and knowledge transfer that aid Forensic Science Service Providers (FSSPs) in addressing and overcoming emerging challenges.

During this presentation, attendees will learn about the FTCOE's partnership with Marshall University Forensic Science Center (MUFSC) and other FSSPs to develop a no-cost emergency response toolkit to support the immediate deployment of rapid DNA instrumentation for mass disaster events. This toolkit aims to furnish FSSPs with adaptable, essential resources for the preparation and deployment of rapid DNA instrumentation to identify unidentified human remains.

This toolkit is the product of four key development phases. First, material was exchanged with agencies experienced in mass disaster response planning (e.g., Palm Beach Sheriff's Office) to establish a foundation for the toolkit. Second, a gap analysis was conducted through questionnaires and interviews with past deploying agencies (e.g., Miami Dade Police Department, Butte County Sheriff's Office). Third, mock scenarios were developed to identify disaster-specific resources (e.g., Kentucky State Police, Lee County Sheriff's Office) necessary for a comprehensive toolkit. Finally, the development team participated in a mass disaster exercise conducted by the Kentucky State Police, utilizing the mock scenario developed via this study.

Through the development of this toolkit, engagement with early adopters assisted with identifying five key deficiencies and areas for improvement: Strategy, Infrastructure, Database (e.g., Laboratory Information Management System) Integration, Collaboration, and Training. These gaps were specifically addressed in the development of this toolkit, enhanced with disaster-specific resources for versatile deployment. Validation of the toolkit's efficacy was demonstrated during a mock mass disaster exercise, showcasing its practical implementation in disaster response.

By the end of this presentation, attendees will gain foundational resources for adopting or enhancing an emergency response plan that is compatible with their agencies' current infrastructure, offering valuable insights and tools for agencies at any stage of their emergency response preparedness process.

Rapid DNA; Mass Disaster; Unidentified Persons

B208 Standards Development Activities in Trace Materials

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Learning Objectives: 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.

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.

Abstract Text: 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 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 2024, the Trace Materials Subcommittee has 24 published and 6 proposed standards on the OSAC Registry. Work is on-going for additional documents making their way through the subcommittee, OSAC Registry, and ANSI/ASTM processes.

The following (new or updated) standards were added to the OSAC Registry in the past year:

Fibers:
Standard Guide for Forensic Analysis of Fibers by Infrared Spectroscopy
Standard Guide for Forensic Examination of Fabrics and Cordage
Standard Guide for Forensic Examination of Dyes in Textile Fibers by Thin-Layer Chromatography
Standard Guide for Microscopical Examination of Textile Fibers

Geology:
Standard Practice for Use of Color in the Visual Examination and Forensic Comparison of Soil Samples
Standard Guide for Collection of Soils and Other Geological Evidence for Criminal Forensic Applications
Standard Guide for Forensic Analysis of Geological Materials by Powder X-Ray Diffraction

Hair:
Standard Practice for Training in the Forensic Examination of Hair by Microscopy
Standard Guide for Forensic Examination of Hair by Microscopy

Physical Fit:
Standard Guide for Forensic Physical Fit Examination

Polymer:
Guide for Using Micro X-Ray Fluorescence (μ-XRF) in Forensic Polymer Examinations.

Published standards that have been reapproved and remain on the OSAC Registry while going through the ASTM 5-year review process or waiting for OSAC Registry approval include:
Standard Guide for Forensic Paint Analysis and Comparison
Standard Test Method for Forensic Comparison of Glass Using Micro X-ray Fluorescence (μ-XRF) Spectrometry
Standard Guide for Using Infrared Spectroscopy in Forensic Paint Examinations
Standard Guide for Fourier Transform Infrared Spectroscopy in Forensic Tape Examinations

Published standard eligible for the OSAC Registry:
Test Method for Determination of Trace Elements in Soda-Lime Glass Samples Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry for Forensic Comparisons (revised).

Proposed standards added to the OSAC Registry:
OSAC 2022-S-0017, Standard Guide for Microspectrophotometry in Forensic Fiber Analysis
OSAC 2022-N-0018, Standard Practice for a Forensic Fiber Training Program
OSAC 2022-S-0019, Standard Guide for Forensic Examination of Fibers
OSAC 2023-N-0005, Standard Practice for Training a Forensic Glass Practitioner
OSAC 2023-N-0011, Standard Practice for a Physical Fit Analysis Training Program
OSAC 2024-S-0015, Standard Guide for Evaluating Physical and Optical Characteristics in Forensic Tape Examination and Comparison.
OSAC 2023-N-0027, Standard Guide for Forensic Trace Evidence Recovery

Documents going through the ASTM process:
WK72932 Standard Guide for Forensic Glass Analysis and Comparison

Documents under development:
Standard Guide for Interpretation and Reporting in Forensic Comparisons of Trace Materials.
Standard Practice for the Forensic Analysis of Geological Materials by Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectrometry.
Standard Guide for Polarized Light Microscopy of Soils and Geological Materials for Forensic Applications.
Standard Guide for Forensic Examination of Textile Damage
Standard Guide for Forensic Examination of Fibers and Textiles Using Fluorescence
Standard Terminology Relating to Forensic Analysis of Trace Evidence Materials
Standard Guide to Raman Spectroscopy in Forensic Polymer Examinations
Standard Guide for Using X-ray Diffraction in Forensic Polymer Examinations

Subcommittee priorities for new documents and research needs for the community to address will be discussed during the presentation.

Standards; Trace Analysis; Criminalistics

B209 Differentiating Soil by Color and Mineralogy for Soil Comparisons: An Experiment Using Soil From North Carolina

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Learning Objectives: This presentation will summarize the use of soil color, mineral grain identification, and X-ray diffraction in forensic soil examinations and test the spatial variability of the properties at a range of spatial scales in effort to document what differences may be expected and those that may be used to exclude a common source of material.

Impact Statement: This presentation could inform the forensic science community about the unique qualities of soil trace evidence and the interpretation of results within soil comparison examinations.

Abstract Text: **Goal of Study:** To test the potential application of novel examination methodology in forensic soil comparisons, surface soils were collected in triplicate (1m apart) from 30 locations in North Carolina with paired sites with distinct vegetation cover (n=180). The experimental design is primarily aimed at assessing the potential use of E-DNA in soil comparison (presented in companion presentation), organic rich soils present at half the sites. However, this presentation assesses the similarities and differences observed among these samples using methods commonly used in soil examinations (color, mineral identification by Polarized Light Microscopy [PLM]).

COLOR: Soil color was measured in CIE L*a*b* space, both of the bulk soil and a fine-grained fraction to allow quantification of color difference. The derived metric, ΔE00, corresponds to perceptible color difference, where a color difference value < 1 will be imperceptible, and a value of > 10 is similar to exceeding the faint soil color contrast category used the United States Department of Agriculture (USDA) Soil Survey.[1,2] Approximately 3% of triplicate soil samples showed color differences of ΔE00>10 in the fine fraction (6% in bulk), meaning that on small spatial scales soil color is relatively similar (few false exclusions), and these errors could be further mitigated by thorough collection of known exemplars, whereas soil color differences between paired sites (collected 12 to 490 m apart) exceed this ΔE00 >10 threshold 21.8%, 18,1 % and 27.8 % of the time for bulk color, fine fraction color or the combination, respectively. Comparing all 180 soils, 41.9% (bulk) and 56.2 % (fine fraction) have ΔE00 >10. This indicates that color alone can differentiate among many soil samples and is useful as an initial screening method.

MICROSCOPY: Soil (1.6–6.6g) was washed to mount mineral for examination by PLM. Most mineral types present at >~1 % modal abundances not helpful at differentiation among locations as all samples included, feldspar, and opaque grains (two locations had other minerals >1% abundance). The trace grains on slides containing ~13,000 grains were assessed for "drop out" of low abundance mineral types among triplicate soils. The maximum number of grain-types observed in 1 slide with zero observed in from soil 1m away was 14 (biotite). The three mineral types with the highest grain count with dropout from an adjacent soil were biotite, chlorite and kyanite, all which have perfect cleavage. This high grain count with "drop out" could result from grain fracture along cleavage planes during sample preparation. The mean and median of grain counts with dropout are two and three respectively, suggesting caution should be given for the absence of a single mineral type observed in trace amount in one sample and absent in another. The presence of at least one unique grain type in all three samples at one site absent from all three samples at its paired sites occurred in 14 of 30 locations, whereas these mineral differences occur in 81% of comparisons when including all 60 sites.

The combination of color and mineral grain presence improves the capacity to differentiate soils. In this study, 92 percent of the sites could be differentiated by comparing average color with the unique presence/absence of a mineral type among all three sub site samples. There may be other notable characteristics allowing the differentiation of soils from these sites, for example, biological components, morphological features (grain size distribution, grain shape, grain surface texture, zoning, inclusions), or minerals in the clay-sized fraction of the soil.

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2. Soil Color Contrast, Soil Survey Technical Note 430-SS-2, May 2023.

Soil Analysis; Microscopy; Minerals

B210 Soil Discrimination by Particle-Correlated Raman Spectroscopy

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Learning Objectives: After attending this presentation, attendees will learn about the discrimination capability of Particle-Correlated Raman Spectroscopy (PCRS) for soil samples.

Impact Statement: This presentation will impact the forensic science community by evaluating the discriminating potential of PCRS for the differentiation of soil samples using varied statistical approaches, including hypothesis testing and chemometric methods.

Abstract Text: Forensic soil analysis has had a rich history of providing valuable information for investigating criminal events. However, many modern forensic laboratories have stopped performing this analysis due to the perception that it is either too time consuming or labor intensive. Further complicating the issue is the view that many forensic soil analytical methods are subjective due to their reliance on feature comparisons. PCRS is a new method that may address both of these concerns by providing automated and objective analysis and comparison of sample mixtures. PCRS is an integrated technique that combines automated image analysis with Raman spectroscopy. Particle imaging determines particle size and shape distributions for each component in a sample, yielding detailed morphological information (e.g., circularity, area). At the same time, Raman spectroscopy can probe the molecular chemistry of specific particles of interest. In forensic soil analysis, PCRS is therefore able to non-destructively identify the types of minerals present and provide morphological information about individual mineral grains. Particle size distributions can be generated for the entire sample or for each mineral present, along with quantitative information on the relative amount of each type of particle.

Mineral counts and morphological properties are used as the basis for classification and comparison of Raman-identified particles. The discrimination potential of PCRS was explored using a variety of statistical methods from data collected from topsoil samples collected in triplicate from 30 different locations in the northeast United States. First, error rates were investigated using traditional comparative statistics (i.e., hypothesis testing) with eight match criteria. The match criteria tested consisted of confidence intervals based on range, range +0.00005 , mean +1 standard deviation, mean +2 standard deviations, t-test at 95% confidence, t-test at 99% confidence, mean +0.0001, and mean +0.0002 (Miller criterion). The resulting match criteria were evaluated based on their Type 1 (false negative) and Type 2 (false positive) error rates, with the preferred criteria being one that minimizes both. The particle size and other morphological property distribution comparisons were evaluated using a two-sample Kolmogorov–Smirnov test, due to its sensitivity to differences in both location and shape of the empirical cumulative distribution functions of the two samples. Multivariate statistical methods were explored for the discrimination of PCRS mineral data, which included creating and testing models with Partial Least Squares-Discriminant Analysis (PLSDA), Support Vector Classification (SVC), and Hierarchical Cluster Analysis (HCA). Ultimately, this research provides statistical evidence for the discriminatory power of minerals and their morphologies for the classification and source identification of soil samples.

PCRS; Raman Spectroscopy; Discrimination

B211 An Elemental Analysis of Glass Powder on Spent Projectiles

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Learning Objectives: The attendee will be able to explain how to implement a method to analyze the elemental composition of glass in powder form.

Impact Statement: This presentation will impact the forensic community by adding an analytical method that could support or exclude scenarios proposed during the reconstruction of a shooting incident.

Abstract Text: In forensic investigations of cases involving shootings, the reconstruction of the trajectory of a bullet is of relevance ,particularly when considering the transfer of material from intermediate targets.[1,2] Glass fragments are among the traces that can be useful to analyze on recovered bullets. Glass fragments have been long recognized as useful trace evidence; they are easily transferable, and broken glass can give a timing and location of an event (the moment the glass was broken, and which glass was broken). The glass evidence embedded in a spent projectile, usually in powder form, can be associated with potential sources or exclude other sources, testing the proposed trajectory of the projectile.

Work previously presented provided independent evidence for shooting reconstructions through comparative analysis of glass evidence using the elemental composition and optical properties of the glass. However, the sample size and complex matrix excluded the direct use of the gold standard for float glass Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS). This study explored the robustness of a shorter modified method to analyze glass powder by LA-ICP/MS based on American Society for Testing and Materials (ASTM) 2927-23. This modified method also included the use of a simple signal integration algorithm for the automated processing of the shorter time resolved signal, minimizing operator bias. The advantages and disadvantages of the analysis using these modifications will be discussed.

The set of samples consisted of simulated casework samples from vehicle glass (float soda-lime) prepared by shooting through different glass panes at typical angles found in vehicles. The spent projectiles were recovered from a loose Kevlar receiver box. The proposed source glasses were then compared to the glass powder embedded on recovered spent projectiles. Because of the ablation cell configuration, it was possible to mount the spent projectiles directly in the cell for ablation. Standard reference materials included CFGS2 for calibration and NIST SRM 1831, and National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 612 as verification standards. All the elemental profiles were compared using the criteria in ASTM standard methods E2927-23. In our limited universe situation, where the number of sources were few, known, and distinguishable by chemical composition, we were able to accurately associate the recovered traces to the corresponding source.

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Trace Analysis; Glass; Python

B212 Data Fusion From Infrared, Elemental, MSP, and Raman Analysis Techniques to the Maximization of the Efficiency of the Analytical Sequence for the Forensic Examination of Paint Evidence

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Learning Objectives: After attending this presentation, attendees will gain knowledge about the advantages of combining analytical data from different spectroscopic methods when applied in the context of automotive paint examinations for interpretation purposes.

Impact Statement: This presentation will impact the forensic science community, with emphasis for trace evidence examiners, by exploring various data fusion methods to obtain a data sets of discriminating features in the light of addressing interpretive aspects (i.e., source attribution questions) following comparative examinations of paint specimens.

Abstract Text: Forensic paint examinations are conducted following an analytical sequence that typically starts with microscopical methods followed by methods of instrumental analysis. The type and number of methods utilized during analyses primarily depends on the quality and quantity of unknown specimens recovered in each case (i.e., multilayered fragments yield more analytical information than abrasive smears resulting from abrasions).

While comparing sets of recovered specimens and reference samples, data obtained from the various techniques are usually evaluated sequentially by technique, in the sense that when exclusionary differences are noted between compared sets for a given technique, then there is no need to proceed with further testing and the examiner typically reports an exclusion. In cases where the analyst cannot differentiate the compared sets with a method, a subsequent one is applied until all relevant and available methods have been applied. When the compared sets cannot be distinguished at the end of an analytical sequence, an overall evaluation of the data is made to typically address the question of a common source between them.

While evaluating the question of a common source, a critical facet is the estimation of the chance to randomly observe another coated object in a population of interest that exhibits the same properties as those observed within the questioned specimens as a function, for example, of the observed microscopic features *and* infrared profile *and* elemental profile. Currently this process is carried out subjectively and to date any effort made to assist practitioners with the implementation of objective statistical or probabilistic approaches has been limited to the consideration of data from methods taken in isolation. Hence, this project aimed to maximize the efficiency of analytical schemes for forensic paint examinations by evaluating various data fusion approaches applied to infrared, elemental, Microspectrophotometric (MSP), and Raman analysis techniques.

The study focused on the definition of the features of interest detected with the proposed techniques, the evaluation of each technique to reliably detect minor components resulting from a heterogenous paint formulations, and the identification of dependencies or redundancies of analytical information collected from the different analytical techniques. Different sample sets were used to study different aspects of the contribution of the utilized techniques (e.g., sensitivity, selectivity, or redundancy), including different brands of spray paint samples of commonly encountered colors, binary mixtures of refinishing automotive basecoat paints, and binary mixtures of interior architectural paints featuring color desaturation with white and black components. Different low-level and mid-level data fusion approaches were applied including sparse Linear Discriminant Analysis (sLDA) and Random Forests within each instrument and combined the classification results with majority voting. It was observed that the "fused" or combined results gave high levels of performance—in terms of classification accuracy compared with the individual classification results—thus demonstrating the effectiveness of data fusion. Functional Data Analysis (FDA) was also investigated as a means of reducing the data dimensionality before applying classification methods.

This approach proved to be remarkably efficient and offers a novel approach to both analyzing spectral data and performing data fusion. These methods appeared to perform better than the typical data fusion methods consisting of Multiblock classification by means of Common Components and Specific Weight Analysis (CCSWA) and Sequential and Orthogonalized Partial Least Squares (SO-PLS) regression.

Trace Analysis; Paint; Criminalistics

B213 Wait, Hot Cooking Oil Turned Your Shirt From Green to Orange?

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Learning Objectives: After attending this presentation, attendees will learn and see the effect heat, cooking oil, and solvents can cause on colored fabric. They will also learn different tests to determine what could cause the color damage.

Impact Statement: This presentation will impact the forensic science community by presenting methods to determine the effects that heat, cooking oils, and solvents can have on the dye of a garment.

Abstract Text: In June 2023, a Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF) Special Agent Certified Fire Investigator (CFI) received a request for assistance from a local district attorney's office. They were preparing to prosecute a case where the defendant was accused of intentionally burning his girlfriend. At the time of arrest, the defendant stated to police that his girlfriend burned herself during an altercation that occurred between the two of them while she was cooking at their stove with "hot oil." The victim stated at the time that the suspect poured Isopropyl Alcohol (IPA) over her and lit her on fire using a cigarette lighter. However, in the months since the incident, she recanted her statement, even writing a letter to the judge stating that she had in fact, burned herself with "grease." Investigators and prosecutors were now left with competing hypotheses as to what occurred.

The available evidence included digital images of the victim, her garments, and the scene after the altercation. As seen from the images, the victim had discernable skin burns on her right shoulder, right side of her neck, and her right wrist. The images of the damaged clothing (a bra and a tunic) appeared to correspond to the same areas. The remaining images did not reveal any signs of heat damage to the apartment. In addition, ATF investigators could not conduct a fire scene examination, since they were not notified from the onset and several months had elapsed since the incident.

The CFI reached out to the ATF Forensic Science Laboratory-Atlanta (FSL-A) for assistance. Traditional fire debris testing was not a viable option as the suspected accelerant would no longer be present on the garments. Examination of the damage to the tunic would require a non-traditional examination. The tunic appeared to have dye discoloration and possible thermal deterioration of the green cotton. Ultimately, the fact in question was whether the damage to the clothing was caused by hot cooking oil or ignited isopropyl alcohol. A series of examinations were conducted on the fabric to determine if the question could be answered.
The following findings were noted:

- Visual examination of the clothing revealed no grease/oil stains.
- Testing revealed neither IPA nor cooking oil at room temperature discolored the garment from green to various shades of orange as was observed in the original damaged area. The cooking oil does leave a greasy patch that is slightly darker than the surrounding area.
- When the tunic is subjected to hot vegetable or canola cooking oil (up to 235°C), the fabric does not discolor like the original damaged area. Nor does it deteriorate or char. References cite a range of 177-232°C as preferred to fry most foods.[1,2]
- In controlled temperature dry heat examinations, fabric discoloration began to appear when subjected to 275°C for 90 seconds; however, the intensity of discoloring was not consistent with the thermal damage that was observed on the tunic. At 300°C, the fabric completely discolored in two minutes time; however, no charring or total deterioration was present.
- When 91% IPA is splashed on the garment and ignited with a disposable lighter, the garment immediately burns, discolors, and deteriorates. When cooking oil is splashed on the garment and ignited with the same lighter, the garment is slow to burn and chars heavily with minimal discoloration.

The results of this testing support the premise that the damage observed on the shirt is consistent with originating from an ignited solvent such as IPA and inconsistent with a hot or ignited cooking oil source. The presentation will deep dive into the process that was used to reach these conclusions.

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Trace Analysis; Textiles; Fabric Damage

B214 Skeleton in the Stream: Unraveling the Murder Mystery of the River's Silent Witness

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Learning Objectives: The attendees will learn about the various challenges a forensic examiner faces during the autopsy of the dead bodies recovered from the water. They will also learn about the multiple factors that can hinder the determination of the cause of death, time since death, and identification of the deceased in similar cases. Overall, attendees will have a deeper understanding of the complexities of examining skeletal remains recovered from water environments. They will be equipped with practical skills, updated knowledge, and a comprehensive approach to conducting thorough and accurate forensic examinations in such cases.

Impact Statement: The presentation will enhance the community's understanding of drowning deaths and the complexities associated with it. The presentation will also emphasize recognizing and interpreting postmortem changes due to prolonged water exposure. This will assist forensic pathologists in making accurate time-of-death estimations and understanding the decomposition process in aquatic environments. By elucidating the forensic challenges and potential pitfalls in water-related autopsies, the presentation will aid forensic pathologists and legal professionals in understanding the complexities involved. This can help them make more informed decisions in legal proceedings and criminal investigations. The presentation will serve as an educational resource for forensic science students and professionals, providing them with updated knowledge and best practices during the examination of the dead bodies recovered from the water. This can contribute to the overall advancement of forensic education and training programs.

Abstract Text: Disposing of a dead body to conceal a crime involves various illegal and unethical methods, including throwing the body in water, burial, cremation, chemical dissolution, dismemberment, and concealment in structures or vehicles. Water disposal is a common method in India due to the widespread availability of water resources. Examining dead bodies recovered from water poses several challenges for forensic examiners, complicating the determination of the cause of death, time since death, and identification of the deceased.

In the case presented, divers accidentally recovered a skeleton from a riverbed while searching for the body of a relative involved in another case. The divers left the skeletonized body on the riverbank, where it was discovered by a villager, who then alerted the police.

An autopsy examination revealed the remains to be those of an elderly female who had been murdered by ligature strangulation. Her body was disposed of in the river, with her hands tied, likely to conceal the crime. Efforts are ongoing to identify the victim through DNA profiling and by cross-referencing the details with missing person reports of a similar age and sex in the state.

This case illustrates the forensic challenges and potential pitfalls in water-related autopsies. It aims to help forensic pathologists and legal professionals understand the complexities of such cases, aiding in more informed decision making in legal proceedings and criminal investigations. Additionally, it will serve as an educational resource for forensic science students and professionals, providing them with updated knowledge and best practices for examining bodies recovered from water. This can contribute to the overall advancement of forensic education and training programs.

This case also highlights the importance of meticulous forensic examination and the need for standardized protocols when dealing with water-recovered bodies. The ongoing efforts to identify the victim underscore the critical role of DNA profiling and collaboration between forensic and law enforcement agencies. This knowledge can improve the accuracy and efficiency of investigations, ultimately aiding in the pursuit of justice.

Drowning; Death Investigation; Forensic Analysis

B215 Chemometric Approaches for Investigating Multiblock Hyperspectral Data in Forensic Biological Evidence Analysis

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Learning Objectives: In this presentation, attendees will gain insights into the advanced application of multiblock hyperspectral imaging techniques for forensic analysis of biological fluids. Specifically, they will learn how these techniques, utilizing X-Ray Fluorescence (XRF) data alongside Visible and Near-Infrared (VNIR) and Short-Wave Infrared Reflectance spectra (SWIR), enable selective and non-destructive detection of dehydrated biological traces. The study focuses on two primary objectives: first, to effectively identify and spatially localize various biological fluids on substrates such as wood; and second, to distinguish between specific fluids like blood, urine, semen, and saliva deposited along different times. Through the implementation of chemometric methods, including Principal Component Analysis (PCA) and Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS), the presentation demonstrates how these tools enhance the recognition and differentiation of biological fluids based on their unique chemical signatures. This approach not only highlights the practical application of hyperspectral imaging in forensic investigations but also underscores the critical role of data fusion strategies in overcoming challenges posed by complex sample matrices and degradation effects commonly encountered in forensic scenarios.

Impact Statement: This presentation will significantly impact the forensic science community by showcasing the transformative potential of multiblock hyperspectral imaging techniques in the analysis of biological fluids at crime scenes. By demonstrating the efficacy of integrating XRF data with VNIR as well as SWIR, the study offers a novel approach to detecting and differentiating dehydrated biological traces non-destructively and with high selectivity. The application of advanced chemometric methods further enhances the accuracy and reliability of identifying specific fluids like blood, urine, semen, and saliva, crucial for forensic investigations. This innovative methodology not only expands the forensic toolkit but also sets a precedent for future research and practical applications in crime scene investigation, ultimately advancing the field's capabilities in solving complex cases involving biological evidence.

Abstract Text: In the field of forensic science, traditional techniques face significant obstacles when analyzing and identifying biological fluids, such as complex sample matrices and potential degradation effects.[1,2] As a result, there is an urgent requirement for novel methods capable of identifying hidden biological evidence specifically and without causing damage during forensic investigations. This research investigates the features of a modern multiblock platform (IRIS, XGLab SRL – Bruker Nano Analytics) that acquires XRF data in addition to VNIR (380–1,100nm) and SWIR, (1,100–2,500nm) simultaneously. This platform, when used together with advanced chemometric techniques, seeks to accurately recognize dehydrated biological fluids. Different biological fluids such as blood, urine, semen, and saliva from various donors were placed on wood samples and examined at different time intervals for two main purposes: identifying the location of the fluids on surfaces and distinguishing between different types of fluids. Utilizing the diverse hyperspectral data blocks, a multivariate approach using PCA and MCR-ALS was used to process each block and the combined fused arrays, leveraging their complementary information. The chemometric strategies employed proved highly effective in generating detailed chemical maps that facilitated the recognition and discrimination of various biological fluids. By integrating XRF, VNIR, and SWIR spectra with sophisticated algorithms like PCA and MCR-ALS, the study achieved a comprehensive understanding of the spectral signatures unique to each fluid type. A critical aspect of the study was the utilization of a low-level data fusion approach, which synergistically combined information from multiple spectral domains to enhance differentiation accuracy. This approach was particularly pivotal in scenarios where similarities in vibrational bands and elemental compositions posed challenges, such as distinguishing between urine and saliva samples. This research not only underscores the potential of advanced hyperspectral imaging and chemometric techniques in forensic investigations but also provides novel insights and methodologies to enhance the field's capabilities in the analysis of biological evidence. Moving forward, these findings hold promise for refining forensic protocols, improving evidence processing efficiency, and ultimately strengthening the forensic community's ability to provide accurate and scientifically robust conclusions in criminal investigations involving biological fluids.

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Imaging; Machine Learning; Crime Scene Investigation

B216 A Layered Analytical Method for Detecting Clandestine Human Burials

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Learning Objectives: After attending this presentation, attendees will understand how using Light Detection And Ranging (LiDAR) and a Soil Vapor Probe (SVP) sampling-layered analytical method can be deployed prior to excavations during clandestine human burial investigations.

Impact Statement: This presentation will impact the forensic science community by providing a methodology that uses LiDAR and SVP technologies to facilitate the process of excavation and is based on the qualitative and quantitative nature of the Volatile Organic Compounds (VOCs) that can be detected in soil resulting from human decomposition. This new methodology will enable Law Enforcement (LE) to use another "tool" in their arsenal when faced with these investigations.

Abstract Text: The use of "tools" such as Ground Penetrating Radar (GPR) and canines has provided inconsistent success rates over the years and as such LE is always looking to scientists for assistance to improve their investigative abilities and build support for search warrants.[1-4] Given the LE quandary, the use of soil vapor probes for sampling the VOCs in the vicinity of a suspected clandestine burial related to a missing persons cold case out of Washington in 2023 will be discussed. In this case, there was a requirement to search 71 acres and to locate buried victims. This research will show results from this wide search area case using LiDAR and a non-invasive SVP VOCs collection strategy followed by an Environmental Protection Agency (EPA) number 15 Total Organic (TO15) Gas Chromatography/Mass Spectrometry (GC/MS) analysis.

VOCs were collected using a non-invasive SVP sampling strategy along with a layered scheme to cover a wide search area. A drone with LiDAR capability was used to reveal potential SVP locations for the 71-acre search area. Several SVPs were strategically placed based on the LiDAR data. Each SVP consists of a 1" by 0.5"-diameter fine mesh screen located near the tip of a 0.5"-diameter steel rod that is positioned several feet underground and connected to the surface via an inner Teflon tube. Evacuated canisters connected at the surface to the Teflon tube extract collected VOCs through a flow controller while reaching atmospheric pressure. Each canister was then subjected to a TO15 GC/MS VOCs analysis protocol that is based on trapping and thermal desorption. The TO15 EPA methodology employs a VOC concentrator for vapor phase trapping. The concentrator is desorbed to the GC via a 60-meter fused silica capillary column directly connected to the source of a quadrupole mass spectrometer. The system is calibrated using a modified direct dynamic calibration system with a fixed volume sample loop to meter in the internal standard to the air stream for quantitation.

For this wide search area scene, the LiDAR indicated 11 potential SVP locations. Over three days, 33 samples were acquired along with a few scattered SVPs and other samples that appeared on the surface as interesting. All samples were analyzed via quantitative GC/MS TO15 via the JMP multivariate statistical analysis software. The results indicated a highly unusual soil VOCs pattern relative to what would be expected in a control soil sample and therefore provided credence to a source of human decomposition. The analysis indicated that only 2 of the 11 locations yielded VOC data indicative of human decomposition. There were several compound classes detected, including: alkenes, alkanes, aldehydes, aromatics, sulfides, chlorinated compounds, and ketones. The classes of compounds were grouped and compared across the samples and controls. Sites were identified from the compound comparisons and were investigated resulting in the successful location and subsequent recovery of the homicide victims.

Field execution of SVP sampling and analysis of the VOCs detected for the successful recovery of homicide victims in a one-year cold case will be presented.

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Human Decomposition; Soil Vapor; VOCs

B217 Standards Development Activities Related to Ignitable Liquids, Explosives, and Gunshot Residue

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Learning Objectives: 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.

Abstract Text: 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, IL, E, and GSR standards development activities will be discussed. These include:

1. Recent standards that have been added to the OSAC Registry:

- ASTM E2451-21 Standard Practice for Preserving Ignitable Liquids and Ignitable Liquid Residue Extracts from Fire Debris Samples
- ASTM E3196-21, Standard Terminology Relating to the Examination of Explosives
- ASTM E3253-21, Standard Practice for Establishing an Examination Scheme for Intact Explosives
- ASTM E3329-21ε1, Standard Practice for Establishing an Examination Scheme for Explosive Residues
- 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:

- OSAC 2021-N-0009, Standard Practice for the Collection and Preservation of Organic Gunshot Residue
- OSAC 2022-S-0002, Standard Practice for the Identification of Compounds Related to Organic Gunshot Residue (oGSR) by Gas Chromatography/Mass Spectrometry (GS/MS)
- OSAC 2022-S-0003, Standard Practice for the Identification of Compounds Related to Organic Gunshot Residue (oGSR) by Liquid Chromatography/Mass Spectrometry (LC/MS)
- OSAC 2022-S-0023, Standard Practice for the Forensic Analysis of Explosives by Polarized Light Microscope
- OSAC 2023-N-0010, Standard Practice for the Collection of Primer Gunshot Residue (pGSR) Particles from Clothing, Vehicles, and Other Inanimate Objects using Scanning Electron Microscopy (SEM) Stubs

3. Published standards from ASTM that have yet to go through the Registry approval process:

- 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
- ASTM E1386-23 Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Solvent Extraction
- ASTM E3197-23 Standard Terminology Relating to the Examination of Fire Debris
- ASTM E3391-24 Standard Terminology Relating to Gunshot Residue Analysis (GSR)
- ASTM E3284-23 Standard Practice for Training in the Forensic Examination of Primer Gunshot Residue (pGSR) Using Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry (SEM/EDS)
- ASTM E1388-24 Standard Practice for Static Headspace Sampling of Vapors from Fire Debris Samples

4. Documents currently in development at the OSAC or by the SDO:

- REVISION to ASTM E2997-16 Standard Test Method for Analysis of Biodiesel Products by Gas Chromatography/Mass Spectrometry (under revision as WK78732)
- REVISION to ASTM E2998-16 Standard Practice for Characterization and Classification of Smokeless Powder
- REVISION to ASTM E2999-17 Test Method for Analysis of Smokeless Powder by Gas Chromatography-Mass Spectrometry and Fourier-Transform Infrared Spectroscopy
- REVISION to ASTM E3255-21 Standard Practice for Quality Assurance of Laboratories Performing Chemical Analysis of Ignitable Liquids and Ignitable Liquid Residues (Annex)
- REVISION to ASTM E1588-20 Standard Test Method for Primer Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry
- Standard Practice for Training in the Forensic Examination of Fire Debris
- Standard Practice for the Characterization of Solid Oxidizer/Fuel Explosive

- Standard Practice for Expert Opinions on the Interpretation of Primer Gunshot Residue (pGSR) Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry (SEM/EDS)
- Standard Practice for Forensic Explosives Analysis Training Program
- Standard Practice for Reporting Results and Opinions of Ignitable Liquids Analysis
- Standard Practice for Reporting Results and Opinions of Explosives Analysis

Standards; Fire Debris Analysis; Gun Shot Residue

B218 The Validation of a Rapid GC/MS Method for Forensic Fire Debris Screening Applications

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Learning Objectives: After attending this presentation, attendees will learn about a validated method for rapid Gas Chromatography/Mass Spectrometry (GC/MS) screening of fire debris samples. Capabilities and limitations of rapid GC/MS will be presented as they relate to each validation component. Attendees will also learn about an associated validation package developed for forensic applications of rapid GC/MS that is available for use when adopting the technique.

Impact Statement: This presentation will impact the forensic science community by demonstrating a fully validated rapid GC/MS method available for forensic fire debris screening, consisting of nine validation studies to evaluate the performance of the analytical system. The work is part of a larger validation template developed for forensic applications of rapid GC/MS. The template is available for use as written or can be modified to fit a laboratory's needs, ultimately intended to reduce the barrier of implementation of rapid GC/MS in practicing forensic laboratories.

Abstract Text: The detection of ignitable liquids in fire debris samples is most commonly achieved through GC/MS analysis to identify compounds consistent with a reference liquid. Due to lengthy GC/MS analysis times required for these complex samples, the utility of screening techniques can be beneficial to preliminarily determine sample contents prior to confirmatory analysis. Rapid GC/MS is a screening technique gaining interest due to its fast separation capabilities, high sensitivities, and MS detection.[1,2] Its utility has been demonstrated for several areas of forensic analysis, including ignitable liquid applications.[3,4] However, for it to be adopted in practicing forensic laboratories, validation is required to demonstrate its suitability for the intended purpose(s). Currently, standardized validation protocols do not exist across the forensic chemistry community, especially for trace evidence analysis. Validations themselves can be time-consuming to perform, and the process of developing new protocols can serve as a barrier to implementing new techniques. Thus, demonstrated validations according to pre-defined plans can help ease this burden.

This work presents the complete validation of rapid GC/MS for ignitable liquids according to a template developed specifically for the system. Nine components were studied to gauge analytical performance (i.e., selectivity, matrix effects, precision, accuracy, range, carryover/contamination, robustness, ruggedness, and stability), and results were compared to established acceptance criteria. Single- and multi-compound test solutions were utilized, consisting of compounds present in common ignitable liquids. Based on the results from each study, a comprehensive determination of system performance specifically for ignitable liquids was achieved.

Results for seven of the nine validation components met the associated acceptance criteria. For example, precision, robustness, and stability were evaluated by assessing variation in retention times and mass spectral search scores for each test compound. In all cases, percent Relative Standard Deviations (% RSDs) were below the 10 % acceptance criteria for both metrics. Limits Of Detection (LODs) were determined, ranging from 0.0630µg to 0.138µg across seven compounds, comparable to LODs determined for similar compounds using traditional GC/MS. Ruggedness was evaluated by assessing instrument performance after varying parameters external to the instrumental method. While the greatest variability was observed when analyses were performed by a different analyst, only 3% RSD was observed, which was still well below the 10 % acceptance criteria.

Limitations to the system were identified when evaluating selectivity and accuracy. Isomer differentiation for three isomer series was assessed to measure selectivity. Differentiation was achieved for some, but not all, isomer species studied, and was particularly difficult for early-eluting compounds. Accuracy was assessed by comparing compounds identified in mock burn samples analyzed by rapid GC/MS and traditional GC/MS. Due to the complexity of such samples, complete agreement of compounds detected between the two techniques was unable to be obtained. However, these results were expected due to the timescale of the rapid (1.63min) versus traditional (31min) temperature programs and are important for understanding the extent of the system's capabilities.

In total, rapid GC/MS was successfully validated for ignitable liquid applications. Additional studies have focused on verification using solid phase microextraction as an alternative sampling and injection technique. This work is part of a full validation package developed for forensic applications of rapid GC/MS. The package includes plans with developed procedures, materials, and acceptance criteria for all components and is designed for use as written or can be modified to fit a laboratory's analytical needs. Overall, this work demonstrates the utility of rapid GC/MS for forensic fire debris analysis to provide an informed analytical approach and highlights the utility of the developed validation package to ultimately reduce the barrier of implementation of such technology.

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GC/MS; Ignitable Liquids; Validation

B219 An Application of the Expert Algorithm for Substance Identification (EASI) to Predict ASTM E1618-19 Ignitable Liquid Classes From Gas Chromatography/Mass Spectrometry Data of Ignitable Liquid Residues

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Learning Objectives: After attending this presentation, attendees will understand the benefits of using a statistical modeling method for the classification of ignitable liquids into their American Society for Testing and Materials (ASTM) E1618-19 classes. Attendees will also gain an understanding of how ions from volatile compounds in ignitable liquids correlate during weathering and permit effective prediction of ignitable liquids.

Impact Statement: This presentation will impact the forensic science community by discussing the benefits of the statistical model and how it works. Understanding this model and its foundations will aid analysts to make more informed decisions on the presence of ignitable liquids in casework samples.

Abstract Text: Arson investigators require reliable and objective methods to interrogate fire debris for the presence of ignitable liquid residues. Although standard methods like ASTM E1618-19 exist for such procedures, these methods typically do not rely on quantitative measures but instead rely on the subjective judgment of fire debris analysts.[1] To provide a more objective means to interpret fire debris evidence, we examined the ability of the EASI to identify ignitable liquid classes from the Total Ion Spectrum (TIS) of Gas Chromatography/Mass Spectrometry (GC/MS) data files.[2,3] TISs have been promoted by others to help remove variability caused by the GC dimension.[4] This work focused on modeling and identifying gasoline samples from the other ignitable liquid classes.

EASI first assumes that there must be correlations and anticorrelations between variables (*m/z* abundances) in the TIS of a gasoline sample as it evaporates. For example, abundances of fragments like *m/z* 91 and 105 from volatile aromatic should correlate strongly with one another and should both decrease with the extent of weathering. EASI determines the correlation matrix between all the covariates, then uses mixed stepwise selection to build multivariate general linear models of each of the 15 most abundant ions in the TIS. Each dependent ion is sequentially modeled using the remaining ions as covariates.

The TISs of 64 gasoline samples in the training set were first preprocessed using two different methods and two different methods of normalization. The accuracy of the 15 spectral predictions per sample were evaluated using both Pearson Product Moment Correlations (PPMCs) and the Mean Absolute Residual (MAR). These measures were then used for binary classification (e.g., gasoline present or not present) and assessed using Receiver Operating Characteristic (ROC) curves. MARs produced the most accurate spectral predictions, as determined by evaluating the Area Under the ROC curves (AUROC). More specifically, the greatest AUROC of 0.937 was observed for MAR using ions selected according to fragments in ASTM E1618-19 and normalized by summing TISs to one. Most of the false positives were caused by samples from the aromatic class, which is understandable given the chemical similarity between aromatic liquids and gasoline. EASI was also compared to the Mahalanobis distance as a binary classifier. The AUROC of the Mahalanobis distance using ions selected according to fragments in ASTM E1618-19 and normalized by summing TISs to one was 0.571—slightly above the useless "guessing" threshold AUROC value of 0.50. The lower AUROC for Mahalanobis distance indicates that EASI is a more effective means to model and classify ignitable liquids into their ASTM E1618-19 classifications than more naïve statistical approaches that depend on clustering.

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Arson; Gasoline; Mass Spectrometry

B220 Revealing Gunshot Residue Flow and Deposition Behaviors Using Laser Sheet Scattering, High-Speed Videography, Atmospheric Particle Analysis, and Spectrometric Analytical Techniques

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Learning Objectives: By attending this presentation, attendees will understand the flow and deposition patterns of Gunshot Residue (GSR), the effects of firing conditions on the GSR plume and resulting deposition, and the differences in GSR exposure risk to a shooter, bystander, or passerby.

Impact Statement: This study will impact the forensic community by demonstrating the behavior of GSR under different environmental conditions associated with activity-level hypotheses that can become relevant in assessing the evidence in the courtroom. This study utilizes a comprehensive and modern analytical approach that includes laser sheet scattering and videography, two real-time atmospheric sampling methods, and two analytical techniques for the visualization and analysis of GSR. The results from this study are expected to expand our understanding of the movement and deposition mechanics of GSR when considering various firing conditions and in different scenarios involving a shooter, bystander, or passerby individual.

Abstract Text: During the discharge of a firearm, GSR is released in highly variable patterns and directions, which are heavily dependent on the environmental conditions of the firing event. Because these residues are prone to deposit in the vicinity of the firing, information surrounding the generation and dispersion of the chemical components and resulting GSR are of interest to forensic investigations. However, the complexity of GSR production and variable diffusion factors make the interpretation of GSR evidence challenging. For instance, it can be hypothesized that the presence of GSR on the hands of a person of interest indicates the person fired a gun, was in contact with a gun but did not fire it, or was in the vicinity of the shooting during or after the firing. From a criminal justice perspective, elucidating which of these hypotheses is more likely is paramount for the fact finder, yet it is complex for a forensic scientist to evaluate the probability of observing the GSR evidence under some of these competing propositions. The main reason for this challenge is the lack of knowledge-base and technology to distinguish the processes of transfer and deposition of GSR under various circumstances. This study employs a novel multi-sensor and multi-instrument approach to enhance the current understanding of GSR flow, deposition, and airborne persistence.

In this study, particle sizing systems and custom-made atmospheric samplers with the ability to determine in real-time the density of airborne particles were employed in indoor, outdoor, and vehicular environments to simulate shooting events in enclosed residential areas, open outdoor settings, and drive-by shootings. Particulate matter < 2.5µm (PM2.5) was measured simultaneously by nine custom-made sensors to evaluate the GSR plume's dynamic flow after discharging the firearm. Concurrently, color and high-speed videography combined with laser sheet scattering were used to reveal the flow of GSR to the naked eye under various controlled experimental conditions. The combination of visual techniques has brought forth novel advances to aid in the visualization and interpretation of GSR deposition mechanics and evaluate the effects of different firing procedures (i.e., make and caliber of firearm, airflow, number of shots fired, slide position, enclosed and open spaces, relative locations from the firing point).

Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) were used as complementary tools to confirm the elemental (Pb, Ba, Sb) and chemical (2-nitrodiphenylamine, 4-nitrodiphenylamine, Akardite II, diphenylamine, and ethyl centralite) makeup of residues detected by or in proximity to the sensors. This study compared different behaviors and risks of exposure of organic and inorganic residues (OGSR and IGSR). GSR was found to diffuse rapidly to fill an enclosed space, and settling can take up to three hours in enclosed rooms with limited airflow. However, in an open (outdoor) environment, the resulting GSR cloud was found to be easily and quickly (< 60s) swept away by even a light breeze (< 0.5mph). From a forensic perspective, LC/MS/MS allowed differentiation of OGSR present on a shooter's hand from those on a bystander or passerby, while SEM/EDS revealed a bystander or even passerby following a shooting event is more prone to receive exposure to IGSR and offered critical information regarding the flow and properties of the IGSR particles. Exposure to IGSR on the skin of a vehicle passenger and deposition of vehicle surfaces was also more likely than OGSR. The combination of these sampling and visualization tools provides breakthrough knowledge on the movement and redistribution of inorganic and organic compounds during shooting that can lead to recommendations for safety regulations, sampling, collection, and evidence interpretation, while the videography performed in this study offers a never-before-seen look into the movement and deposition dynamics of GSR.

Gun Shot Residue; Laser Sheet Scattering; Video Analysis

B221 An Assessment of the Transfer and Persistence of Gunshot Residue in Simulated Arrest Scenarios Analyzed by Screening and Confirmatory Methods

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Learning Objectives: After attending this presentation, attendees will understand the risks of exposure to Gunshot Residue (GSR) in mock arrest scenarios under firearm handling and firing situations with different levels of contact between the arresting personnel and the arrestee.

Impact Statement: This presentation will impact the forensic community by demonstrating how screening and confirmatory methods can be combined for a more encompassing understanding of GSR transfer and persistence in arrest situations. The study provides three levels of contact and different transfer situations to assess the transfer and persistence from arresting personnel to the arrestee and vice versa. These results help with understanding GSR and its behavior in these situations, which can lead to recommendations to minimize risks of unintended contamination.

Abstract Text: GSR evidence interpretation can be difficult due to the complex nature of their generation and depositions, which can be further compounded by post-shooting transfer and persistence factors. With over 50,000 deaths and injuries occurring due to firearm violence in the United States, there is a need for information on how GSR can help answer investigative questions and provide relevant information to other parts of the criminal justice system.[1] The GSR discipline has identified the need to understand these mechanisms for both Inorganic and Organic Gunshot Residue (IGSR and OGSR) as per the Organization of Scientific Areas Committee (OSAC) Research Needs.[2-4] IGSR is typically studied with the standardized method using Scanning Electron Microscopy Energy Dispersive X-ray Spectroscopy (SEM/EDS) due to its inherent capabilities of classifying particles by morphology and elemental composition.[5] Other confirmatory methods like Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) have been proposed to include OGSR in the analytical workflow, and standard practices are being drafted to assist forensic agencies with future adoption. Additionally, recent research has investigated employing screening methods for mobile GSR analysis to help with triage from the crime scene to the laboratory and improve turnaround times. The benefits of OGSR analysis and screening technology are to enhance understanding of the entirety of GSR and its interpretation in situations where exposure to GSR may be challenged.

Our research group has developed and transitioned to mobile screening methods for IGSR and OGSR analysis by Electrochemical Detection (ECD) and Laser-Induced Breakdown Spectroscopy (LIBS).[6-8] The goal of this study is to help fill the gaps in general knowledge of GSR transfer and persistence in arrest scenarios. For this study, three degrees of activity were investigated to simulate real-case situations where a low, medium, or high contact arrest was performed between the officer and the Person Of Interest (POI). For each arrest, three different transfer scenarios were performed where the POI had recently fired and then been arrested, the officer have recently fired then arrested the POI, and the officer have recently handled their firearm and then arrested the POI. The POI and officer were sampled before and post-arrest. Each combination of arrests and transfer scenarios was replicated five times for a total of 180 samples collected during the study. To gain a comprehensive picture of IGSR and OGSR in these scenarios, screening by ECD and LIBS was followed by confirmatory analysis by SEM/EDS and LC/MS/MS.

The results demonstrate that IGSR and OGSR behave differently, and their transfer and persistence vary with the level of contact, activities, and exposure to GSR before the arrest. In general, OGSR was less likely to secondary transfer than IGSR, and there was a decrease in GSR detected with increased activity during the arrests. In low-contact situations, IGSR and OGSR are more likely to persist after the arrest of the person who had recently fired a gun than in medium and high-resistance arrests. While performing the same low-contact arrest, IGSR persisted more than OGSR on the individual who had recently handled or fired their weapon. These findings can help the decision-making process in future OGSR analysis adoption to inform which screening and confirmatory methods to prioritize to save time and cost. Recommendations during arrest protocols and post-arrest collections are discussed in this presentation.

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Gun Shot Residue; Simulated Arrests; Interpretation

B222 Standard Development Activities in Seized Drug Analysis: The Current Status and Challenges

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Learning Objectives: 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.

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.

Abstract Text: 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/Criminalistics Subcommittee 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 standard documents regarding the identification of marijuana, reporting drug analysis results and opinions, evidence handling practices, the use of color tests, structural similarity assessments, and others. 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 this past year. In addition, the changes being implemented to assist in the efficiency of the standard balloting process will be discussed.

A brief look at some of the other activities related to seized drug standards will also be discussed. Furthermore, the process for the revisions of standards once they are added to the OSAC Registry and how those revisions are balanced with the need to draft new standards is handled by the Seized Drugs Subcommittee.

Seized Drugs; Standards; Forensic Analysis

B223 Tracking the Emergence of Medetomidine, Tetracaine, and Other Substances as Adulterants in Philadelphia, Pennsylvania, Recreational Opioid Markets

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Learning Objectives: Through attending this presentation, attendees will examine the evolving landscape of recreational drug adulteration in Philadelphia, with a focus on the decrease in xylazine prevalence and the emergence of new adulterants in the opioid market. The audience will be able to assess changes in adulteration as evidence of the adaptability of drug markets in response to legislation, with significant implications for public health and safety.

Impact Statement: This presentation will impact the forensic science community by describing a timeline of changes in a recreational drug market, emphasizing the need for surveillance programs that can rapidly identify emerging substances of concern.

Abstract Text: In 2020, the recreational drug market in Philadelphia, PA, became the epicenter of an outbreak of overdoses attributed to fentanyl adulterated with xylazine. Xylazine, an animal tranquilizer not approved for use in humans, acts as a central nervous system depressant. Xylazine is now frequently sold in combination with fentanyl and intended to enhance or prolong the effects of the opioid; however, its use has been associated with necrotic wounds and other health risks. Despite this risk, xylazine has proliferated through various recreational opioid markets. By 2021, xylazine was present in over 90% of recreational opioids from Philadelphia.

Xylazine-adulterated opioids have proliferated in recreational drug markets across the United States. In response, the United States Congress introduced the Combating Illicit Xylazine Act in March 2023, which would classify xylazine as a Schedule III drug at the federal level. In May 2024, Pennsylvania passed legislation classifying xylazine as a Schedule III drug, joining several other states in criminalizing the possession and use of the drug.

Soon afterward, the Novel Psychoactive Substance (NPS) Discovery program at the Center for Forensic Science Research and Education (CFSRE) began recording a decrease in the percent composition of xylazine in fentanyl products and a decrease in the percentage of xylazine-positive recreational opioid samples from the state of Pennsylvania. The percentage of fentanyl samples containing xylazine dropped from 98% in Q1 2024 to 85% in Q2 2024. This marked the first quarter in which the percent positivity dropped below 98% since Q1 2023. Q2 2024 also marked the Pennsylvania when we began detecting two new adulterants previously not observed by our laboratory in the PA recreational opioid supply: medetomidine and tetracaine. These adulterants were detected in 25% and 27%, respectively, of the opioid samples from Pennsylvania tested at the CFSRE in Q2 2024. This sudden rise in new adulterants, coinciding with the decrease in positivity for xylazine, suggests that one or both may evolve into a potential replacement for xylazine as an adulterant in recreational opioids in Pennsylvania.

Medetomidine is a more potent alpha-2 agonist than xylazine and exists as a mixture of the pharmacologically active dexmedetomidine enantiomer and the inactive levomedetomidine enantiomer. Dexmedetomidine is approved for both human and veterinary use, while levomedetomidine only appears in veterinary use. Its emergence in the Philadelphia drug supply as a fentanyl adulterant corresponded with a large-scale overdose outbreak, marked by increased sedation and severe bradycardia, as noted by clinical colleagues. Surprisingly, medetomidine was present in larger amounts relative to fentanyl and xylazine in some samples tested; however, overall ratios of the drugs varied.

Tetracaine is a local anesthetic like lidocaine but has rarely been observed with recreational drugs. Like xylazine and medetomidine, it is also a veterinary drug. Lidocaine is used as an adulterant in cocaine and less commonly in fentanyl to add bulk weight. Lidocaine and tetracaine intoxication have been associated with bradycardia and other negative cardiovascular effects. In our experience, the combined use of tetracaine and cocaine is rare and its combined use with opioids has not previously been reported. Currently, neither medetomidine nor tetracaine are explicitly scheduled in the United States.

Real-time surveillance findings of medetomidine and tetracaine represent the kind of information that can be provided by drug-checking programs. The decreasing abundance of xylazine and the simultaneous emergence of previously unreported adulterants shows that recreational drug markets respond quickly to legislation and remain versatile, dynamic, and increasingly toxic.

Medetomidine; Drug Checking; Adulterants

B224 A Systematic Study of Liquid Chromatography in Search of the Best Separation of Cannabinoids for Potency Testing of Hemp-Based Products

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Learning Objectives: After attending this presentation, attendees will gain knowledge in achieving the best separation of cannabinoids for potency testing of hemp-based products by either Liquid Chromatography/Diode Array Detector (LC/DAD) or Liquid Chromatography/Electrospray Ionization/Tandem Mass Spectrometry (LC/ESI/MS/MS).

Impact Statement: This presentation will first impact the forensic science community by enabling crime labs to effortlessly select an appropriate LC separation for potency testing of hemp-based products by either LC/DAD or LC/ESI/MS/MS. It will further impact the forensic science community by allowing the regulatory agencies to easily create a standard method for potency testing of hemp-based products.

Abstract Text: Since the 2018 Farm Bill legalized hemp products with a Delta 9-Tetrahydrocannabinol (D9-THC) concentration of not more than 0.3% on a dry weight basis, a variety of hemp-based products, including plant materials, concentrates, vape cartridges, tinctures, edibles, topicals, and pet treats, entered the United States market. Potency testing of hemp-based products is meant to quantify all the major cannabinoids in a sample, so that its quality and safety can be determined. So far, the maximum number of cannabinoids that were quantified in hemp-based products for the purpose of potency testing was 18.[1] Although many methods have been published for potency testing of hemp-based products, only five LC/DAD, four LC/ESI/MS/MS, and two LC/ESI/MS methods were developed for 14 and more cannabinoids.[1-11]

For potency testing of hemp-based products by LC/DAD, baseline separation of 18 cannabinoids, (i.e., CBC, CBCA, CBD, CBDA, CBDV, CBDVA, CBG, CBGA, CBL, CBLA, CBN, CBNA, CBT, D9-THC, D9-THCA, D8-THC, THCV, and THCVA) was successfully achieved using two sequential Poroshell 120 EC-C18 columns (stationary phase: dimethyl-octadecyl (-OSi(CH3)2C18H37); A solvent: 0.03% HCO2H + 0.5 mM NH4HCO2 (pH 2.97); B solvent: acetonitrile; mobile phase: 77.5% (v/v) B; flow rate: 0.45mL/min) within 18.0-min. Baseline separation of 18 cannabinoids was also achieved using two consecutive Raptor ARC-18 columns (stationary phase: diisobutyl-octadecyl (-OSi(*i*Bu)2C18H37); A solvent: 0.03% HCO2H + 0.5 mM NH4HCO2 (pH 2.97); B solvent: acetonitrile; mobile phase: 75.0% (v/v) B; flow rate: 0.5mL/min) within 15.0-min. Baseline separation of 18 cannabinoids, except for the CBCA/CBLA pair, was also achieved using a Cortecs Shield RP-18 column (stationary phase: RP-carbamate (-OSi(CH3)2C3H6O(CO)NHC12H25); A solvent: 0.02% HCO2H + 1 mM NH4HCO2 (pH 3.15); B solvent: acetonitrile; mobile phase: 65.0% (v/v) B; flow rate: 03 mL/min) within 20.0-min. For potency testing of hemp-based products by LC/ESI/MS/MS, baseline separation of all isomers of the 18 cannabinoids was successfully achieved using the same Cortecs Shield RP-18 column (A solvent: 0.01% HCO2H + 1 mM NH4HCO2 (pH 3.38); B solvent: acetonitrile; mobile phase: 70.0% (v/v) B; flow rate: 0.5mL/min) within 7.0-min. The two separations by the Cortecs Shield RP-18 column can be used together for potency testing of hemp-based products by LC/DAD. Baseline separation of 18 cannabinoids was further achieved using an Ascentis Express RP-Amide column (stationary phase: RP-amide (-OSi(CH3)2C3H6NH(CO)C15H31); A solvent: 0.01% (v/v) HCO2H + 4 mM NH4HCO2 (pH 3.80); B solvent: acetonitrile; mobile phase: 65.0% (v/v) B; flow rate: 0.5mL/min) within 24.5-min.

For potency testing of hemp-based products by LC/DAD, the two RP stationary phases were better choices due to shorter run times. Between Poroshell 120 EC-C18 and Restek Raptor ARC-18, the longer run time by Poroshell 120 EC-C18 was due to higher backpressure and consequently lower flow rate, which would not be a problem for Ultra High-Pressure Liquid Chromatography (UHPLC) systems. Other similar columns with either dimethyl-octadecyl or diisobutyl-octadecyl stationary phase are likely to achieve similar separation under optimized LC conditions. For potency testing of hemp-based products by LC/ESI/MS/MS, the RP-carbamate stationary phase was the best choice. However, when columns with RP-carbamate stationary phase other than Cortecs Shield RP-18 are considered, the short chain length of Cortecs Shield RP-18 should be taken into account. The RP-Amide stationary phase was the least preferred for potency testing of hemp-based products by either LC/DAD or LC/ESI/MS/MS because some acidic cannabinoids had high retention factors and imperfect peak shapes.

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Cannabinoids; Hemp; Liquid Chromatography

B225 Sample Preparation Procedures at NIST for the Determination of Δ9-THC and Its Isomers in Commercial and Seized Cannabis Vape Products by GC/MS

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Learning Objectives: After attending this presentation, attendees will learn about the Gas Chromatograph/Mass Spectrometry (GC/MS) method at the National Institute of Standards and Technology (NIST), the differences in sample preparation for both methods, and comparisons of Δ9-Tetrahydrocannabinol (Δ9-THC) isomer levels between commercial and seized cannabis vape products.

Impact Statement: This presentation will impact the forensic science community by providing a summary of sample preparation procedures used at NIST for GC/MS measurements of THC isomers in commercial and seized cannabis vape products. This presentation will help inform the forensic science community of challenges encountered by the sample matrix for the determination of Δ9-THC and its isomers. NIST has developed these procedures with forensic laboratories in mind to help them distinguish seized cannabis vapes as legal or illegal products in the future.

Abstract Text: Hemp was removed from the United States Drug Enforcement Administration controlled substance list with the passage of the Agriculture Improvement Act of 2018 (Farm Bill), which defined hemp as *Cannabis sativa* containing 0.3 % or less of Δ9-THC. The passage of new legislation resulted in a significant increase in hemp farms in the United States, bringing forth an explosion of different *Cannabis*-derived finished products. One of the more common products in various forms is *Cannabis* vapes such as e-liquid solutions, oil additives, and disposable cartridges. Additionally, hemp manufacturers started to convert Cannabidiol (CBD) -rich hemp flower into other cannabinoids such as Δ8-tetrahydrocannabinol (Δ8-THC) and other THC isomers. Forensic laboratories have primarily been measuring only Δ9-THC in seized *Cannabis* flowers/buds by GC/MS. Some forensic laboratories have started to express interest in having access to reliable sample preparation and analytical methods for seized *Cannabis* products.

This presentation will expand on previous studies optimizing a sample preparation procedure that was originally developed for the determination of 11 cannabinoids (e.g., Δ9-THC) in hemp oil samples by Liquid Chromatography-Photodiode Array (LC-PDA).[1,2] The revised procedure for LC-PDA uses approximately 0.1g of vape sample mixed with 25mL of methanol and shaken for 1min. The methanolic sample is filtered and further diluted with methanol through a 10-fold and 100-fold sample dilution prior to analysis. The goal of the new research presented here was to adapt a similar approach for GC/MS measurements of Δ9-THC while also expanding to include other THC isomer vape products, including Δ7-THC, Δ8-THC, four stereoisomers of Δ10-THC and *exo*-THC. The new sample preparation procedure included varying the mass of the sample and dilution factors. This procedure was validated for GC/MS by a single laboratory at NIST for 10 commercial oil products, 25 commercial vape products, and 25 seized vape products. Approximately 50 % of the commercial oil products contained levels of Δ9-THC too low to measure by GC/MS because of the necessary dilution factors of 100 times to minimize the oil matrix being injected in the GC/MS instrument. All the commercial and seized vape products could be analyzed by GC/MS because the sample matrix is primarily comprised of components generally measured in tobacco e-cigarettes. Comparisons will be presented for the mass fractions of Δ9-THC in the *Cannabis* products by both LC-PDA and GC/MS methods. Distinct advantages will be highlighted for each of the methods in response to chromatographic co-elution with Δ9-THC from other THC isomers.

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Cannabis; Tetrahydrocannabinol; Vape

B226 The Development of a Δ9-THC Colorimetric Breathalyzer Using 3D Printing Manufacture

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Learning Objectives: This presentation will demonstrate the development of a Δ9-Tetrahydrocannabinol (Δ9-THC) breathalyzer for the detection of recent cannabis use on the site. After attending this presentation, attendees will better understand the mechanism of the colorimetric reaction applied to detect Δ9-THC and the application of this reaction to a support base that works as a prototype for a colorimetric-based Δ 9-THC breathalyzer.

Impact Statement: This presentation will impact the forensic science community by showing the development of the foundational mechanism for the development of a portable colorimetric device to detect Δ9-THC in breath for the early detection of driving under the influence of marijuana.

Abstract Text: According to the 2021 World Drug Report, cannabis continues to be one of the most widely used drugs worldwide. Currently, in the United States, 31 states and the District of Columbia have legalized non-medical cannabis. The 2018 Farm Bill defines hemp as a cannabis plant or finished product containing 0.3% or less decarboxylated Δ9-THC and removed hemp from the controlled substances list. As more and more products containing Δ9-THC are becoming available, concerns about the public health consequences of cannabis use have increased.

As a result of Δ9-THC ability to impair performance and decrease the ability of drivers, and the current lack of correlation between driving impairment and Δ9-THC concentrations in blood, the necessity of a quick and portable detection method to identify Δ9-THC in non-invasive samples from drivers is imperative. Due to the popularity of alcohol breathalyzers, breath was chosen as the matrix for the Δ9-THC detection. The concentration of Δ9-THC in breath is estimated to be 250 parts per trillion (ppt), a challenging detection limit. However, the Δ9-THC concentration in the breath may be higher since some of the generated Δ9-THC during smoking will be carried by aerosolized particles and deposited on the lung tissues. These particles can be removed by exhalation and be detected in breath. The goal of this project was to develop a cartridge based on a colorimetric reaction for the creation of a breathalyzer for the detection of Δ9-THC in breath samples.

The development of the Δ9-THC breathalyzer was based on the application of an additive manufacturing solid device made by 3D printing. Five different 3D polymerizable resins and three different cannabinoid-specific dyes were analyzed. Resins were treated with varying concentrations of Fast Blue B, Fast Blue BB, and Fast Blue RR dyes, followed by treatment with 10µg of Δ9-THC for the optimization of best resin/dye/dye concentration (1, 2, 4, and 6% w/w) set. Cartridges were printed using an Anycubic 3D printer. Color formation was captured using a ballistic microscope coupled to a high-resolution camera and the intensity of the color was analyzed using the software ImageJ. The solubility of the dye in the resin, the analytical signal obtained, the stability of the dye, and the influence of the shape of the cartridge were evaluated.

Five resins commercially available were tested, including the AnyCubic 3D resin suggested by the printer vendors. The white Creality 3D resin showed the best signal for the same concentration of Δ9-THC among all resins. The solubility of the dyes also showed to be better in the Creality resin when compared to the other four resins tested. Among the dyes, Fast Blue B and BB at 1% (w/v) showed the best results with higher signals than the signals given by Fast Blue RR. The cartridge shape was shown to be relevant to the stability of the reaction. A circular shape is the target shape due to the air collection system that will be attached to the cartridge to form the breathalyzer prototype. A current validation of the parameters is being performed and preliminary results showed the capacity of the cartridge to react positively to a concentration of 0.01µg of Δ9-THC. Achieving lower concentrations is extremely important as the concentration of Δ9-THC in exhaled air is described to be in the range of 1ng/30 L of exhaled air.

The results obtained in this work are the initial foundation needed to construct a reliable semi-quantitative breathalyzer device to be applied in Driving Under the Influence of Drugs (DUID) monitoring in the United States.

3D Technology; Cannabis; Driving Under the Influence

B227 Psilocin Derivatives in Edibles: Issues, Extraction Tips, and Case Studies

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Learning Objectives: After attending this presentation, attendees will have learned about the emerging trend of psilocin derivatives in counterfeit mushroom edibles. Attendees will also learn of extraction techniques to avoid false psilocin positives and see examples from real casework.

Impact Statement: This presentation will provide a unique perspective through case studies into the real-time complications that occur in drug analysis. Analysts will receive information and tips for extracting psilocin derivatives from complex matrices.

Abstract Text: Proper identification of chemical substances is the crux of the controlled substance discipline. Identification of illegal substances often requires the analyst to have an understanding of laws that govern their jurisdiction. Statutes can be worded in ways such that a particular drug is illegal, but analogs and derivatives are de facto legal. It is also possible that the process of testing for a substance can have the unintended consequence of converting a compound leading to erroneous results.[1]

Psilocybe "magic mushrooms" are popular hallucinogens due to the activity of psilocin. While directly ingesting the basidiocarps is the common route of administration, mixing mushrooms into edible matrices such as chocolate is a popular means.[2] Online searches lead to a plethora of retailers claiming to sell confectionaries containing these or sometimes other hallucinogenic mushrooms like fly agaric.

Derivatives of psilocin, such as o-acetylpsilocin, have increasingly appeared in edibles advertised to contain *psilocybe* throughout the country.[3,4] These items have made their way to the Texas Department of Public Safety (DPS) as well. Our analysis of these cases showed large peaks for psilocin derivatives accompanied by minor peaks attributed to psilocin. The issue presented is two-fold. Many of the submitted retail edibles specifically listed legal or non-psilocin compounds as their active ingredients. Therefore, the presence of either psilocin or its derivatives demonstrate the existence of counterfeits that can further complicate the judicial process.

The second, more pressing issue is conversion. Literature has shown strong base extractions used to isolate drugs of interest can unintentionally convert psilocin derivative into psilocin.[5] Further, the Weber exam, a color test to detect psilocin, was found to give false positives when only o-acetylpsilocin was present. Combined, the conversion phenomena and false Weber results can lead to erroneous reporting of psilocin where only a derivative is present. This is especially salient if jurisdictions only charge for psilocin and not related derivatives.

The Texas DPS lab system allows flexibility to analysts when choosing extraction techniques. These range from complex and time-consuming to simple and efficient. For this study, cases with confirmed psilocin or psilocin derivative from the past decade were examined. The majority of cases had a positive Weber exam. The case review found that simple extractions produced the lowest-quality TICs with many coeluting compounds obscuring analytes of interest. Conversely, complex extractions showed very clean TICs. None of the DPS utilized techniques, fortunately, demonstrated conversion as seen in literature. Our use of moderate acid and base solutions as opposed to extreme pH solutions may account for this.

To verify this assertion, DPS extraction techniques were compared to a strong acid technique on o-acetylpsilocin standard. Strong base extraction was not tested as literature already demonstrated conversion. For the first DPS technique, standard was dissolved in 1 M sodium carbonate base solution (pH ~10.1) and extracted into chloroform. For the second, standard was extracted into 0.1 N HCl acid solution (pH ~1), sat overnight, then pH shifted with the base solution and extracted into chloroform. Neither technique showed conversion of o-acetylpsilocin into psilocin. For the strong acid technique, standard was dissolved in 2 N HCl solution (pH ~0.1), sat overnight, then pH shifted with the base solution and extracted into chloroform. This technique showed almost 100% conversion of o-acetylpsilocin into psilocin. Conversion via deacylation at the Gas Chromatograph/Mass Spectrometry (GC/MS) inlet was ruled out as the temperature was too low. Acylated compounds are more thermally stable in general, hence the use of acylating derivatizing agents for thermally sensitive compounds. Regarding the Weber exams, it is hypothesized that the HCl acid step is converting derivative into psilocin leading to false positives.

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Psilocin; Mushroom; Chocolate

B228 The Application of the Expert Algorithm for Substance Identification (EASI) to Differentiate Tandem Mass Spectra of 2,3-MDMA and 3,4-MDMA

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Learning Objectives: In this presentation, attendees will learn that variance that exists in the branching patterns of replicate mass spectra is not random, but highly correlated, and can be modeled and used to effectively discriminate between compounds of notoriously high spectral and structural similarity, such as 2,3-MDMA and 3,4-MDMA.

Impact Statement: This presentation will provide the forensic community with an algorithm that, in theory, can cope with the variance that exists within and between mass spectrometers. The flexible algorithm is capable of extrapolating from data collected on one instrument to assess the spectral behavior of 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 in the absence of reference spectra on the same instrument.

Abstract Text: In most existing library search algorithms, a query spectrum is compared to a library of discrete exemplar spectra of standards. In our discussions, the average spectrum of hundreds of replicate spectra of a substance is considered to be the consensus or exemplar spectrum to which the individual replicates or any query spectra can be compared. One problem with this consensus approach is that even when conditions are kept as constant as possible, inter-day spectral abundances can vary by up to ±20%. This variance is even more pronounced when collecting tandem mass spectra at different collision energies or on a different instrument. Such variance increases the difficulty in differentiating between compounds with similar mass spectra. EASI takes an informed approach that accounts for the different sources of variance by modeling the correlations within replicate spectra.[1,2] Our central hypothesis is that the relative abundances of mass spectra fragments are not independent but highly correlated. This hypothesis is supported by unimolecular fragmentation theories like Rice–Ramsperger–Kassel–Marcus (RRKM) and confirmed by Pearson correlation matrices of replicate spectra for each compound.

For this presentation, 300 replicate spectra were collected each for 2,3-MDMA and 3,4-MDMA at eight different collision energies on a triple-quadrupole mass spectrometer equipped with a Direct Analysis in Real Time (DART) ionization source. The total number of spectra was 4,800. The replicate spectra were randomly distributed to create a training set for General Linear Modeling (GLM) and an equal-sized test set for unbiased assessment. Since the branching patterns vary at different collision energies, the 30 most abundant mass fragments across all collision energies were selected as variables for the linear models. The variables were entered in a stepwise addition method to produce the simplest models that explained the most possible variance in each fragment ion abundance. Typically, the amount of explainable variance in each ion's abundance at a fixed collision energy was greater than 90%. The models from each training set were used to predict each compound's fragment abundance. The measured and EASI-predicted abundances are evaluated using similarity and dissimilarity metrics, such as the weighted dot-product and mean absolute residual. Each metric is then used as a binary classifier to generate a Receiver Operating Characteristic (ROC) curve to determine the true positive rate and false positive rate over a range of threshold values. The Area Under the Curve (AUC) is then calculated to assess the performance of each metric and its supporting model.

In line with previous work, the residuals from the EASI models were typically four times smaller than those from the consensus-based approach at each collision energy, and EASI had a significantly higher overall accuracy than the consensus-based approach.[1,2]

This presentation will discuss more details on data processing, model building, evaluation, and accuracy performance.

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Seized Drugs; Novel Psychoactive Substances; Tandem Mass Spectrometry

B229 Standardizing and Implementing Direct Analysis in Real Time-Mass Spectrometry Methods Throughout DEA Laboratories

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the multi-phase and multi-year process undertaken to standardize and implement the rapid analysis technique of Direct Analysis in Real Time-Mass Spectrometry (DART-MS) throughout the Drug Enforcement Administration (DEA) laboratory system.

Impact Statement: This presentation will impact the forensic science community by providing a comprehensive overview of the necessary steps completed in order to: (1) select the appropriate instrument, (2) standardize and validate fit-for-purpose analysis methods, (3) transfer the technology and methods to multiple laboratories, and (4) successfully train the forensic analysts who will use the methods during active casework.

Abstract Text: Since its introduction to the scientific community back in 2005, the ambient ionization technique of DART-MS has been successfully applied across a variety of forensic fields, including analysis of drugs, inks, gunshot residue, paints, explosives, etc.[1,2] One of the most common commercially available systems combines a DART ionization source with a high-resolution time-of-flight MS system. The main advantage of such a configuration is its ability to achieve fast analysis while also facilitating the preliminary identification of New Psychoactive Substances (NPS) via determination of their elemental chemical formulae. However, adoption of the technique by forensic laboratories has been hindered by factors such as its high cost and maintenance, the need for knowledgeable operators, the inability to perform formal tandem MS analyses, and difficulties analyzing mixtures, among others.

This presentation will describe a dual-configuration liquid chromatography/DART triple quadrupole MS system and the advantages of implementing this simpler and more affordable configuration within routine seized-drug analytical schemes. Adoption of this technology included the creation of 12-second analysis methods for both non-targeted full-scan spectral acquisition and targeted tandem (MS/MS) analyses. Method variations included automated use of sampling cards and glass capillaries as well as the use of manual sampling. The accuracy of the spectra generated was successfully evaluated using the National Institute of Standards and Technology (NIST) 2020 MS/MS libraries.

After validation and standardization at the original laboratory in Virginia, eight additional dual-configuration systems were procured and installed throughout DEA laboratories in New York, Maryland, Florida, Illinois, Texas, California (two laboratories), and the DEA Training Academy. A 3-day training program was designed and delivered to forensic chemists at each of these locations. The training included a two-hour lecture covering background on soft ionization techniques and tandem mass spectrometry. This also provided opportunities for open discussions regarding the analytical information that can be obtained from using these techniques in comparison to the more routine GC/MS systems. The main part of the training consisted of hands-on exercises that covered: how to perform a non-targeted analysis; how to customize and execute a targeted MS/MS analysis at the optimized collision energy; how to evaluate and process data using standardized instrument reporting templates; and how to perform library searches. Dedicated technical/quality personnel at each of the laboratories were trained on how to switch ionization sources from Electrospray Ionization (ESI) to DART, how to develop in-house spectral libraries, and how to complete and document routine performance verification procedures. This latter group of analysts were also responsible for performing and documenting the verification of the methods at each of the receiving laboratories. During the last day of training, at least two DART-MS "champions" were competency tested at each of the laboratories and these analysts (along with the Quality Assurance supervisors) were then responsible for testing and documenting the competency of their colleagues during the weeks following the training.

This report will present an overview of the method validation, verification, implementation, and training activities completed (including illustrations of some of the training materials used), and the lessons learned throughout this multi-year process.

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Seized Drugs; Direct Analysis in Real Time (DART); Validation

B230 The Development and Validation of the Global Uniform Analysis and Reporting of Drug-Related Substances (GUARDS) Method

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Learning Objectives: After attending this presentation, attendees will know how the Global Uniform Analysis and Reporting for Drug-Related Substances (GUARDS) method was developed, validated, and implemented throughout Drug Enforcement Administration (DEA) laboratories. This qualitative non-targeted Gas Chromatography/Mass Spectrometry (GC/MS) method, designed to standardize drug analysis within DEA laboratories, is now available for implementation throughout the seized drug community at large.

Impact Statement: The forensic science community will benefit from this presentation by learning about the various method parameters optimized during development, the challenges faced, the performance characteristics evaluated, and the chromatographic advantages that GUARDS offers. Using hydrogen as a carrier gas to combat the global shortage of helium, the method was developed to also produce increased sensitivity by using a narrow-bore capillary column and modified tune parameters.

Abstract Text: In response to the continual emergence of Novel Psychoactive Substances (NPS) and the ongoing opioid epidemic, GUARDS was designed to offer a GC/MS screening method that would allow for consistent analysis and reporting of both commonly encountered drugs as well as emergent compounds. With each DEA laboratory using GUARDS, data can be compared nationwide and also allow the early reporting of regionally detected NPS and trends. Under current jurisdictional requirements, reporting of analysis findings varies throughout municipal, county, state, and federal laboratories. This disparity in reporting requirements sometimes results in some laboratories not reporting lower-scheduled or non-controlled drugs such as fentanyl or xylazine when found in mixtures containing a higher scheduled drug,like heroin. GUARDS was envisioned to address these and other national reporting discrepancies by providing laboratories with a single, uniform method that would also facilitate interlaboratory comparisons.

This presentation will describe the aspects of qualitative method validation used by the DEA lab system as well as explain the development of the method which consisted of evaluation of three different narrow-bore column phases (DB-1, DB-5, and DB-35), numerous column lengths, and temperature ramp programs. Over 90 method iterations were assessed and compared and system suitability was monitored using numerous sets of forensically relevant close-eluting compounds, including fentanyl analogs and their positional isomers. The initial method validation process involved evaluation of selectivity, repeatability (short-term precision), and reproducibility (long-term precision). Results from these tests—and additional testing focused on critical-resolution compound pairs—also provided the basis for revising the acceptance criterion used throughout DEA laboratories for retention time comparisons during routine casework. After validation was completed, the method was transferred to two regional laboratories (ruggedness partners) for additional verifications. As of this writing, the method has been successfully transferred, verified, and implemented on over 25 additional instruments across ten regional and satellite laboratories. Furthermore, the method was recently shared with another federal agency, where it has been successfully verified and adopted. This presentation will include a summary and discussion of the data collected during the validation process as well as the data obtained beyond validation and across the other instruments' verifications, highlighting the figures of merit, such as a less than 3% Relative Standard Deviation (RSD) among tested compounds across multiple instruments, that this new screening method offers to the seized-drug community.

Seized Drugs; GC/MS; Method Development

C1 A Framework for Systematic Error Mitigation for Digital Evidence

Eoghan Casey, PhD, University of Lausanne, Lausanne, Vaud*

Learning Objectives: Participants in this session will learn about the Digital Evidence Weakness Taxonomy (DEWT), which organizes potential sources of error in the digital forensic process and provides a systematic support for error mitigation analysis as defined in the American Society for Testing and Materials (ASTM) E3016-18.[1] The DEWT provides a common language to discuss effective approaches to reducing weaknesses in digital evidence and increasing reliability of forensic results and expert opinions.

The DEWT knowledge base will be freely available to everyone, helping practitioners, attorneys, developers, and researchers improve their understanding of problems in digital investigations.

- Practitioners can use the DEWT to help avoid potential problems in their work.
- Attorneys can use the DEWT to assess their cases for weaknesses.
- Agencies can use the DEWT to determine the most common sources of error and implement mitigations.
- Developers can use the DEWT to test their solutions for bugs and other issues.
- Researchers can use the DEWT to find gaps that require further study.

The objective of the DEWT is to increase trust and transparency in digital investigations and resulting digital evidence and expert opinions.

Impact Statement: This presentation will inform the forensic community of the range and scope of potential errors in digital forensic science and provide a systematic approach to prioritizing and mitigating these errors.

Abstract Text: The increasing complexity and diversity of computer technology and forensic methods make it difficult for practitioners, attorneys, developers, and researchers to know about every potential problem that can weaken digital evidence. Furthermore, criminals are exhibiting a variety of concealment behaviors that make it more difficult to find digital evidence of their activities. These challenges increase the risk of failures, errors, mistakes, and misinterpretations, undermining the primary purpose of forensic science to produce trustworthy understanding of digital evidence to support decision making in legal contexts. There is a pressing need for a knowledge base that describes potential weaknesses at each phase of the digital forensic process. The weaknesses include errors in digital forensic tools, incompleteness, misinterpretation, and inaccuracy related to the existence, alteration, association, or corruption of digital evidence.

This session presents the DEWT, a digital forensic community initiative to organize potential sources of error in the digital forensic process and to support error mitigation analysis. Specifically, the DEWT provides a systematic support for ASTM E3016-18, Standard Guide for Establishing Confidence in Digital and Multimedia Evidence Forensic Results by Error Mitigation Analysis.[1] The DEWT provides a common language to discuss effective approaches to reducing weaknesses in digital evidence and increasing reliability of forensic results and expert opinions. For each category of weakness, the DEWT outlines associated subcategories, mitigations, detection methods, and references to related resources. The DEWT can be used to highlight and mitigate weaknesses in a given item of digital evidence in a repeatable and transparent manner.

This session demonstrates how practitioners, attorneys, developers, and researchers can use the knowledge base, and developers will learn how to reference specific errors they have mitigated in their tools. Planned work will be discussed, including operationalizing the knowledge base and creating test datasets to increase visibility and understanding of errors at each abstraction layer.

Reference:

1. *Standard Guide for Establishing Confidence in Digital and Multimedia Evidence Forensic Results by Error Mitigation Analysis* (ASTM E3016-18).

Digital Evidence; Error; Mitigation

C2 NoSQL Database Forensics: An Analysis of Redis Logs

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Learning Objectives: This presentation focuses on advancing the field of database forensics, with a specific emphasis on NoSQL databases where there is currently less research.

Impact Statement: This presentation will significantly impact the forensic science community by providing insights into NoSQL database forensics using the Redis server as an example. The presentation highlights potential sources of information in the database logs and the potential challenges in the analysis process.

Abstract Text: Many of the advances in database forensics have focused on relational database systems. However, with the changing landscape of data management, non-relational (NoSQL) database systems, particularly key-value stores, are increasingly essential for managing large amounts of data due to their simplicity and efficiency. This study utilized Redis, an in-memory key-value store, as a case study to demonstrate some of the challenges in database forensics examinations, with a focus on NoSQL systems. Drawing from many of the existing works in database forensics, logs play a critical role in database examinations, providing essential information about database operations and security incidents.[1,2] From a forensics standpoint, insights from these logs are vital for identifying security breaches, unauthorized access, and other suspicious activities that help piece together data and event reconstruction.

We hypothesize that while Redis provides basic logging and supports Access Control Lists (ACLs) for managing user permissions, there is a limited understanding of its logging behavior, particularly about how much information can be gathered about unauthorized command executions. To better understand this limitation, we conducted a detailed examination of Redis's default logging capabilities, focusing on different log levels debug, verbose, notice, and warning, and the events they record. We designed and executed a series of experiments to observe Redis' logging behavior under various scenarios, including both authorized and unauthorized command executions. By utilizing Redis' ACLs, we simulated unauthorized command attempts to evaluate Redis' ability to detect and log these events. Through these experiments, we also aimed to gain insights into Redis' operations and how it can serve as a potential source of information for forensic investigations, given the increasing use of key-value stores in application areas such as caching, session, user profile management, messaging queues, and publisher/subscriber systems.

Our evaluation uncovered several key findings. Debug-level logs provide detailed insights into the database's contents, such as the number of keys and their structure, which are crucial for debugging but lack context about ongoing operations. Verbose logs offer periodic snapshots of the database's status without additional context, while notice-level logs give critical information about the server's configuration and operational status but not detailed data changes. Warning logs highlight potential issues, such as memory overcommitment, without offering detailed diagnostic information or resolution steps. The ACL logs record user activities and the context of command executions but do not specify the outcomes or any errors. The MONITOR command logs real-time command execution along with client information but lacks user identification, command outcomes, error messages, performance data, and details about session durations or security incidents.

These findings advance knowledge on the forensics analysis of Redis databases and build on current works, which have focused on high-level experimentation to understand the data file structures in Redis.[3] Our experiments draw insights from the structures of the Redis data files in analyzing the logs for artifacts and limitations. The study provides a basis for identifying and acquiring artifacts from forensic analysis from Redis.

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2. Fasan, O. M., and Olivier, M. (2012). Reconstruction in database forensics. *In Advances in Digital Forensics VIII*, pp273-287. Springer.
3. Xu, M., Xu, X., Xu, J., Ren, Y., Zhang, H., & Zheng, N. (2014). A Forensic Analysis Method for Redis Database based on RDB and AOF File. *Journal of Computers*, 9(11), 2538-2544.

NoSQL Database Forensics; Reconstruction; Key-Value Stores

C3 Reimagining the Forensic Pipeline Out of Necessity

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Learning Objectives: The learning objective of this presentation is to introduce and describe a large-scale case study of the aftermath beyond triage and recovery from an incident in a large corporate setting. When presented with a large number of projects with potentially tainted data, the team constructed innovative and practical forensic workflow and file sanitization procedures.

Impact Statement: This presentation will impact the forensics community by discussing the aftermath of incident discovery and response. How does an organization protect the clean portions of its enterprise from data that was potentially exploited as part of a cyber incident? How can this be extended to help investigators that may need to deal with potentially tainted or malicious files from a forensic image?

Abstract Text: In April 2024, The MITRE Corporation discovered they were subject to an intrusion into one of its research and prototyping networks. MITRE's security team immediately began an investigation, cut off known access vectors to/from the threat actor, and brought in third-party Digital Forensics Incident Response teams to perform an analysis alongside the company's in-house experts. Prior to the incident, the impacted networks contained a significant number of actively used projects. While investigative activities may have yielded indicators of unauthorized accesses to projects and files, it was determined that it would be impossible to gain a full understanding of the actor's actions on the network to exfiltrate or impact files due to the potential for undiscovered capabilities and malware. MITRE faced the question: What can be done to get projects back online with a high assurance that any restored data was not compromised?

MITRE has a long history of developing solutions for cross-domain challenges, but usually these types of solutions deal with a limited number of file types and are designed, developed, and tested with customer requirements provided in advance.[1-3] This incident was treated as a novel cross-domain challenge involving hundreds of known and unknown file types, each of which could be compromised through unknown adversary malware. The team could not rely solely on traditional Anti-Virus (AV) solutions, given that AV products are not 100% effective in identifying malware.

During the presentation, the team will unveil a new forensic pipeline that was built, tested, and used to aid in the aftermath of MITRE's cyber intrusion event. The team will provide strategies, recommendations, and lessons learned for others that may be similarly impacted. Topics will cover forensic file type handling, data transfer, automated analysis, and reporting. For example, text files (e.g., configs, code, free text, etc.) proved to be a complex challenge when ensuring that no executable data would traverse from our "dirty" to "clean" networks. We will also describe procedures used for other popular file types to ensure that malware could not be embedded in those formats.

We will also discuss a custom physical one-way transfer solution that was developed to confine undiscovered malware that may be attempting to break out of our "dirty" network. As Stuxnet has demonstrated, removable media can be exploited to jump air gapped networks.[4] The solution kept all data on-net, which became paramount for speed and reliability during automated workflow processes. The capability also mitigated the potential for malicious processes to laterally move across boundaries using most modern internet protocols. We will discuss, in practical terms, these added innovations that enabled MITRE to migrate projects to "clean" environments and reestablish trust in our data in a relatively short amount of time.

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3. Lex Crumpton and Charles Clancy "Infiltrating Defenses: Abusing VMware in MITRE's Cyber Intrusion" *MITRE-Engenuity Blog* https://medium.com/mitre-engenuity/infiltrating-defenses-abusing-vmware-in-mitres-cyber-intrusion-4ea647b83f5b
4. https://en.wikipedia.org/wiki/Stuxnet.

Forensic Pipeline; Incident Recovery; One Way Transfer

C4 Bias in Bytes: Practitioner Awareness in Digital Forensics

Chris Shennan, MSc, Liverpool John Moores University, Liverpool, England, United Kingdom*

WITHDRAWN

C5 A Forensics Investigation of the Social Networking Application: Tencent QQ on iOS and Android

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Learning Objectives: This presentation will reveal the forensically recoverable artifacts from a popular social networking application, Tencent QQ, with a variety of functions, including device connection, file editing and transferring, built-in camera, shared documents, payment and service, real-time location sharing, contacts, QQ group activities, messages withdrawal, conversation history, QQ zone, payment, private information preserving and protecting.

Impact Statement: This presentation can aid digital forensic investigators in a thorough analysis in their cases where QQ application is used. What's more, it can serve as a foundational reference for other researchers. They can build on our findings to further explore Tencent QQ and apply similar techniques to other applications. Recovered artifacts can inform the enhancement of existing forensic tools that can automatically extract and analyze Tencent QQ artifacts, thereby improving investigations' efficiency. Researchers and educators can use your findings as case studies or training materials, which can assist new forensic analysts to handle data from social networking applications.

Abstract Text: Tencent QQ, founded in 1999, stands as one of the world's most widely used instant messaging applications, with a peak user base of around 900 million in 2016.[1] Despite the rise of similar applications like Instagram, Discord, and WeChat, QQ continues to hold its ground, particularly among young adults in China. As of December 2023, QQ still boasts nearly 600 million users, with the majority under 25 and only 0.9% between the ages of 26 and 35.[2,3] According to a report by QuestMobile, QQ remains the most popular instant messaging app among young people in China.[4] Given its extensive user base and features, mobile forensics on QQ is crucial. One representative issue concerning QQ is that it includes a payment feature called CAIFUTONG, used for personal and business transactions, which has unfortunately attracted scammers, particularly in Southeast Asia. These fraudsters exploit QQ to contact victims and extort money, leading to significant and often irrecoverable losses. This ongoing issue underscores the need for comprehensive forensic research into QQ's functions to enhance security measures and protect users from fraud.

While there have been numerous forensic studies on QQ since 2009 focusing on its memory functions, instant messaging capabilities, and PC version, many of its newer features have not been fully explored. To address this gap, we conducted a comprehensive forensic analysis of QQ's latest functions on Android and iOS devices. This includes an examination of device detection, file editing and transferring, the built-in camera, shared documents, payment and service functions, message withdrawal, real-time location sharing, contacts, QQ group activities, conversation history, QQ Zone, and private information preservation. This study has uncovered security-critical evidence related to payment functions, location data, identity information, chat histories, multimedia files, and contact information.

This presentation will delve into the recovered artifacts from Tencent QQ by examining its features carefully. The research is designed to assist investigators in fully harnessing the forensic recoverability of QQ's updated functionalities. Gaining insight into the types of evidence that can be retrieved and understanding the scope of recoverable data is crucial for addressing current knowledge gaps. These artifacts are crucial for uncovering evidence in criminal investigations and tackling issues related to cybercrime as well as safeguarding personal and corporate security. By preserving and analyzing digital evidence, mobile forensics are essential for protecting individuals and organizations from various security threats.

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Mobile Forensics; Social Networking; Tencent QQ

C6 A Story of Obsession: Data Analysis of a Stalker's Mobile Phone Device

Nicole R. Odom, MSFS, University of Wisconsin-Madison Police Department, Medex Forensics, Richmond, VA*

Learning Objectives: Through this presentation, attendees will learn about the immense amount of data stored in a wide variety of applications on mobile phones and how this information provided detectives at the University of Wisconsin-Madison's Police Department (UWPD) substantial documentation of a stalker's mental state and resulting actions.

Impact Statement: This presentation will impact the forensic science community by providing a detailed breakdown of phone usage and explaining how, through in-depth analysis, several independent data points can be connected to relay an individual's mindset and behavior both via actions performed directly by the user or those passively recorded by a digital device.

Abstract Text: In early 2024, a resident of Madison, WI, was repeatedly contacted and physically approached by a former partner, leading them to file an incident report of stalking with the UWPD. Through the resulting investigation, the offending party's mobile phone device was acquired via consent. A thorough review of the suspect's device revealed that the claims of stalking were well-founded and were, in fact, much more in-depth than originally anticipated.

In this technological day and age, it is commonplace for much of the public to not only own a mobile phone device, but to also carry it on their person at all times. Given all the various applications running on these devices, and the fact that software developers often strive to collect and categorize as much data as possible about their users, there is a vast amount of data being created and stored about an individual on any given day. This only continues to increase exponentially as smart phones advance, adding a variety of hardware sensors and robust operating systems capable of all sorts of communication methods, search utilities, location capabilities, and multimedia functionalities.

Due to this overwhelming amount of content potentially obtained from each device, it can be easy to extract and produce everything in a report without providing any context to the data; however, this case study will highlight the importance of our responsibility as digital forensic examiners to thoroughly review and correlate the information presented to us and to transform vast quantities of data into evidence that has solid probative value. By breaking the data down into small chunks of relational information, it is possible to effectively tell the story of a stalker solely through their digital footprint, outlining their thought process from the initial breakup to breaking point to arrest in a way that is irrefutable.

Mobile Device; Digital Evidence; Psychological Behavior

C7 ImageRec: A Tool for Android In-Memory Code Reconstruction

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Learning Objectives: The audience will learn about Android runtime design and how that can aid the recovery and reconstruction of in-memory code. Additionally, the presentation will provide a detailed comparison between the in-memory structures used for Code management from Android 8-14, which is invaluable to the field of mobile memory forensics. Finally, the presentation will provide a case study of how the proposed tool was used to recover code that was not available at installation and had no footprint on the disk.

Impact Statement: From a digital forensics standpoint, this tool can impact the community in code recovery, which can then be used to identify the underlying structure of application logic and, thus, facilitate data and user activity recovery.

Abstract Text: Android application developers generally withhold loading their complete code from Google Play to protect against reverse engineering and IP theft, opting instead for a skeletal version that is replaced or updated upon installation. Moreover, apps may load dynamic classes at runtime that only leave a footprint in the transient memory. This challenge of the potential lack of a complete code base in secondary storage and/or marketplace can significantly negatively impact mobile forensics and malware analysis. Addressing these challenges, we propose ImageRec—a tool designed to recover and reconstruct an Android-loaded code base from a target process's memory image. ImageRec works by navigating the runtime object to locate the OAT File Manager pointer, which leads to a set structure containing addresses of OAT Files associated with the process. By traversing each OATFile structure, ImageRec focuses on retrieving the file name and the embedded OAT dex files' storage locations in memory, enabling the recovery of all developer's loaded dex files and any boot images used by the runtime environment.

Our evaluation of the recovery path for Android Dex files from Android 8-14 showed that the same structures are maintained for this purpose. However, we observe a notable shift in the recovery offsets for some internal structures. We also assessed ImageRec by analyzing the Facebook process memory, successfully recovering the entire app code base, including the Instant Game code. This enabled a detailed examination of the design and operation of these games within Facebook. Our analysis revealed that the games are hosted within a WebView object, with settings like setAllowFileAccess, setMixedContentMode, setJavaScriptEnabled, addJavaScriptInterface, and setAcceptThirdPartyCookies enabled by default. Notably, these settings cannot be disabled by users, raising potential concerns regarding user control and security. From a digital forensics standpoint, this tool is instrumental in recovering code, which can then be used to identify the underlying structure of application logic and thus facilitate data and user activity recovery.

Android; Cyber Forensics; Code

C8 The Reliability of Confidence Factors in iPhone GPS Data for Forensic Applications

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Learning Objectives: From this presentation, participants will learn how to rigorously validate the accuracy of geolocation data extracted from iPhones, focusing on the reliability of Apple's confidence factors. By applying statistical analysis and comparing Global Positioning System (GPS) points to a known reference point, participants will gain measurable skills in assessing the precision of geolocation data, which is essential for forensic investigations and legal proceedings.

Impact Statement: This presentation enhances the forensic science community's competence by providing methodologies to validate the accuracy of geolocation data extracted from iPhones. It translates this competence into action by offering practical steps for forensic examiners to assess and verify the precision of GPS data in real-world scenarios. Stakeholder outcomes are positively impacted as legal professionals and investigators gain reliable data to support or challenge evidence in court, ensuring accurate and just judicial decisions.

Abstract Text: In modern digital forensics, the ability to accurately validate geolocation data extracted from smart phones is paramount, particularly in legal proceedings where the integrity of evidence is scrutinized. This study focuses on the analysis of geolocation accuracy of digital forensic data extracted from an iPhone. Specifically, it examines the reliability of the confidence factor assigned by Apple to each GPS point by comparing the recorded locations to a known reference point. The necessity for precise validation of GPS data in legal context arises from the critical role this information can play in establishing alibis, tracking movements, and corroborating or refuting testimonies. Inaccurate geolocation data could lead to wrongful conclusions, affecting the outcomes of investigations and judicial decisions.

Geolocation data extracted from iPhones includes latitude, longitude, timestamps, and confidence factors, all of which are stored in various databases within the device. A key database utilized in this study includes Cache.sqlite, which stores detailed records of a device's location history. This database provides a wealth of information but also necessitates rigorous validation methods to ensure the data's accuracy and reliability.

This study establishes and validates an accuracy factor by measuring the distance between the extracted GPS points and a pre-determined reference point and then comparing these distances to the expected values indicated by the confidence factors. This factor quantifies the precision of the geolocation data, providing insights into the effectiveness and reliability of Apple's location services in digital forensic applications. Such validation is crucial for forensic examiners and legal professionals who must rely on this data to construct or dismantle narratives in court. The study aims to enhance the forensic community's understanding of geolocation data accuracy and provide methodologies to ensure its reliability in legal contexts.

Per the Apple developer website, the data variable horizontalAccuracy (referred as confidence factor in this report) is: the latitude and longitude specified by the coordinate property identify the center of the circle, and this value (horizonalAccuracy) indicates the radius of that circle.[1]

Additionally, this study focuses not on the accuracy of the individual GPS data points relative to the actual known GPS location but rather on assessing the reliability of Apple's "horizontalAccuracy" confidence factor in representing the precision of the collected GPS data points. This will be defined by examining whether collected data points fall within each individually reported confidence factor.

Reference:

1. https://developer.apple.com/documentation/corelocation/clvisit/horizontalaccuracy .

Digital Evidence; Forensic Science; Forensic Investigation

C9 Using TikTok Around the World: A Comparison and Updated Forensic Analysis of TikTok on Android and Ios

Xiao Hu, Purdue University, West Lafayette, IN; Umit Karabiyik, PhD*, Purdue University, West Lafayette, IN*

Learning Objectives: By attending this presentation, attendees will be able to understand the main content and storage path of key data in a TikTok application installed on both Android and iOS; learn the updated parts of artifacts compared to previous studies; recognize the characteristics and differences in the information stored in TikTok between different APK versions; and distinguish the performance of commercial and open-source digital forensic tools for parsing TikTok.

Impact Statement: This presentation will impact the forensic science community to further explore the forensic value of TikTok by identifying the types, locations, and analysis methods of artifacts related to TikTok's main features and the new feature, TikTok store. The results of this work cover TikTok versions used in different parts of the world, raising awareness on a larger scale that digital forensics research should evolve more aggressively to keep up with the rapid technological advances in social media platforms.

Abstract Text: TikTok, the online video-sharing social media network, has made a significant impact worldwide. With over 37 million active users in the United States alone, it has become a dominant force in the social media landscape. Dedicated to creating and sharing short videos, TikTok has gained immense popularity.[1] Recently, the platform introduced the TikTok store feature, which collects sensitive data related to users' orders, addresses, and payments. The forensic analysis of TikTok can provide valuable insights into user behavior, data transactions, and potential security vulnerabilities. However, despite its massive user base, TikTok's forensic research capabilities still have room for improvement.

This presentation will illustrate the analysis of TikTok's general information, media, and new shopping features, updating previous research on this app. In the general information section, we will present data about user accounts and apps. In the media section, we will explain how to discover the video content uploaded and watched by users and suggest possible ways to differentiate what users watched. The research methodology used in our study follows the approach described by Fahad E. Salamh et al. in and is further guided by the National Institute of Standards and Technology (NIST), which consists of five phases: data population, data acquisition, analysis and examination, validation, and reporting.[2,3] Since TikTok uses different APKs in different regions, this presentation will also contrast TikTok applications using different APK versions. In our forensic images that will be presented, scenarios include account registration, keyword searches, user conversations, follower additions, video uploading and favoriting, card registration, purchases, etc. In terms of the use of digital forensic tools, we will also report on the results of analyzing our images using both commercial tools such as Magnet AXIOM and open-source tools such as iLEAPP, thus further comparing their effectiveness. This work further refines the forensic analysis of TikTok by providing investigators with information about TikTok-related folder structures and important data paths, offering valuable insights into digital forensic investigations, which are crucial for keeping up with the rapid technological advancements and overall development of social media platforms.

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Forensics; Mobile Device; Data

C10 A Forensic Analysis of Google Fit App on WearOS

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Learning Objectives: Attendees will gain insights into the fundamental challenges and limitations in extracting forensic artifacts from the Google Fit app on WearOS. They will learn how to utilize a novel forensic analysis toolkit specifically tailored for WearOS to automatically detect and analyze forensic artifacts stored on smart watches and data transmitted to paired phones. The presentation will cover the types of data stored and transmitted by the Google Fit app, including health data, location data, and device data, as well as the mechanisms of data transmission between Google Fit and other connected apps, highlighting potential exposure of sensitive information. Additionally, attendees will understand the importance of data storage and transmission for forensic investigations, focusing on the identification and preservation of evidence. The presentation will include a comparative analysis with existing mobile forensic toolkits, demonstrating the effectiveness and efficiency of the new methodology through experimental evaluations. The broader implications for forensic science, application security, and the development of robust forensic analysis procedures will also be discussed.

Impact Statement: This presentation will significantly impact the forensic science community by introducing innovative methodologies for analyzing data from wearable devices, specifically the Google Fit app on WearOS. By providing a comprehensive forensic analysis toolkit tailored for WearOS, the presentation will enhance the ability of forensic investigators to automatically detect and analyze forensic artifacts stored on smart watches and data transmitted to paired phones. This will improve the efficiency and accuracy of forensic investigations, particularly in cases involving data breaches, unauthorized data access, or personal injury claims where health data is critical. Additionally, the insights gained from this analysis will aid in the development of more secure wearable technologies, ensuring better protection of user data. Ultimately, this presentation will contribute to the advancement of forensic science by offering new approaches to uncovering crucial evidence and promoting the development of robust forensic analysis procedures.

Abstract Text: Our detailed analysis uncovers various types of data from the Google Fit app, including health data (such as heart rate and activity levels), location data (Global Positioning System [GPS] coordinates), and device data (sensor readings).[1] Additionally, we examine the data transmission between Google Fit and other connected apps, shedding light on how these interactions can potentially expose sensitive information.

The forensic toolkit identifies and analyzes how data is stored locally on the smart watch and how it is synchronized with paired devices. Health and activity data are frequently transmitted to the user's phone and backed up on Google's servers, facilitating seamless synchronization across devices. This transmission process involves various data types, including user activity logs, health metrics, and sensor data, which are critical for providing personalized fitness insights and recommendations.

Understanding the storage and transmission mechanisms is crucial for forensic investigations as it allows for the identification and preservation of evidence. This kind of analysis is important in the cases involving data breaches, unauthorized data access, or even in personal injury claims where health data can serve as evidence.

Our methodology includes a comparative analysis with existing mobile forensic toolkits and demonstrates the effectiveness of our approach through extensive experimental evaluations. The toolkit significantly expedites the investigation process and enhances the ability of forensic investigators to locate and analyze forensic artifacts with high precision.

The implications of this study extend to the broader forensic science community, developers, and researchers. By understanding the forensic artifacts generated by the Google Fit app on WearOS, stakeholders can better protect user data, improve application security, and develop more robust forensic analysis procedures.[2]

Overall, our findings provide valuable insights into the types of data stored and transmitted by the Google Fit app on WearOS. This analysis not only underscores the importance of securing wearable devices but also highlights the potential forensic implications, given the sensitive nature of the data involved.

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Forensic Analysis; Android; App

C11 Unveiling Sleep Patterns: Insights From Garmin FIT Protocol Data

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Learning Objectives: After attending this presentation, attendees will have a comprehensive understanding of Flexible and Interoperable Data Transfer (FIT) Protocol files designed by Garmin developers, specifically, the forensically relevant data contained in these files. The FIT files collected and analyzed in this study focus specifically on the sleep activity collected by a Garmin smart watch, although these files also keep location, activity, workout, and wellness data. Such data can be used as digital evidence in a law enforcement investigation, for instance, corroborating the alibi that a person of interest was asleep during the time a crime was committed.

Impact Statement: Due to the lack of previous research on the FIT Protocol and its related files, this presentation will be of great interest to the Digital and Multimedia Sciences community, as a deeper understanding of such files obtained from Garmin devices will be discussed.

Abstract Text: The everyday use of smart watches to track health and fitness information has become a common practice among adults in the United States. This is further supported by a study that indicated 25.8% (n = 2,368) of the total United States adults (n = 9,193) wore a smart watch to monitor their health and activity within the past 12 months.[1] Garmin, a popular smart watch brand, has devices that are most commonly used in tandem with a smart phone and the Garmin Connect app. Together, these devices and application collect a plethora of user health-related information, including but not limited to: number of steps, number of flights of stairs climbed, active energy, weight, body fat percentage, Body Mass Index (BMI), heart rate, resting energy, walking and running distance, workouts, and sleep analysis.[2] To effectively store and share such data, Garmin developers designed the FIT protocol, which "defines a set of data storage templates (FIT messages) that can be used to store information such as user profiles and activity data in files."[3] As the health and activity data potentially stored in FIT files could prove to be valuable evidence in a law enforcement investigation, understanding these files is of great importance to the forensic and law enforcement community. Due to the lack of previous research specifically focused on the sleep activity data in these files, this study aims to close the gap and gain further insight by forensically analyzing the sleep activity collected by a Garmin smart watch.

For this study, a Garmin vívoactive4 smart watch was utilized and synced with a Google Pixel 7a smart phone via the Garmin Connect App. Sleep data was populated by wearing the Garmin vívoactive4 while sleeping. As data population is completed, forensic images of the two devices used in this study are being collected. Thus far, two full file system images of the Google Pixel 7a using Magnet GrayKey and Cellebrite Premium have been acquired. In the near future, the researchers aim to collect the following forensic images: a Google Pixel 7a image using Android Logs Events and Protobuf Parser (ALEAPP), a Garmin vívoactive4 smart watch image using MOBILedit Smartwatch Kit, and Garmin Connect App images using Magnet AXIOM Cloud and an Application Programming Interface (API) developed for collecting smart watch cloud data. Upon analysis of the forensic images, the researchers will present the findings during this presentation.

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Garmin Flexible and Interoperable Data Transfer (FIT) Protocol Files; Smart Watch Forensics; Sleep Activity Artifacts

C12 Utilizing Smart Watches in Forensic Science: A Case Study on Verifying Witness Statements and Tracking Drug Ingestion

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Learning Objectives: By the end of this presentation, attendees will gain insight into the value and potential of utilizing smart watch data in forensic science investigations. They will explore how this data can be integrated with witness statements to establish accurate timelines in drug-related cases. Additionally, attendees will become aware of the limitations and ethical considerations of using smart watch data in forensic investigations. This presentation aims to equip attendees with the knowledge and awareness to effectively incorporate smart watch data into forensic practices, ensuring responsible and informed decision-making.

Impact Statement: This presentation will empower the forensic science community to embrace and leverage the potential of smart watch data, leading to advancements in forensic science practices and the enhancement of investigative outcomes.

Abstract Text: The case history revolves around an individual suspected of drug abuse, with suspicions that her non-physician boyfriend injected her with ketamine and attempted resuscitation with multiple epinephrine injections before her death. The investigation team acquired a smart watch worn by the individual during the alleged incident. This smart watch, equipped with sensors measuring heart rate, motion, and other relevant parameters, provided critical data. Through meticulous analysis, investigators aimed to identify the probable time of drug injection. Additionally, witness statements from those present during the incident were collected, detailing the sequence of events, the presence of drug paraphernalia, and the individual's behavior. The goal was to compare the smart watch data with witness statements to establish a consistent and accurate timeline.

The findings of this case study underscored the utility of smart watch data in forensic toxicology investigations. The smart watch data revealed an increase in heart rate and expected motion patterns during the suspected time of drug injections. These findings were further validated by witness statements, which described the individual's behavior and the presence of drug paraphernalia during the same timeframe.

The collaborative approach of integrating smart watch data with witness statements proved instrumental in establishing a more precise timeline of the drug injection. This method not only strengthened the reliability of forensic investigations but also enhanced the admissibility of evidence in legal proceedings.

Despite the promising results, this study has several limitations. The accuracy of smart watch data can be influenced by factors such as device quality, placement on the body, and user activity levels. Witness statements are inherently subjective and may be affected by memory recall biases or personal perceptions. The reliance on a single case study limits the generalizability of the findings. Further research with larger sample sizes and multiple cases is essential to validate this approach. Ethical concerns regarding privacy and data security must also be addressed to ensure the responsible use of personal technology in forensic investigations.

This case study highlights the immense potential of utilizing smart watch data in forensic toxicology to determine the time of drug injection. The collaboration between smart watch data and witness statements offers a comprehensive and corroborative approach for establishing accurate timelines in drug-related cases. This research underscores the importance of integrating emerging technologies, such as smart watches, into forensic investigations to enhance evidence collection and analysis efficiency and accuracy. As technology evolves, its role in forensic science may oscillate between being a tool for transformation or tyranny, emphasizing the need for ethical considerations and rigorous standards in its application.

Scene Investigation; Mobile Device; Forensic Analysis

C13 DarkWeb Investigations Using Offensive Security Techniques

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Learning Objectives: After attending this presentation, attendees will better understand the DarkWeb inner workings and implement several advanced cyber investigation techniques in this environment to identify malicious servers and users and de-anonymize services.

Impact Statement: This presentation will impact the forensic science community by providing extensive detail about DarkWeb investigations, enabling forensic analysts to uncover information that would not be possible through traditional techniques.

Abstract Text: The DarkWeb is a network environment, within the internet, that implements different protocols and massive encryption to protect its users data and provide extreme privacy. The access to these networks requires special software and their content is very difficult to index.

Several kinds of DarkWeb exist; the best known and most popular is The Onion Route, known as Tor.[1] This network uses multiple nodes before packets reach the final destination. Each of these nodes implements a level of cryptography in the application layer of the OSI Model and only knows the previous and next node, thus keeping the anonymity of this traffic. In this way, it also acts as a Virtual Private Network (VPN), protecting this data and hiding the source of these connections. The use of traditional investigation techniques for discovering information such as IP addresses, geolocalization of services, and user tracking are completely ineffective in these environments.

Despite this type of technology having been developed to assist with privacy and security, aimed at protecting freedom of expression in repressive regimes, for instance, what we are witnessing in reality is an increase in illegal activities on these networks. Currently, with technological advancements and the ease of using these tools, criminals are migrating to the DarkWeb. Ransomware groups, child pornography, drug trafficking, arms trade, and terrorism are examples of how these networks are being utilized.

Due to the encryption techniques implemented, new forms of cyber investigation must be applied by law enforcement agencies to collect and identify these malicious services.

Advanced offensive security techniques (hacking) can usher in a new era in the field of cyber intelligence and digital forensics. The use of social engineering can be employed for the de-anonymization of users in these environments. Vulnerabilities in web applications are a means for identifying the real IP address of a malicious server or for the remote collection of relevant cyber evidence, such as databases and internal operating system files.

Indexing services for devices such as Shodan and Censys can be used as means to de-anonymize servers on the Dark Web.[2,3]

This presentation will showcase practical case studies demonstrating how these techniques and tools are crucial for the discovery of information and identification of criminals in the most hostile environments on the internet.

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DarkWeb; Offensive Security; Investigation

C14 The Combination of Non-Obvious Links in Online Fraud Investigations

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Learning Objectives: Attendees will learn how to detect non-obvious links between fraud investigations using approximate matching of pseudonyms and how to combine multiple non-obvious links to help analysts work with big data. We will discuss the decision-making process of working with many investigations and dealing with an important number of links between them.

Impact Statement: This presentation demonstrates how digital traces can contribute to forensic intelligence and discusses the challenges of working with a large quantity of linked data. Based on real-world data, this research aims to provide actionable methods to assist field investigators and engage the research community in discussions about the relevance of detected links.

Abstract Text: This research concentrates on finding and combining non-obvious links between high-volume internet crimes to produce forensic intelligence. Non-obvious links are drawn using approximate matching of pseudonyms created by criminals. Perpetrators can easily switch to different online accounts or platforms, effectively changing their digital identity and the traces of their activities. As a result, unique and exact comparisons of digital traces is a limitation for link discovery in online fraud investigations. To overcome this limitation and avoid linkage blindness, it is necessary to use near similarity comparison of distinctive digital traces. Investigations usually involve many digital traces in each case, drawing multiple non-obvious links between them. Combining those weak links enables the detection of related investigations. With thousands of reported online frauds each year, this approach helps analysts prioritize important investigations involving many linked offences.

The first aspect of this work is focused on the detection of non-obvious links, using non-exact matching between pseudonyms. Various algorithms can be used to compute string similarity and a first step was to examine how these methods perform on short strings and how they can be used to produce sound and actionable intelligence in online fraud investigations. Preliminary results showed good performance on detecting similar pseudonyms but that a significant number of new links were detected.

The second part of this study examines the need for better and more transparent methods to combine and prioritize multiple non-obvious, or weaker, links. When faced with many investigations and thousands of kinks, it may be difficult for an analyst to decide where resources should be dedicated. The decision-making process of evaluating the combination of links found using near-similarity computation is an integral part of this work. Understanding the process leading to the detected links and their ranking is essential to manage investigative resources.

Methods to compute pseudonym similarity, the combination of non-obvious links, and the decision-making process surrounding these methods in the investigation of online frauds will be discussed in this presentation. The targeted audience is practitioners and academics of any level.

Forensic Intelligence; Approximate Matching; Cyber Forensics

C15 Principles of Locality of Reference: A Proposal to Reduce the Scope of Forensic Analysis in Cases Occurring in the Cyber Environment

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WITHDRAWN

C16 Is It a Transformation? Is It Tyranny? Is It Even Cost Effective? The Value of Independent Software Testing Your Technology

Marc Canellas, JD, PhD, Maryland Office of the Public Defender, Alexandria, VA*

Learning Objectives: The objectives of this presentation are to: (1) understand why independent software testing is valuable to forensic laboratories when purchasing and implementing new technologies; (2) understand what independent software means, the processes, and the documentation; and (3) understand how the results of independent software testing can inform their testimony in courts and the admissibility of evidence generated by software.

Impact Statement: From this presentation, attendees will better understand what to ask of developers when purchasing technology, what to do when they are making sure the technology works for them, and how to leverage these methods to ensure results of reliable technology are admissible in court.

Abstract Text: Forensic science is now a software-based discipline. DNA now has probabilistic genotyping. Fingerprints have the Automated Fingerprint Identification System (AFIS). Ballistics have the National Integrated Ballistic Information Network (NIBIN). Cell phone analysis and Global Positioning System (GPS) location are awash in readers and analyzers. As this conference asks: Are these technologies tools for transformation or tyranny? And as labs spending the money may ask: Are these technologies cost effective? This presentation answers that the only way to answer these questions is through independent software testing—the universal method that is used to ensure the effectiveness and safety of everything from our medical systems and nuclear power plants to mobile apps and games.1-3 Given the rise of software in forensic science, the community has increasingly interacted with software testing: testing prior to acquisition or implementation (e.g., before labs adopt STRmix) and responding to calls for testing by the White House, proposed federal legislation, and defense attorneys.4,5 Despite the rise in discussions of software testing, and the interest by forensic labs, this presentation will answer fundamental questions that have been left unanswered: (1) What is independent software testing? (2) What are the documents that labs should require before purchase from the developer and produce after their testing? (3) What are its implications for the questions of reliability?

First, software testing involves the processes of Verification and Validation (V&V), which are intended to show that a system both conforms to its specification (verification) and that it meets the expectations of the system customer (validation). This V&V process is foundational for any software system, including things as big as nuclear systems and as small as mobile games. Then, depending on how much risk is acceptable, there may be additional steps or required independence of the testers in order to prevent conflicts of interest. We know this from our daily lives: We don't trust students to grade their own homework, where all that is at stake is a GPA. This presentation will synthesize this understanding through a review of the industry standard documents like Institute of Electrical and Electronics Engineers (IEEE) 1012 Standard for System, Software, and Hardware Verification and Validation, the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC)/IEEE 12207 Software Lifecycle Processes and Activities, and National Aeronautics and Space Administration (NASA) Procedural Requirement 7150.2D Software Engineering Requirements, and software engineering textbooks and handbooks.6-10

Second, prior to labs purchasing or relying on software, they should require documents to show that proper software testing was done. This includes the requirements document that provides complete details on how the system should behave. The verification in V&V is the process of making sure that the final system meets these requirements. The goal is that if the requirements are met, then the customer should be satisfied. There is also the V&V (or test) plan, which documents the steps the tester plans to take to ensure that the requirements (including what level of independence is necessary) and the customer are satisfied. One of the final documents is the acceptance test documentation that describes the final testing, making sure that the customer is satisfied with the software.

Third, when software-generated evidence is used in court, these documents are essential for understanding the evidence's reliability. Under Frye, independent software testing is the generally accepted method of determining any piece of software. Under Daubert, documented independent software testing will, by definition, address questions of standards and testing, while the results of the testing will generate meaningful error rates.

Ultimately, independent software testing is a long-established method that can assure the forensic science community that they are purchasing and implementing reliable software to transform the criminal legal system in positive way towards justice and away from tyranny.

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Artificial Intelligence; Software; DNA

C17 The Limitations of Digital Forensics Tools for Database System Examination

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Learning Objectives: The goal of this presentation is to highlight some of the yet-existing limitations of digital forensics tools in database systems examinations. The presentation focuses on a selected set of tools and compares their capabilities for database forensics analysis.

Impact Statement: This presentation will impact the forensic science community by focusing attention on the challenges in database forensics and highlighting the limitations of existing tools as well as how the capabilities of the tools can be improved.

Abstract Text: Over the past decade, there have been advances in the definition of processes for database forensics and data reconstruction methods.[1,2] Many have also highlighted several challenges relating to the use of digital forensics tools for database analysis. However, it is important to focus on each challenge in relation to the goals of database forensics analysis to better understand the limitations of the existing tools and changes that may be necessary to address these challenges.

In this work, we focus mainly on some of the common tools that can be used in the database forensics process, such as Autopsy, ILEAPP, ALEAPP, and FTK imager. We evaluated the usability of these tools for some of the processes in database forensics by conducting several experiments, involving custom database setups and using dumps from different devices, including mobile phones.

Our analysis shows that when examining database files, many tools are unable to extract Binary Large Objects (BLOBs) such as images, videos, and other multimedia objects from the data file. Also, in many cases, it was impossible to view database files based on their associated applications. Although most tools support searching within an overall file system, the ability to search or sort within records and fields in data files was absent in many tools. For mobile devices, the use of location information stored in databases on the devices as well as the ability to parse common database file type into a viewer was incorporated into many tools. In terms of reconstructing data deleted from a database, only a subset of the tools could recover the information from the disk. In some cases where data is recovered, that data can no longer be associated with the database. In other cases, only the unallocated memory space was identified and the data had to be manually recreated. In addition, dependent database files such as Write-Ahead Log (WAL) files and Shared Memory (SHM) files could not be extracted at all or in a way that preserved their dependency in many cases.

Our experiments also considered some of the known challenges of database forensics with respect to tools to evaluate how well some of the challenges have been addressed. The complexity of database systems and the interconnection of different database objects are some of the major challenges discussed in the literature. We note that while the retrieval of database files, especially in relational databases, can be done with some existing tools, complex structures such as indexes, schemas, and triggers still require a manual process to retrieve them. Also, processes such as handling database file formats that are not commonly used, capturing database memory, and handling encrypted information are all still particularly challenging when using existing tools for database systems. Although our experiments did not explore all possible digital forensics tools, these findings provide insights into many aspects of tool development that are still required for database forensics examinations.

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Database Systems; Digital Investigations; Tool Analysis

C18 Standards Development Activities in Digital and Multimedia Forensic Science

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WITHDRAWN

C19 Facial Identification Scientific Working Group Update

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WITHDRAWN

C20 An Update on the Status of a Facial Identification Certification Program

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Learning Objectives: To update attendees regarding the status of a Facial Identification (FI) Certification Program and the challenges/hurdles the developers face throughout the process. The presentation will cover, at a high-level: (1) requirements necessary for creation of a new certification program, (2) challenges to achieve "accreditation level" approval, (3) events and incidents that have heightened interest in a certification program, (4) stakeholders in the forensic community that are tracking the program's progress, and (5) anticipated launch window and expected initial participation.

Additional elements include: (1) a better comprehension of FI as a forensics discipline, (2) more insight into the certification program development process, (3) understanding the increasing need for highly qualified facial examiners and reviewers, and (4) seeing the growing interest in FI from the stakeholder community.

Impact Statement: Despite the fact that FI as a forensic discipline has a relatively short history (the Facial Identification Scientific Working Group [FISWG] was founded in 2009), the increased accuracy of Facial Recognition (FR) algorithms has increased the spotlight on the practice as well as the demand for law enforcement use. Professional courses of instruction and a valid, accredited certification program are a logical next step for this discipline.

Abstract Text: Law enforcement use of FR technology remains a controversial subject, but there is growing support for use of FR as part of an investigative enterprise pursuant to assisting in the identification (and potential apprehension) of perpetrators of criminal activity. In order to ensure the highest level of accuracy and mitigate potential misuse of this technology, training, proficiency, and certification of FR system end-users will be essential for acceptance from the legal community, the media and the general public.

This "Update on the Status of a Facial Identification Certification Program" is intended to further inform the forensic science community and other stakeholders regarding how and when a program of testing will be made available to facial identification professionals. Interest in the status of this effort reaches the highest levels of state and federal government organizations as there is general consensus that by establishing competency and proficiency assessments through vetted and valid testing programs, there will be significant improvement in the effectiveness of the overall FR system enterprise. As FR algorithms improve, the accuracy tends toward more challenging comparisons of possible source exclusions and inclusions. Just as Automated Fingerprint Identification Systems (AFIS) require a well-trained and experienced fingerprint examiner to adjudicate the algorithmic results of a search, an automated face identification system (or a multimodal biometric system that includes a facial algorithm) requires similarly trained and skilled facial examiners and facial reviewers to assess and compare resulting face image probe searches against a controlled capture known face database. Additionally, like latent print examiners, facial examiners are expected to work with extremely challenging comparisons. Often these unknown images are results of closed-circuit and/or surveillance camera stills with poor lighting, less than optimal pose, and often exhibit other image quality artifacts. Properly training and tested practitioners will understand how these challenges to comparison can be addressed and overcome. The presentation will include: (1) a summary of training and experience requirements to be eligible for certification testing, (2) a list of the reference materials necessary to prepare for testing, (3) a description of the practical and written tests, and (4) a breakdown of the two levels of certification (Reviewer and Examiner).

Facial Identification; Certification; Law Enforcement

C21 Distinguishing Head Poses From Camera Angles in Non-Frontal Images for Automated Face Identification

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Learning Objectives: After attending this presentation, attendees will understand the challenges encountered by state-of-the-art face processing algorithms. They will become familiar with performance results obtained from images taken with varying head poses and camera angles.

Impact Statement: This presentation will impact the forensic science community by illustrating how variations in head pose. caused by the subject's head movement and camera angle, resulting from changes in the camera's position, affect automated face identification.

Abstract Text: Changes in view have been identified as key factors that influence a person's physical appearance captured in images, consequently diminishing the performance of automated identification systems.1 The angle of view is a result of two distinct positions: head pose and camera angle. Head pose is intrinsic to the subject, referring to the orientation of a person's head relative to their body. It can be described in three main directions: roll (motion toward the right/left shoulder), yaw (right to left), and pitch (up and down), although any combination of these directions is possible, limited only by the subject's range of motion. Camera angle, by contrast, is extrinsic to the subject, referring to the position and orientation of the camera relative to the subject. It includes the height, distance, and angle at which the camera is positioned when capturing an image, often dictated by the camera's purpose (surveillance, communication) and the circumstances while in use (visibility). Both factors change the appearance of the face but in different ways. Head pose alters the alignment, visibility, and appearance of facial features. In contrast, camera angle changes the perspective and proportions. In studies of automated face identification, the effects of head and camera orientation have typically been considered together without further distinctions. However, with the rise of surveillance using drone-mounted cameras, where non-stationary camera angles collide with the subject's behavior, it has become crucial to properly distinguish between their separate effects on automated identification. Additionally, distinguishing between head pose and camera angle is essential when comparing 2D and 3D images, where the angle of view is simulated by rotating a 3D face.

This study tests the influence of view angle, separated into head pose and camera angle, on automated face processing, which includes face detection, view estimation, and face identification. The sample consisted of 145 participants, 74 males and 71 females of European descent, aged between 19 and 87 years. For each participant, an array of 75 photographs varying in head poses, camera height (from 0.87m to 2.24m), and angles (from 0° to ±145°) at a constant camera-to-subject distance of 0.83m was collected. In total, the dataset comprises 10,955 images with a resolution of 4208x3120 pixels. Additionally, each subject's 3D facial appearance was captured using the Vectra 3D imaging system. Head poses were determined according to a 3-point plane constructed using the right and left tragion and the intersection between the assumed line connecting the right and left infraorbital margins and the mid-sagittal plane.

The study tested selected algorithms for face detection (OpenCV, RetinaFace, YOLOv8 Face) and view angle estimation (MediaPipe, SPIGA, FLAME). For face identification, the performance of two open-source algorithms, ArcFace and MagFace, was explored.2,3 Dissimilarity scores were recorded for each pair-wise intra-/inter-person comparison. Additionally, facial typicality/uniqueness scores were computed as standardized Procrustes distances between a subject's 3D face and the mean. The performance was evaluated using Receiver Operator Curve (ROC) curves and likelihood ratios. To explore the effects of head and camera views, angles were expressed as sine and cosine values and treated as covariates.

The results revealed significant variation in the performance of face detection and angle view estimation algorithms when subjected to different head poses and camera angles. They also validated the need to address head poses and camera angles separately. ArcFace consistently underperformed compared to MagFace. Furthermore, the algorithms varied in how they handled intra-person and inter-person comparisons, which notably impacted their overall performance. Our study underscores the importance of thoroughly understanding algorithms and their performance under various real-world conditions to enhance the transparency of automated face identification in forensic settings and address the black-box problem.

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Facial Identification; Automated Algorithms; Head Poses

C22 A Global Interlaboratory Study: Assessing Consistency of Source Opinions for Face, Hands, and Clothing Image Pairs

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Learning Objectives: After attending this presentation, attendees will: (1) understand the source opinion categories provided in Organization of Scientific Area Committees (OSAC) Proposed Standard 2022-S-0001; (2) consistency of source opinions for face, hands, and clothing image pairs among both forensic practitioners and laypersons; and (3) considerations when conducting a global interlaboratory study.

Impact Statement: This presentation will impact the forensic science community by demonstrating of the value of OSAC developed standards for practical use in digital and multimedia forensics. This is the first verbal opinion document for comparisons to come out of OSAC and provides a framework for numerous digital/multimedia disciplines. Thus, it is imperative that practitioners who complete image comparisons have a standard that is straightforward, applicable to diverse subjects, and, when followed, permits reproducible results.

Abstract Text: Forensic image comparison is an assessment of the correspondence between features in questioned items depicted in images and either questioned or known objects or images for the purpose of rendering an expert opinion regarding identification or elimination. Images that are sent to forensic laboratories for comparison include but are not limited to faces (and other body parts/areas), vehicles, weapons, clothing, luggage, furniture, landscapes, and buildings. Trained forensic practitioners are tasked with determining whether there is sufficient support to conclude either common source or different source.

To harmonize the opinion categories used across the digital multimedia forensic disciplines for image comparisons, a task group consisting of individuals from the Digital/Multimedia Scientific Area Committee (DMSAC) of the OSAC for Forensic Science developed OSAC Proposed Standard 2022-S-0001, Standard Guide for Image Comparison Opinions. While this proposed standard was extensively developed with input from a broad range of United States-based forensic practitioners, the practical utility to reproducibly apply the opinion categories in 2022-S-0001 has not been assessed. To ensure this standard meets the OSAC program goal of developing technically sound standards and promoting their adoption through the forensic community, an interlaboratory study focused on 2022-S-0001 was conducted using face, hands, and clothing images. Two cohorts of individuals were recruited to participate: (1) forensic practitioners who have been determined to be competent by their respective agency, organization, or other entity, and currently conduct forensic casework on face, hands, or clothing image comparisons; and (2) laypersons composed of individuals trained in forensic science but not image comparisons, undergraduate/graduate forensic science students, and the general public.

Machine learning approaches were used to identify 100 face, hands, and clothing image pairs from over 125,000 images licensed to the North Carolina State from the University of Notre Dame Computer Vision Research Laboratory. Subject matter experts subsequently selected a total of 20 image pairs for each image category that included varying source (i.e., same or different) and difficulty (i.e., easy or hard comparison). Qualtrics was used to disseminate a survey to over 60 forensic practitioners and 100 laypersons and collect participants responses. This presentation will present the results on the distribution, reproducibility and accuracy of source opinion categories (as specified in 2022-S-0001) selected when completing image comparisons. Additionally, we will assess whether results are correlated to the participant cohort (e.g., forensic practitioner, laypersons), image subject (face, hands, or clothing), and the level of relevant expertise/training.

OSAC Proposed Standard; Image Comparisons; Digital and Multimedia Forensics

C23 An Evaluation of Interpolation Methods and Their Impact on Resizing Images: Is the Nearest Neighbor Algorithm the Best Solution in All Situations?

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Learning Objectives: Many video and image forensics training organizations have taught and currently teach when resampling images/videos (enlargement, line duplication, correcting pixel dimensions and aspect ratios, etc.) that the nearest neighbor algorithm may be the most accurate and reliable method to "forensically" conduct this process in certain circumstances. Many forensic practitioners in the Digital and Multimedia Evidence (DME) field contend this resampling algorithm is the only "valid" approach to produce accurate and reliable data for use in forensics when the resampling algorithms are applied to preserve the original image pixel details and identify small and/or discrete objects (e.g., weapons in hands, tattoos, license plate characters, etc.). Although this method is being taught and recommended for specific forensic purposes, very little if any independent and objective scientific research has been conducted to evaluate these statements and this approach. This research was conducted to start this scientific discussion and to evaluate these claims using carefully designed experiments and a novel approach to resampling test images. Various statistical techniques were used to evaluate the results.

Impact Statement: This work used a novel scientific method to generate various known test images containing specific content (down-sampling). These test images were generated in a very careful and specific process such that the resampling algorithm used to resize (up-sample) the test images to the original pixel dimensions (size) could be compared to the original starting test image in a statistical manner (quantitative analysis) to assess any difference the algorithm had introduced in the process. We compared the interpolated image to the original image to assess the statistical differences between the images and which algorithm was most similar and dissimilar to the original unaltered test image.

Abstract Text: Digital forensic laboratories receive video and image data in a wide variety of formats, pixel dimensions, and levels of compression. Many of these files are from Digital Video Recorders (DVRs), body-worn cameras, mobile devices, and other surveillance systems. Many of these recording systems generate data in a proprietary format or do not conform to standard video and/or image pixel dimensions (height x width) and aspect ratios (width divided by height). Many times, this data is in a non-standard aspect ratio, and this must be corrected to accurately represent the real-world scene as it was captured. This involves resampling the data in the image/video typically into larger standard dimensions and aspect ratios. This resampling is accomplished by interpolation algorithms.

For many years, forensic practitioners have been taught that the nearest neighbor algorithm may be the most accurate and accepted method to accomplish this task. In this research, we reviewed the common and generally accepted interpolation algorithms available to forensic practitioners and tested them on known data to evaluate the pros and cons of each approach. Several difference statistics were used to evaluate the changes to the base image through the interpolation process to assess how similar the resampled data is to the original. Several methods of evaluating the effect of the interpolation algorithms on the resized data were used to assess the mathematical similarities of each interpolation approach. The final analysis involved applying these techniques to real-world image data to determine if there are situations where one resampling algorithm is more or less appropriate based on the statistical analysis of these differences. Various statistical models were applied to attempt to determine the meaning and interpretation of these differences and what variable(s) may be at play, if any. This is the first step in scientifically evaluating the various resampling approaches to see if there are any differences and potential impacts.

Digital Evidence; Forensic Imaging; Forensic Science

C24 Identifying Social Media Video Provenance Through Forensic Structural Analysis

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Learning Objectives: Attendees will gain a clear understanding of how social media platforms alter video files upon upload, leaving unique digital "signatures" that can be used to verify the provenance and authenticity of video evidence. This knowledge will enhance their ability to investigate the provenance of video files.

Impact Statement: This presentation will equip the forensic science community with advanced methods for identifying the provenance of video files altered by social media platforms. Practitioners will have a more informed approach to authenticating digital evidence, ultimately leading to more accurate and reliable investigative processes.

Abstract Text: This study explores the hypothesis that Social Media Platforms (SMPs) introduce unique, detectable alterations to video files, which can be utilized to verify their provenance. As digital media increasingly plays a critical role in forensic investigations and legal proceedings, understanding how SMPs modify video files is crucial for maintaining the integrity of digital evidence. This research aims to identify and analyze these alterations to enhance forensic methodologies.

The study employed in-depth forensic structural analysis to examine video files before and after their upload to various SMPs. The primary objectives were to determine whether video files undergo structural changes upon upload, to identify and catalog these changes, and to assess the consistency of these alterations across different devices and operating systems. Additionally, the research sought to discover whether these changes could be used to establish a unique, repeatable "signature" for each SMP.[1]

A diverse dataset of video files was collected from several SMPs, including TikTok, Facebook ,and Instagram, using various devices and operating systems. Forensic analysis focused on metadata changes, such as file size and codec information, as well as patterns in structural alterations, including processing and conversion artefacts. Metadata parsers, such as ExifTool, were used to extract the relevant metadata for collation and comparison.

The results revealed that SMPs impose distinct and consistent modifications on video files. These modifications include metadata removal, varying degrees of compression, and specific encoding practices. These findings confirm the hypothesis that each SMP introduces unique digital "fingerprints" that can be detected and used to trace the video's origin. This aligns with previous research, which have similarly found and utilized these "fingerprints" in their investigations.[2-4]

This research addresses significant challenges in digital forensics by providing a methodological framework for identifying SMP-specific alterations. The unique markers identified for each platform enable forensic experts to more accurately determine the provenance of video evidence, even when traditional metadata is missing or manipulated. This capability is critical in verifying the authenticity of video evidence, a growing concern given the proliferation of manipulated media online.[5,6]

In conclusion, this study contributes to the field of digital forensics by enhancing the tools available for video provenance verification. By establishing a database of identifiable SMP-induced artifacts, forensic analysts can improve their ability to authenticate video evidence and support the integrity of judicial processes. This advancement not only strengthens forensic methodologies but also provides a foundation for future research in social media forensics, addressing the growing challenges posed by digital media manipulation.

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Digital Evidence; Social Media; Video Analysis

C25 The Application of Likelihood Ratios to Audio Authentications: Selected Preliminary Results

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Learning Objectives: Attendees will learn the following:

- What likelihood ratios are
- Why they are considered the most logical form of evaluation of evidence within forensic science
- A proposed method for the evaluation of audio authentication examinations using likelihood ratios
- Some selected results pertaining to the within-source variability of a selection of parameters
- Some selected results pertaining to the between-source variability of a selection of parameters
- Some selected evaluations using likelihood ratios based on the within and between source variability

Impact Statement: The purpose of an audio authentication examination is for the examiner to opine on the authenticity of a recording. The current method recommended within guidance published by the Scientific Working Group on Digital Evidence (SWGDE) is to conclude whether a recording is consistent or inconsistent with an original recording. The research presented will provide a method and some preliminary results using likelihood ratios rather than the currently recommended method. The motivation behind the study is to push the audio authentication discipline forward by using a format that is widely accepted as the most logical method for evaluation of evidence within forensic science

Abstract Text: Although there are various methods for reporting when addressing the question of source problem within forensics, for example, verbal levels of support, categorical match/non-match, numerical scores and likelihood ratios, it is the latter that is considered by some to be the most logical method.[1-3] This is evidenced by its widespread use and success when opining on the source DNA trace evidence.

The reporting method currently recommended for audio authentications is the use of 'consistent with,' inconsistent with,' or no conclusion, based on the premise that if an audio recording is observed to be consistent with an original, the possibility of editing having occurred can be refuted.[4,5] This method only considers a single proposition and does not, therefore, communicate the significance of the observations. In order to do so, an alternative proposition would need to be introduced to inform the trier of fact how consistent or inconsistent the observations are within a relevant population. This is an element that likelihood ratios resolve, as two propositions are fundamental to their use.[6]

After attending the presentation, attendees will learn the motivation for the study, the overarching method and the methods to consider the three segments into which the research has been broken down, namely: within (or intra) source variability, between (or inter) source variability, and the evaluation of results using likelihood ratios. They will also learn of difficulties in applying likelihood ratios when inferring the source of an item that is mass-manufactured and a proposed definition of source that inherently reduces within-sample variability while increasing between-sample variability.

The research forms part of a PhD study relating to the use of likelihood ratios for audio authentication evaluation, which is currently at its halfway stage.

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Score Likelihood Ratios; Evidence Evaluation; Multimedia

C26 Keeping Up With the Times: Are Standards Meeting the Needs of Rapidly Expanding Technology in Forensic Multimedia Analysis?

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Learning Objectives: After this presentation, attendees will have an understanding of the challenges and capabilities that technological advances have created within forensic multimedia examinations. Additionally, attendees will learn how the work being conducted on standards and best practices within the Video/Imaging Technology & Analysis (VITAL) Subcommittee at the Organization of Scientific Area Committees (OSAC) and at the Scientific Working Group on Digital Evidence (SGWDE) is being used to address the changing forensic multimedia needs arising from technological developments.

Impact Statement: This presentation will impact the forensic science community by offering information on the ongoing efforts that support and address the ever-expanding technological developments within multimedia forensics. Information on the current work efforts within the OSAC VITAL Subcommittee and SWGDE will be highlighted.

Abstract Text: Forensic multimedia analysis has long been impacted by and intertwined with technological advancements. From the invention of the digital camera in the 1970s to the expansion of synthetically generated media today, challenges are presented to the forensic multimedia community on how to incorporate and efficiently address the rapid evolution of multimedia technology. Such developments have expanded this field through the creation of new tools and capabilities available to examiners; however, when such technology is analyzed and employed in a forensic setting, boundaries must be established to ensure high-quality, technically sound standards and best practices are employed.

Advancements in image capture and video recording have improved the quality and accessibility of multimedia evidence and enhanced the capabilities available to forensic multimedia examiners. The prevalence of high-resolution cameras, the increased reliance on smart phones, the widespread use of surveillance systems, and the pervasiveness of social media have led to an exponential increase in the volume and complexity of multimedia data available. Furthermore, significant impacts to digital and multimedia analysis have been introduced through the use of tools powered by Artificial Intelligence (AI) and Machine Learning (ML). The acceptance and use of such tools has blurred the lines between what is possible with imagery and what should be done to ensure imagery is not deceptive. The expansion of the capabilities and work volume, as well as ambiguity in the application of new technology, present new questions and challenges demonstrating the need for up-to-date standards and best practices for handling multimedia evidence in forensic settings.

Organizations, such as the OSAC VITAL Subcommittee and the Scientific Working Group on Digital Evidence (SWGDE), both focus on developing and prompting standards and best practices to support examiners in their analysis of multimedia evidence. As the development of new technologies will continue, such standards related organizations must be continually prepared to update their documents while simultaneously emphasizing the importance of validation, reproducibility, and transparency in forensic processes. These organizations must address the challenges for defining standards and best practices that quickly adapt to the changing needs of the forensic multimedia community.

Up-to-date standards and their implementation are essential to ensuring the sound practice of forensic science across all disciplines, including digital and multimedia sciences. However, rapidly expanding technology presents a challenge to both examiners conducting multimedia analysis as well as the organizations developing standards and best practices. Ensuring the reliability, validity, and admissibility of multimedia evidence in this dynamic environment requires continuous adaptation and adherence to current, relevant standards and best practices.

Multimedia; Standards; Imagery

C27 Detecting Generative AI Through the Structural Analysis of Multimedia Files

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Learning Objectives: After attending this presentation, attendees will understand how file structural analysis can be utilized to detect multimedia files created or modified through use of generative Artificial Intelligence (AI). As a result, attendees will also develop an understanding of possible limitations when it comes to the identification of synthetic media and the associated ethical responsibility of industry leaders.

Impact Statement: This presentation will impact the forensic science community by exploring novel techniques for the authentication of multimedia files. Specifically, this presentation will compare the output of a variety of popular generative AI software at the structural level.

Abstract Text: There has been a mass influx of synthetic media generation and multimedia modification utilizing AI, making it more important than ever to be able to reliably and effectively identify and apply context to media files. AI is an extremely useful tool for a wide number and variety of industries but can present strong ethical concerns when it comes to engendering trust in a multimedia file, especially in an era where generative AI is becoming much more readily available to the general public. Although many market leaders in multimedia production are taking measures to show AI-generated or modified content, current approaches are not reliable since they lack any independent authenticity analysis.[1,2] Many current methods utilized to identify or label AI media online require the user to self-identify their AI-assisted content, which places a lot of trust in content creators to self-identify generative AI.

Many detection and analysis tools, forensic and open-source alike, claim to be able to detect generative AI through probability analysis. While this may initially appear helpful, these "black box" methods are unable to be validated by digital forensic examiners or consumers and often provide percentages of probability for a given observation with little to no explainability. This study aims to provide a reliable and reproducible method for identifying synthetic or manipulated media produced from generative AI.

For this work, several of the most popular generative AI software were utilized to both create multimedia files and modify existing content. The produced multimedia files were analyzed at the structural level utilizing a file format forensic approach to apply context[3], both through provenance and generational history, to allow for a transparent method for evaluating synthetic media that is both repeatable and meets legal admissibility standards.[3,4]. Additionally, ethical concerns and considerations regarding possible multi-generational implications (i.e., structural normalization) will be discussed.

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Artificial Intelligence; File Format Forensics; Multimedia

C28 Artificial Intelligence for Image and Video Forensics: Mine of Information or Minefield?

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Learning Objectives: Attending this presentation will help attendees understand the difference between AI, machine learning, and deep learning. Through simple examples, they will see the impact of training data bias on forensic applications. Finally, they will discover the opportunities and pitfalls of deep learning-based techniques for video forensics.

Impact Statement: The presentation aims to increase awareness of the pitfalls of using deep learning techniques for video enhancement and analysis. Attendees will learn to weigh the explainability of an AI method and will learn the importance of implementing bias-mitigation techniques in the use of AI systems. Correct handling of AI techniques can significantly impact justice and security.

Abstract Text: This presentation consists of three parts and discusses the opportunities and challenges of using AI-based technologies for image and video forensics, specifically focusing on modern deep-learning solutions.

The first part introduces some terminology related to machine learning and distinguishes investigative use from evidentiary use and image enhancement from image analysis. It then discusses how typical deep-learning techniques compare against the accuracy, reproducibility, and explainability requirements, showing with a simple yet insightful example that training data can dramatically impact the result of a super-resolution system based on AI. Finally, it proposes some general guidelines for using AI for video forensics to foster discussion at the end of the presentation and during the conference.

The second part shows the results of research conducted by the authors on using AI for three common tasks:

1. AI-based image super-resolution to aid humans with face identification.[1] The experiment compares the performance of humans when presented with AI-scaled faces with those obtained using bicubic-scaled faces. Results show that the improvements brought by AI super-resolution in recognition rate are not significantly different from those brought by bicubic interpolation. The results of a similar study focusing on fully automated face recognition will also be mentioned.[2]
2. AI-based reading of severely degraded license plates. The experiment evaluates the ability of a neural network trained on synthetic license plates to decipher real license plates, showing results obtained from an extensive dataset of 2,400 real plate images of varying difficulties.[3]
3. AI-based detection of deepfakes, specifically Generative Adversarial Network (GAN) -generated images. The developed method ranked second in an international challenge.[4] The presentation will also highlight some interesting properties of diffusion-model deepfakes that can help their detection even with traditional analysis tools.

The third and final part mentions some cases presented in the news in which the jaunty use of AI-driven methods led to mistakenly arrested people and discusses a recent court case that has challenged the suitability of AI for video enhancement.

After attending the presentation, attendees will better understand the opportunities and pitfalls of using deep learning-based methods for forensic and investigative applications.

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Artificial Intelligence; Deep Learning; Deepfakes

C29 Deepfake Detection: Technology as a Tool for Transformation or Tyranny

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Learning Objectives: Attendees will gain insights into the technical, legal, and ethical dimensions of deepfake detection, empowering forensic professionals to navigate the complexities of this evolving landscape.

Impact Statement: The session will underscore the necessity of a multi-disciplinary approach, combining technological expertise with robust ethical standards to ensure that deepfake detection serves as a tool for transformation, safeguarding truth and justice in the digital age.

Abstract Text: The proliferation of deepfake technology, fueled by advances in generative AI, poses significant challenges and opportunities for forensic science. This presentation at the 2025 American Academy of Forensic Sciences conference explores the dual role of deepfake detection technology as both a transformative tool and a potential instrument of tyranny. Deepfakes, which leverage advanced AI to create highly realistic but fake media, threaten to undermine trust in visual and auditory evidence, complicate legal proceedings, and enable new forms of cybercrime.

This session will delve into the latest advancements in deepfake detection methods, with a particular focus on Photo Response Non-Uniformity (PRNU) analysis, Electrical Network Frequency (ENF) analysis, and other state-of-the-art algorithms and machine learning techniques designed to identify manipulated content with high precision. PRNU, a technique used to trace the unique sensor pattern noise of digital cameras, has emerged as a critical tool in forensic investigations, enabling the detection of synthetic images and videos generated by AI (Informatics Institute) (Netherlands Forensic Institute). Similarly, ENF analysis leverages the fluctuating frequency of electrical grids captured inadvertently by recording devices to timestamp and geolocate video recordings, adding another layer of verification to digital evidence (Netherlands Forensic Institute).

We will discuss the implementation of these technologies in forensic investigations, emphasizing their potential to uphold the integrity of evidence and support justice. The integration of generative AI with forensic methods, such as PRNU and ENF, allows for a comprehensive approach to deepfake detection. For instance, ENF analysis can reveal inconsistencies in the time and location of recordings, thereby identifying discrepancies that suggest tampering (Netherlands Forensic Institute). When combined with PRNU, which identifies the specific device used to capture an image or video, these techniques provide a robust framework for authenticating digital media (Universiteit van Amsterdam) (Netherlands Forensic Institute).

Conversely, the presentation will address ethical concerns and the risk of misuse of deepfake detection technology. Topics include the implications for privacy, the potential for overreach by authorities, and the danger of false positives leading to wrongful accusations. The ethical use of these technologies requires a careful balance between the need for security and the protection of individual rights. By examining case studies and current legal frameworks, we aim to provoke a critical discussion on the balance between technological innovation and ethical responsibility.

Attendees will gain insights into the technical, legal, and ethical dimensions of deepfake detection, empowering forensic professionals to navigate the complexities of this evolving landscape. The session will underscore the necessity of a multi-disciplinary approach, combining technological expertise with robust ethical standards to ensure that deepfake detection serves as a tool for transformation, safeguarding truth and justice in the digital age.

Multimedia; Video Analysis; Deepfakes

C30 Leveraging Artificial Intelligence for Advancements in Forensic Science: The AI4Forensic Lab

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Learning Objectives: The goals of this presentation are to help attendees:

- Understand the impact of AI on forensic science: Attendees will gain insight into how AI technologies are revolutionizing forensic methodologies, enhancing accuracy, and improving the efficiency of forensic investigations.
- Explore key AI applications in forensics: Participants will learn about specific AI applications such as image and video analysis, digital forensics, biometric facial comparison, and predictive modeling, and how these applications are implemented in real-world scenarios.
- Recognize the importance of ethical compliance: The session will highlight the importance of adhering to regulatory frameworks like the AI Act, ensuring that AI technologies are used responsibly and ethically in forensic science.
- Enhance knowledge of man-machine interaction: Attendees will understand strategies to improve collaboration between forensic experts and AI systems, ensuring effective integration and utilization of AI in forensic workflows.

Impact Statement: Attendees will learn how to:

- Identify Bias and Ensure Fairness: The presentation will cover methods to detect and mitigate biases in AI algorithms, ensuring that forensic analyses are fair and unbiased.
- Emphasize Transparency and Explainability: Participants will learn about the critical role of transparency and explainability in AI systems, ensuring that AI-driven conclusions are understandable, trustworthy, and legally defensible.

Abstract Text: The application of AI in forensic science holds transformative potential, promising unprecedented advancements in accuracy, efficiency, and scope of forensic investigations. The AI4Forensic Lab is at the forefront of this evolution, dedicated to integrating cutting-edge AI technologies into forensic methodologies. This abstract outlines the Lab's pioneering research, key projects, and the profound implications of AI in forensic science, with a specific focus on the AI Act, biometric facial comparison, man-machine interaction, bias prevention, transparency, and explainability. Forensic science has long been reliant on meticulous manual analysis, often constrained by human error and time-intensive processes. The AI4Forensic Lab aims to revolutionize this field by leveraging AI to enhance various aspects of forensic analysis. Our multidisciplinary team, comprising experts in AI, computer science, and forensic science, is working on developing and implementing AI-driven solutions that address critical challenges in forensic investigations, while adhering to regulatory frameworks such as the AI Act.

The primary objectives of the AI4Forensic Lab are to develop AI algorithms capable of analyzing forensic data with high accuracy, automate forensic processes to reduce the time and effort required for investigations, and improve the interpretation of complex forensic data using advanced AI techniques. Additionally, we aim to facilitate real-time decision-making in forensic scenarios and ensure ethical compliance by aligning our AI developments with the AI Act.[1] Central to these efforts are transparency and explainability, ensuring that AI-driven conclusions are understandable and trustworthy. Performance evaluation is conducted thoroughly to ensure the reliability, accuracy, and applicability of AI models in real-world forensic scenarios. We collaborate closely with law enforcement agencies, legal experts, and forensic practitioners to ensure the practical integration of AI solutions into forensic workflows. Enhancing man-machine interaction is a key focus, ensuring seamless collaboration and effective use of technology in investigations. Additionally, we implement strategies to identify and mitigate biases in AI algorithms, ensuring fair and unbiased forensic analyses. Ensuring transparency and explainability in our AI systems is paramount, as it builds trust and facilitates the understanding of AI-driven conclusions by forensic experts, legal professionals, and the public.

The integration of AI into forensic science offers numerous benefits, including enhanced accuracy, reduced workload for forensic experts, and faster resolution of cases. As the AI4Forensic Lab continues to advance its research, we anticipate significant breakthroughs that will shape the future of forensic science. Our ongoing efforts aim to make AI an indispensable tool in forensic investigations, ultimately contributing to the improvement of the criminal justice system. By adhering to the AI Act and focusing on ethical AI usage, we ensure responsible and transparent application of AI technologies. Transparency and explainability will remain central to our research, ensuring that AI-driven forensic methods are clear, interpretable, and legally defensible.

The presentation will cover several key topics, including the development and implementation of the AI4Forensic Lab's research agenda, the impact of AI on various forensic disciplines such as image and video analysis, digital forensics, biometric facial comparison, and predictive modeling. Additionally, we will discuss the ethical considerations and regulatory compliance, particularly in relation to the AI Act, strategies for enhancing man-machine interaction, and efforts to prevent biases in AI algorithms. Furthermore, we will showcase our current projects and funding initiatives, including work on deepfakes, data hiding, speech analysis, and financial data analysis. The broader context of our collaboration with national and international institutions will also be highlighted, along with future funding possibilities and research directions. Special attention will be given to the topics of transparency and explainability, illustrating how our AI systems ensure clear, interpretable, and justifiable outcomes.

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Artificial Intelligence; Accuracy; Research

C31 The Development of a Responsible AI Framework Suitable for Forensic Science Needs

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WITHDRAWN

C32 Attitudes Toward and Barriers to Mental Health Support for Digital & Multimedia Forensic Examiners

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Learning Objectives: After attending this session, attendees will have a better understanding of digital and multimedia forensic examiners' attitudes toward counseling and barriers to receiving mental health support from being exposed to disturbing media as part of their job.

Impact Statement: The presentation will impact the forensic science community by explaining the mental health stigma perceived by digital & multimedia forensic examiners, impacting their decision to receive counseling/treatment. It will also explain their attitude toward mental health support

Abstract Text: Digital evidence is estimated to be a factor in about 90% of criminal cases.[1] Digital & multimedia forensic examiners work on a variety of criminal cases, such as burglary, child sexual exploitation, school shootings, police-involved shootings, rape, fraud, and drug-related offenses.[2] These investigations usually involve more than one type of digital device (e.g., mobile phone, computer, Global Positioning System [GPS], video surveillance) and mobile phones are the most-seized device in criminal investigations.[3]

We now have more audio, video, and image evidence of the actual crime itself than ever before. As a result, digital & multimedia forensic examiners are often requested to review or enhance probative media to identify the actors or clarify the context of the situation, all of which may involve repeatedly viewing or hearing heinous acts (e.g., homicide, child sex abuse, torture).[4] Research shows that digital forensic examiners experience high levels of stress due to the nature of their jobs involving exposure to disturbing media, including anxiety, poor psychological well-being, secondary traumatic stress, and emotional burnout.[5-9] This study aimed to understand digital & multimedia forensic examiners' attitudes toward and experiences with barriers to counseling and mental health support.

Ninety-four digital and multimedia forensic examiners from the United States completed an anonymous, internet-based survey assessing a needs analysis for mental health support. Participants self-reported their current primary role as either an image, audio, or video forensic examiner. In addition, participants reported whether they currently worked as an investigator or detective. Most digital forensic examiners reported multiple primary roles: image (n = 50), audio (n = 27), or video forensic examiner (n = 56). In addition, 53 worked only as a Digital Forensic Examiner (DFE), whereas 41 were both digital forensic examiners and investigators (DFE + DET). Results compared their attitudes toward and experiences with barriers toward mental health support based on their self-reported primary roles. Of the 94 digital forensic examiners, 36% personally sought counseling due to work-related stress. Mental health support was required for only 28% of respondents. However, 50% of participants believed counseling should be required.

Several barriers toward mental health support were reported by the digital & multimedia forensic examiners. Image analysts were more likely to report: "I don't know where to get help" (r = .27), "There would be difficulty getting time off work for treatment" (r = .24), "I find it difficult to talk about" (r = .37) compared to non-image analysts. Audio forensic examiners were more likely to report: "It is difficult to get an appointment" (r = .19) and "I find it difficult to talk about" (r = .19) compared to non-audio analysts. Video forensic examiners were also more likely to report that they have difficulty getting an appointment (r = .19) compared to non-video examiners. Finally, there was a small effect size in that individuals working both as DFE + DET were more likely to report that "It might affect my promotion" (r = .15) compared to individuals who only worked as DFEs.

Additional findings and suggestions for future research and policy implications regarding mental health services for digital and multimedia forensic examiners will be discussed.

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Mental Health Support; Digital Forensic Examiner; Multimedia Forensic Examiner

C33 Emerging Ethical and Technical Challenges in Digital Profiling

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Learning Objectives: Attendees will learn how new technologies can both enhance and complicate digital behavioral analysis. Using victimology, case linkages, and the interpretation of digital artifacts, the use of these technologies will be examined through an ethical lens. Attendees will learn techniques directly applicable to these areas in addition to being provided ethical considerations for applying behavioral analysis to traditional digital forensics work.

Impact Statement: This presentation will bring together evidence-based concepts from psychology and digital forensics and will present challenges from recent criminal cases in these areas. Because digital profiling is a relatively new topic area, this presentation will provide an introduction to the field for many attendees through the coverage of selected real-world technical challenges and solutions in addition to the ethical considerations in applying them.

Abstract Text: After attending this session, attendees will have a better understanding of the risks and limitations associated with behavioral analysis based on digital forensics and data analytics. This will be presented by examining both human and technology factors using current digital investigative case trends within the context of an ethical framework.

This session will review the intersection of digital forensics and data science based on current and emerging tools and techniques, including Artificial Intelligence (AI) and big-data-based approaches. The ethical considerations when using these tools and techniques and the limitations present as a result of examiner bias as well as issues with the underlying technologies will be examined through a behavioral analytical lens.

While digital forensics has matured as a discipline, new challenges have arisen that were not present even a few years prior. AI has provided new opportunities to conduct rapid reviews and assessments, but many of the implementations have poorly understood technical limitations and lack traceability. Additionally, AI tools may exacerbate examiner confirmation bias if used improperly. Finally, generative AI deepfakes and inpainted images and videos may hinder victim identification or mislead investigators if identified as “ground truth” in examinations.

Similar to the challenges presented at an individual device or case level, linkages between cases can be inappropriately made or overstated, leading to inefficient deterrence or even improper attribution. Digital artifacts used in link analysis, including IP address and phone numbers, need to be understood in the context of Voice over IP (VoIP) recycling, carrier grade network address translation, Virtual Private Networks (VPNs), and other technological countermeasures. Similarly, an understanding of current trends in cybercriminology, including the outsourcing of expertise for functions such as botnets and money laundering, can lead to improper conclusions that disparate criminal networks are co-conspirators if not taken into account when utilizing graph databases. Finally, the blending of subjects and victims (e.g., a romance scam victim that becomes a money mule) needs to be incorporated into these analyses when performing targeting.

In addition to the technological challenges, human bias remains present and can be exacerbated through an overreliance, improper use, or misunderstanding of the limitations of digital tools and data analytic approaches. Ways of ensuring that personnel understand the limitations of digital forensics approaches, particularly those using data science and AI (including concepts such as base rates, sensitivity, and specificity) will be covered. Accountability and oversight of digital investigative personnel, and training of those personnel to recognize bias in themselves and their work, are equally essential and systemic improvements that can be operationalized and will be presented.

Artificial Intelligence; Digital Evidence; Behavioral Science

C34 Exploring Fantasy Enactment and Self-Disclosure Themes in Offender-to-Offender Communication: A Comparative Study of Adult and Peer-to-Peer Groomer Digital Evidence

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Learning Objectives: In this session, attendees will learn about the dynamics of offender-to-offender communication in peer-to-peer grooming, a growing form of child sexual exploitation and abuse globally. Additionally, they will learn about the distinctions in communication patterns between adult groomers and minor groomers in these scenarios.

Impact Statement: This presentation will impact the forensic community by providing an in-detail case study involving peer-to-peer grooming cases. There are many aspects of peer-to-peer grooming that are yet to be studied, and this presentation will aid forensic professionals to better understand and identify peer-to-peer grooming from a psychological, technological, and forensic standpoint.

Abstract Text: According to a study published in the Journal of the American Medical Association Network Open, one in six United States children experience online sexual abuse.[1] With a growing number of cases, the concept of Harmful Sexual Behavior (HSB) is becoming more prevalent. HSB is considered any sexual action that is outside of what is safe for a young person's stage of development. HSB contributes to the growing number of peer-to-peer grooming cases, and the level of aggression and violence can be seen in these peer-to-peer grooming cases. Research suggests aggression and glorification of rape can be tied to the prevalence of rape and aggressive sex that is shown in media.[2]

While prior work has examined online sexual abuse processes occurring between an adult and a minor, peer-to-peer grooming between minors has recently garnered attention. This case study compares the differences in fantasy enactment and negotiation in offender-to-offender communication in both minor peer-to-peer grooming and adult online sexual grooming scenarios, which have yet to be studied. This case study covers two separate cases: one in which adult offenders discuss online sexual abuse and one in which minor offenders discuss online sexual abuse. The adult offender cases include two chats between two adults regarding child online sexual grooming, while the peer-to-peer grooming covers ten chats between two minors. This case study analyzes the differences in fantasy enactment and self-disclosure themes between adult offenders and peer-to-peer groomers to gain a deeper understanding of these dynamics in offender-to-offender chats involving child sexual abuse. By understanding these dynamics, researchers and law enforcement can develop more effective intervention and prevention strategies tailored to the specific communication patterns and motivations of different offender groups. With more than 33% of sexual offenses against children being committed by other children, it is important to further understand the fantasy enactment and discussions that take place in peer-to-peer grooming to reduce this statistic.[3]

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Peer-to-Peer Grooming; Online Sexual Grooming; Cyber Crime

C35 Proactive Detection of Child Sexual Abuse Material: Predicting Victim Risk From App Usage, Media Content, and Messaging Behaviors

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Learning Objectives: Attendees of this presentation will have a better understanding of how digital artifacts from a victim's smart phone relating to app usage, media content, and messaging can be of use in a Child Sexual Abuse Material (CSAM) investigation. Further, attendees will gain knowledge on whether such artifacts collected from a minor's (i.e., an individual under the age of 18) smart phone can be used to predict offender risk and victim vulnerabilities in this type of case.

Impact Statement: This presentation will be of great interest to not only the Digital & Multimedia Sciences forensic community but also the entire forensic science community, as information relating to both digital artifacts and CSAM cases will be discussed. Because this study specifically delves into how social networking digital artifacts can play an important evidential role in a CSAM investigation, Law Enforcement (LE) officials who investigate such cases will gain an understanding of the specific information they can obtain from analyzing these artifacts in a CSAM investigation.

Abstract Text: Smart phone usage by the youth community has continued to increase, and minors are using such devices as their main source of communication. A 2018 survey indicated that 89% of teenagers ages 13–17 years had a smart phone, an amount which had doubled since 2012.[1] With the growth in the amount of young people using messaging, social networking, and media-sharing apps by means of their smart phones, the risk of child sexual offenders using such apps to contact, groom, and inquire for CSAM also increases.

In LE investigations, social networking digital artifacts such as the use of social networking websites/apps, the sharing of media content, and messaging behaviors, can offer valuable insight regarding the communication between a child sex offender and their victim. Previous research has indicated the importance of analyzing networking artifacts from the offender's smart phone to better understand child sex offender motivation.[2] Therefore, this study is a continuation of previous research and aims to determine whether such digital artifacts collected from a victim's smart phone can aid in predicting offender risk and victim vulnerabilities in CSAM cases.[2]

For this study, LE investigators from the Tippecanoe County High Tech Crime Unit (HTCU) randomly selected several closed CSAM cases where a forensic image of the victim's phone was present. The research team was then provided with only statistical information (f, %) for various social networking digital artifacts (e.g., list of apps, number of messages) and pornography collection (e.g., % of CSAM, pornography content type), obtained from the forensic image of the victim's device; Investigation details were not disclosed. A comprehensive analysis of the provided data was completed by the research team to predict details about the case (e.g., type of child sex offense, method of contact by the offender), offender risk, and victim vulnerability. Results from the research team's analysis were shared with the HTCU and scored for accuracy by LE.

The results and future implications of the study's findings will be discussed in the future, as the research team is awaiting additional data from the HTCU. Our team aims to receive data from at least 30 CSAM cases.

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Social Networking Digital Artifacts; CSAM Investigations; Digital Forensics

D1 A Study on the Application of a Thermal Mannequin for Estimating a Hypothermia Environment

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WITHDRAWN

D2 Active Air Sampling and Quantitation of Methyl Methacrylate During Controlled Pyrolysis of Poly-(Methyl Methacrylate)

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Learning Objectives: Attendees will learn why Poly-(Methyl Methacrylate) (PMMA) is an ideal polymer for validating analytical instruments like the Fire Propagation Apparatus. They will understand the challenges of air sampling in the fire environment as well as the value in applying and optimizing existing environmental sampling methods to address chemical dangers in fire effluent.

Impact Statement: The forensic science community will gain a deeper understanding of the experimental methods that are employed to measure material properties in the fire environment and how the results are used to validate fire simulations. The methods described here may be applied to study the thermal degradation of other materials and the representative sampling of fire effluent. Future research that addresses material behavior in fire will be valuable for investigators.

Abstract Text: PMMA is a polymer commonly used in fire engineering to evaluate the performance of analytical instruments and validate fire simulations.[1] Its simple structure and well-validated thermal decomposition characteristics make it an ideal compound for fire science simulations. The yield of generation of Methyl Methacrylate (MMA) monomer during the pyrolysis of PMMA has been assumed to be over 90%, but few quantitative samplings and measurements have been done to obtain exact values.[1,2] Active air sampling is a versatile technique used to identify and measure Volatile Organic Compounds (VOCs), Polyaromatic Hydrocarbons (PAHs), and other harmful species in fire effluent.[3,4] In this study, we used active air sampling on sorbent tubes to collect effluents from controlled pyrolysis of PMMA, coupled with gas chromatography to find yield for the generation of MMA under various conditions.

The Fire Propagation Apparatus (FPA) was used to reproducibly pyrolyze black PMMA with constant heat flux at 25, 50, and 75kW/m^2. The effluent was diluted with air and sampled from the duct onto charcoal sorbent tubes for the duration of the pyrolysis. After collection, the sorbent material was transferred to a vial and extracted with carbon disulfide at room temperature. The extract was analyzed for MMA via Gas Chromatography/Mass Spectrometry (GC/MS). A calibration curve was generated using reference standards at various concentrations. MMA was quantified in the effluent with results indicating percent yield greater than 85% for all heat fluxes. No significant difference was observed between heat fluxes. Excellent repeatability was achieved at the desired concentration level with a Relative Standard Deviation (RSD) of 2.3%. The limit of detection and limit of quantitation are 11.53ng and 39.27ng, respectively. The method is capable of adsorbing, separating, and identifying 13 potential VOC and PAH interferences. This work presents a robust method for active air sampling and GC/MS analysis of methyl methacrylate and fire effluent, laying the groundwork for future research into material degradation at high temperatures and the identification of toxins in the fire environment.

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Fire Propagation Apparatus; GC/MS; PMMA

D3 An Examination and Analysis of False Negative in Presumptive Blood Testing Methods on Leather

Sarahy Magdaleno, MSFS, Rutgers University, Somerset, NJ*

Learning Objectives: This presentation will demonstrate the importance of examining and analyzing suspected blood stains on leather and the likelihood of producing false negative results in presumptive testing. After attending this presentation, attendees will better understand that while very dilute bloodstains can produce false negatives, quebracho leather minimally impacts Kastle-Meyer's efficacy.

Impact Statement: This presentation will have a large impact on forensic serology and DNA analysis, given that DNA testing is often reliant on serological testing's results. By demonstrating how certain substrates may interact with reagents in presumptive blood tests, this research can help investigators better understand their results.

Abstract Text: This experimental research assessed the occurrence of false negative results in the presumptive blood testing method, Kastle Meyer, on leather containing quebracho extract using dried bloodstains. Investigators have encountered issues with leather boots containing suspected stains in previous studies.[1] In these previous studies, testing suspected stains with the Kastle Meyer test, a commonly used presumptive blood test in forensic science, resulted in a negative for the presence of blood.[1] Another study suggested that a specific leather component could interfere with the presumptive test screening, causing the issue.[2] This project attempted to confirm whether the outcomes of the previous studies are accurate and reproducible. In the forensic workflow, suspected stains are first tested presumptively. If the test is negative, suggesting that no blood is present, no further testing will be conducted. However, when testing stains on objects containing antioxidant properties and reducing agents, the possibility of a false negative is likely to occur.[1,3] This research is significant because bloodstains, which are vital in the forensic workflow due to the ability to recover a DNA profile from the stain, could be dismissed in a criminal investigation due to negative presumptive test results, potentially leading to the dismissal of crucial evidence.

In the research trials, a positive control (neat blood), a negative control (no blood), and diluted blood (in a dilution series of 1:10, 1:100, 1:1,000, and 1:10,000) were deposited on 11 samples of quebracho leather, left to dry, and tested using the Kastle Meyer test. In order to better understand the impact of quebracho extract on presumptive blood testing, an additional test was conducted using powdered quebracho extract mixed with water. Clothing strips containing neat blood drops were soaked in this quebracho solution and tested with the Kastle Meyer test in different settings. One last experiment was conducted in order to better understand the occurrence of false negative test results on leather boots commonly seen in forensic casework. For this last trial, Timberland brand boots containing drops of neat and diluted blood were set to dry and later tested with the Kastle Meyer test. The results of all three of these experiments showed that diluted bloodstains produce false negatives. However, unlike the results of previous studies, this research suggests that quebracho leather has a minimal impact on Kastle Meyer's efficacy.

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Bloodstains; Leather; Presumptive Blood Test

D4 Failure Analysis of Ink Samples Using Multiple Modes of Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS)

Athena T. Nguyen, BS, Frontier Lab Americas, Houston, TX*

Learning Objectives: In this presentation, attendees will learn how to detect and identify differences in similar polymer materials using Py-GC/MS, a simple and powerful technique for failure and deformulation analysis and reverse engineering. Specifically, attendees will learn about the principles and applications of Py-GC/MS, the capabilities of Frontier Lab's micro-furnace pyrolyzer, and how to apply Py-GC/MS to real-world problems. Attendees will also learn how to perform deformulation and failure analyses using Py-GC/MS, how to interpret the results, and how to connect them to criminal investigations. By the end of the presentation, attendees will be able to apply Py-GC/MS to their own work and research and evaluate its potential for solving complex problems.

Impact Statement: This presentation will significantly impact the forensic science community by introducing a powerful technique for detecting and identifying differences in similar polymer materials, a critical step in failure and deformulation analysis and reverse engineering. Py-GC/MS, combined with Frontier Lab's innovative micro-furnace pyrolyzer, offers a simple and effective solution for analyzing complex materials in criminal investigations. By showcasing the capabilities of Py-GC/MS, this presentation will enable forensic scientists to:

- Solve complex cases involving polymer materials
- Identify and trace evidence in criminal investigations
- Improve their understanding of material properties and behavior
- Enhance their analytical toolkit with a cutting-edge technique
- Advance the field of forensic science with new research and applications

Overall, this presentation will contribute to the advancement of forensic science, helping investigators solve crimes and bring criminals to justice.

Abstract Text: Detecting and identifying differences in similar polymer materials is a crucial step in failure analysis, deformulation studies, and reverse engineering, particularly in forensic science where accurate material identification is critical. Py-GC/MS is a simple yet powerful technique that can aid in this process. Py-GC/MS offers a straightforward solid sample introduction method that expands the capabilities of GC/MS, making it an invaluable tool for analyzing complex materials in forensic investigations. Frontier Lab's innovative micro-furnace pyrolyzer, based on the vertical micro-furnace design, enables comprehensive analysis with minimal sample preparation. The multi-mode micro-furnace pyrolyzer offers various modes of operation, including Evolve Gas Analysis (EGA), Flash pyrolysis (single-shot), Thermal Desorption (TD), and Heart-Cutting (HC), which can be operated in the same system.

In this presentation, deformulation and failure analyses were performed on two ink samples, demonstrating the effectiveness of Py-GC/MS in identifying differences between similar polymer materials in criminal investigations. Following the Frontier's method map, the first step is to perform an EGA using temperature programs. EGA provides information about the thermal temperature behaviors of the sample. The EGA thermogram is then used to determine the next steps in the analysis process. By leveraging EGA-MS and HC modes of operation, detailed information about the differences between the Good and Bad samples was obtained, showcasing the potential of Py-GC/MS in forensic science, failure analysis, and quality control, and highlighting its role in helping investigators solve crimes and bring criminals to justice. Furthermore, Py-GC/MS can aid in the analysis of evidence in cases of counterfeiting, intellectual property theft, and arson, making it a vital tool for law enforcement agencies worldwide. Its cutting-edge technology and versatility make Py-GC/MS an essential instrument in forensic laboratories, enabling experts to analyze a wide range of materials and solve complex cases. With its ability to detect trace evidence and identify unknown materials, Py-GC/MS is a game-changer in forensic science, helping to bring criminals to justice and ensuring public safety.

Laboratory; Chromatography; Failure Analysis

D5 Light It Up! Photoluminescent Lead Detection for Forensic Investigations

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Learning Objectives: Attendees will be informed regarding:

1. Application in Shooting Distance Estimation: Participants will be able to describe how the reagent is used to estimate shooting distances and interpret the luminescent patterns that correlate with various distances.
2. Visualization of Polygonal Patterns: Participants will be able to identify and explain the significance of polygonal rifling patterns visualized using the photoluminescent reagent and their relevance in forensic investigations.
3. Direct Testing Methodology: Participants will be able to outline the procedure for direct testing of lead from Gunshot Residues (GSR) using the reagent and understand the steps involved in ensuring accurate and reliable results. Additionally, the effects this may have for law enforcement will be explored.
4. Comparative Analysis: Participants will be able to compare the novel reagent with traditional lead detection methods, such as sodium rhodizonate. This will highlight the advantages and potential limitations in forensic applications.
5. Future Directions and Innovations: Participants will be able to suggest potential future developments and improvements in photoluminescent lead detection technologies and their possible impacts on forensic science.

Impact Statement: This research introduces a novel photoluminescent lead detection reagent that enhances the accuracy of shooting distance estimations and polygonal pattern visualization. Its application in forensic investigations can significantly improve evidence analysis, providing more reliable results in criminal cases and aiding law enforcement agencies in solving firearm-related crimes. By offering a superior method for detecting and analyzing lead from GSR, this reagent has the potential to advance forensic science practices and contribute to more effective criminal justice outcomes.

Abstract Text: GSR analysis is essential for the forensic investigation of shooting incidents, but it is oftentimes slow, cumbersome, and limited in spatial resolution. Here, a photoluminescent GSR analysis (PL-Pb) method is introduced for instant, spatially resolved detection of GSR with high resolution.[1] Lead dust in GSR reacts into a lead halide perovskite semiconductor that emits bright green light under ultraviolet irradiation. The sensitivity of PL-Pb enables straightforward detection of trace amounts of GSR from ricochet markings, bullet holes, and combustion plumes. We also demonstrate that GSR is transferable with high spatial resolution and preserves fine details such as the polygonal patterns caused by the rifling of a pistol. Moreover, PL-Pb detection yields reproducible GSR patterns for shooting distance reconstruction series. The sensitivity and instant results make PL-Pb suitable for rapid presumptive testing of shooting suspects. Surprisingly, even after washing, GSR can still be detected on the hands of shooters, and GSR is easily found on clothes, shoes, and other objects relevant to a shooting incident.[2] Collectively, the instant results and sensitivity of PL-Pb open unprecedented opportunities for on-site forensic investigations and highlight the potential of perovskite-based lead detection methods for lead-containing, crime-related micro-traces and lead dust in general.

The development of PL-Pb can have a significant advancement in forensic science. This innovative method not only streamlines the process of GSR detection but also enhances the accuracy and reliability of the results. By emitting bright green light upon ultraviolet irradiation, a visually striking and easily identifiable marker for lead residues is possible. This makes it an invaluable tool for forensic investigators working at crime scenes, offering immediate and clear results that can be crucial in time-sensitive situations. The ability to detect GSR even after attempts to wash it off further underscores the robustness, reliability, and sensitivity of this method, making it a powerful addition to the forensic investigator's toolkit.

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GSR; Fluorescence; Crime Scene Investigation

D6 Seeing the Invisible: Flow Visualization and Scientific Imaging Techniques to Support Safety, Security, and Forensics

Edward Sisco, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Matthew Staymates, MS, National Institute of Standards and Technology, Gaithersburg, MD*

WITHDRAWN

D7 Odorant Depletion in Propane Containers

Tim G. Dunn, MS, Dunn Laboratories, Roswell, GA*

Learning Objectives: The objective of this presentation is to bring recognition of the limitation of the odorant in propane gas and the specific conditions by which the integrity may be compromised or non-existent.

Impact Statement: This presentation will improve recognition of the conditions when persons may be unable to detect the presence of flammable concentrations of propane.

Abstract Text: Propane's boiling point is -44°F, though it can be stored and transferred under pressure as a liquid. At the customer's tank, in the top headspace, the vapor that is drawn off is considered the useful fuel. Hence, propane is a Liquefied Petroleum (LP) gas. Propane vapor is heavier than air (1.5 times the density of air), and the flammable limits are 2.15 to 9.6 percent by volume in air. These values are referenced in the National Fire Protection Association (NFPA) pamphlet 58, *LP Gas Code*.[1]

Propane, in its natural state, is considered invisible and odorless. The odorant, ethyl mercaptan in this case, is added solely as a means of detection by smell. With regard to the NFPA pamphlet 58, section 4-2 discusses the requirements for LP gas odorization: "All LP-Gases shall be odorized prior to delivery to a bulk plant by the addition of a warning agent of such character that gases are detectable, by a distinct odor, to a concentration in air of not over one-fifth the lower limit of flammability." An annex to the cited code states that: "Experience has shown that ethyl mercaptan in the ratio of 1.0 pound per 10,000 gallons of liquid LP-Gas has been recognized as an effective odorant." However, as prefaced to this same annex, "It is recognized that no odorant will be completely effective as a warning agent in every circumstance." Simply put, odorant is not a perfect warning system.

Odor depletion in propane containers has been known for many years. When propane is introduced into a new container, or any container that has been out of service for some extended period (where air and moisture have been able to enter), ethyl mercaptan can be depleted chemically by the presence of certain rust matter, such that it will be oxidized to lesser smelling and less volatile disulfides. Rust, particularly red-colored rust, present in a tank will serve as a catalyst for oxidation. Further reduction of the malodorant can occur from physical adsorption, by its affinity to the container wall.

For propane, I have been aware of odorant depletion in new tanks for most of my professional career. At one point in the late 1970s, our engineering firm was approached by a propane supplier, pipeline transporter, and retailer, who told us that it could take as many as seven complete fills before a "reactive" tank became "passivated." In that realm, from their concern, efforts—based upon the size of the containers—were to add larger doses of ethyl mercaptan in an effort to mitigate odorant depletion.

The issues of odorant fade and odorant depletion were main topics some 30 years ago, with papers presented at joint symposiums between the National Propane Gas Association and the Gas Processors Association (GPA). Testing was conducted with part of the thrust being to mitigate the presence of specific iron oxides (red rust). Corrosion inhibitors were also considered.

There are other issues when relying upon odorization as the sole means of warning a person of the presence of fugitive propane. Numerous circumstances have been documented where people have been exposed to flammable concentrations of propane yet have been unable to recognize the fuel by smell. Failure to smell propane by victims was reported in the June 1974 United States Consumer Product Safety Commission publication, *Hazard Analysis—Liquefied Petroleum Gas*.[2] With propane being heavier than air, it will tend to settle in low-lying areas. Clearly, when relying upon an odorant as a sole warning agent, a person's nose must be in the presence of a detectable concentration before they can recognize the dangerous conditions.

References:

1. National Fire Protection Association, pamphlet 58, *LP Gas Code*.
2. 1976 United States Consumer Product Safety Commission publication, *Hazard Analysis—Liquefied Petroleum Gas*.

Propane; Odor; Explosion

D8 Synthetic Routes for the Production of Xylazine: An Analysis of Clandestine Viability

Calvin Keller, PhD, Drug Enforcement Administration, Harper's Ferry, WV*

Learning Objectives: After attending this presentation, attendees will gain a better understanding of the various routes of xylazine synthesis. Furthermore, attendees will learn about the practical requirements of these methods of synthesis. Forensic scientists will benefit from this by learning of new chemicals that could identify the presence of a clandestine xylazine lab.

Impact Statement: This presentation will increase knowledge in the forensic science community of xylazine synthesis. This will enable researchers to better understand how xylazine could be produced in a clan lab, including precursors to watch for, reagents and conditions to be aware of, and what techniques can be considered viable for clandestine chemists.

Abstract Text: Xylazine is a veterinary anesthetic that was never approved for use in humans. It is now used as an adulterant for illicit drugs, including heroin and fentanyl. Xylazine appeared in the illicit drug markets in Puerto Rico in the early 2000s and has since spread throughout the country.[1] As of 2022, it has been found in mixtures with fentanyl in 48 of 50 states.[2] Currently, intelligence indicates that veterinary xylazine is being redirected to drug dealers, who repurpose it for drug use. However, as xylazine purchases are coming under heavy scrutiny, and as nations consider scheduling xylazine, it is imperative to determine if xylazine can be synthesized under clandestine laboratory conditions.

The state of the patent literature with regard to the synthesis of xylazine was analyzed. While a series of routes were observed to synthesize xylazine, all the published routes went through the intermediate 2,6-dimethylphenyl-isothiocyanate. This compound can be converted into xylazine in a two-step one pot reaction. Production of 2,6-dimethylphenylisothiocyanate is varied in the patent literature. However, we determined without any experimentation that thiophosgene was unsuitable for use in a clan lab setting. Furthermore, we felt that carbon disulfide was likely not suited for clan lab usage, but we chose to test its chemistry anyway. We chose the closing step of xylazine production as our initial synthesis, followed by the full synthesis from carbon disulfide. As we attempted this work, a novel route to xylazine was discovered based on profiling data. We added this synthesis to our research objectives.

This presentation will describe the patent methods for xylazine synthesis and our results replicating these syntheses under clandestine laboratory conditions. Furthermore, the novel method of xylazine synthesis not found in the chemical literature will be discussed and analyzed for viability under clandestine settings. The profiling results obtained from these syntheses will be analyzed for the presence of potential marker compounds. A brief discussion of notable impurities of degradation pathways for xylazine will be explored. Concluding remarks will discuss potential chemical combinations in seizures that could indicate the presence of a clandestine xylazine laboratory.

References:

1. Torruella, R.A. "Xylazine (veterinary sedative) use in Puerto Rico" *Subst Abuse Treat Prev Policy 6, 7* (2011). https://doi.org/10.1186/1747-597X-6-7.
2. "DEA Reports Widespread Threat of Fentanyl Mixed with Xylazine," *Public Safety Alerts, Drug Enforcement Administration, US Department of Justice*, https://www.dea.gov/alert/dea-reports-widespread-threat-fentanyl-mixed-xylazine.

Xylazine; 2,6-Dimethylphenylisothiocyanate; 3-Chloropropylisothiocyanate

D9 Forensic Exams of Public Works Contracts: Ethical and Just Methodologies in Pavement Works Investigation

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WITHDRAWN

D10 An Economic Valuation of Environmental Damages Caused by Illegal Artisanal Mining in the Brazilian Yanomami Indigenous Territory

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WITHDRAWN

D11 The Sidney Declaration: Is It a True Revisitation of the Forensic Science Principles?

Veronica Scotti, JD, Politecnico di Milano, Piacenza, Emilia-Romagna, Italy; Alessandro M. Ferrero, MSc, Politecnico di Milano, Milano, Lombardia, Italy*

Learning Objectives: After attending this presentation, attendees will have a critical interpretation of the principles of the Sidney declaration and its interpretation of the forensic science principles.[1] This declaration recognizes that "forensic science deals with a continuum of uncertainties" but does not show how to handle it. This presentation shows that metrology can be a useful tool for dealing with uncertainty in a very efficient way.

Impact Statement: This presentation will impact the forensic science community by discussing the seven principles considered by the Sidney declaration aimed at providing a revisited definition of the forensic sciences.[1] The concepts underpinning this declaration are the unavoidable presence of uncertainty and the need for approaching the forensic activity in a broad scientific sense. The main impact of this presentation is proving that the fundamentals of metrology cannot be neglected by the forensic scientists.

Abstract Text: The Sidney declaration is a recent document aimed at revisiting the essence of forensic science through its fundamental principles.[1] It is one of the numerous interesting documents that have enriched the debate started inside the forensic community by the publication of the well-known National Academy of Sciences (NAS) and **President's Council of Advisors on Science and Technology (**PCAST) documents about the effectiveness and reliability of the forensic sciences in assessing facts.[2,3]

The revisitation proposed by the Sidney declaration is articulated in seven principles that refer, implicitly or explicitly, to two underlying tenets. The first is that the task of forensic scientists is a complex activity that "requires a trained mind with broad science knowledge," while the second is that "forensic science deals with a continuum of uncertainties."

Although there is little to disagree upon on these two points, supported by philosophical, rather than scientific considerations, it is surprising that metrology, and in particular forensic metrology, is not considered among the broad science knowledge, since it is the only science that, by its own nature, can handle and quantify uncertainty and it has been brought to the attention of the forensic community well before the declaration was formulated.[4-6]

One of the main and most important outcomes of forensic metrology is also that of quantifying, in strict probability terms, the risk of making wrong decisions whenever they are based on the results of tests or measurements on traces resulting from an activity.[7]

This paper reconsiders the seven principles of the Sidney declaration under the perspective offered by the forensic metrology and shows how different uncertainty contributions that may affect the analyzed traces can be combined according to the best practice in forensic science and metrology.[7-9] This strict scientific approach allows the forensic scientist to present his or her results with a strictly evaluated probability about how close the obtained result is to the expected true value of the quantity being analyzed and measured.[9] Consequently, the trier of fact is given complete information about the reliability of the identified trace, is in the position of weighing it against other pieces of evidence, and makes a more conscious decision.

The paper discusses also the requirement that the forensic scientists possess a broad scientific knowledge. Modern forensic science spans such a wide range of topics that it is almost impossible that a single individual can cover all of them. Nowadays, a forensic scientist cannot be a single individual, but rather a team of scientists with complementary expertise and a common scientific background on the fundamental tools of science, such as math, statistics, probability, physics, and chemistry. As a consequence of the need to deal with a "continuum of uncertainties," metrology should become part of the background of forensic scientists and also of the trier of fact as well as all users and stakeholders of forensic science.

References:

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2. National Research Council (NRC), *Strengthening Forensic Science in the United States: A Path Forward*, The National Academies Press, Washington, DC, 2009.
3. 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).
4. F. Crispino, O. Ribaux, M. Houck, P. Margot, Forensic science – a true science, *Aust. J. Forensic Sci*. 43 (2–3) (2011).
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9. BIPM JCGM 100:2008: *Evaluation of measurement data – Guide to the expression of uncertainty in measurement (GUM)*. (2008)

Forensic Science; Scientific Evidence; Metrology

D12 A Race Car Accident Analysis

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Learning Objectives: The goals of this presentation are: (1) assessing the car's movement before and during the crash, and the severity of impact; (2) scrutinizing available driving parameters via telemetry data captured by the vehicle's on-board camera: (3) estimating the corner-marshal's position at the time of impact and the likely cause of his fatal injuries; and (4) identifying potential reasons for the driver's loss of control preceding the accident at the track.

Impact Statement: Racing car accidents happen very often during races. But it is very rare that some of those accidents are the subject of a Road Accident Analysis (RAA) Expert opinion. The aim of this real case study is to analyze the movement of the car immediately before and during the crash, the impact severity with the analysis of all available driving parameters by using telemetry data and the vehicle on-board camera. The aim is also to estimate the position of the judge at the moment of impact and the possible cause of his lethal injuries. Also, the driving line of the car was analyzed during previous laps (before the incident) with a conclusion of the possible reason why the driver has lost control over the car in this turn.

Abstract Text: Although racing car accidents are common during races, they seldom undergo forensic analysis (RA analysis). In July 2022, during the CEZ–Endurance Cup International race at the Grobnik racetrack under FIA auspices, a Porsche 911 GT3 CUP racing car veered off track at turn #17 on lap 9, colliding with a safety barrier. While the driver escaped unharmed, a corner-marshal tragically suffered fatal injuries.

Consequently, the Forensic Science Centre "Ivan Vučetić" in Zagreb, Croatia, was tasked with conducting an RA analysis. The analysis aimed to elucidate pertinent facts surrounding the incident using vehicle and track data, a comprehensive description of injuries, and forensic medical examinations, complemented by track and cockpit videos from the Porsche racing car.

The RAA pursued several objectives: (1) assessing the car's movement before and during the crash and the severity of impact (2) scrutinizing available driving parameters via telemetry data captured by the vehicle's on-board camera; (3) estimating the corner-marshal's position at the time of impact and the likely cause of his fatal injuries; and (4) identifying potential reasons for the driver's loss of control preceding the lap 9 accident.

The investigation also sought to address critical questions regarding the race organizer's compliance with FIA regulations concerning organizational and safety standards and the conduct of marshals (mandating three marshals at each corner during training, qualification, and races).[1]

This paper offers a comprehensive analysis of video recordings to pinpoint essential factors contributing to the accident's cause and the resulting casualty. Moreover, it integrates the car's telemetry data, processed with specialized software, and conducts a PC Crash analysis of the event to address the posed questions.

The simulation of the vehicle leaving the track in the curve 9 was made, as well as a simulation of a collision with a safety barrier, whereby the contact of the vehicle/fence with the marshal's body, which was located near the barrier, was also realized. A calculation was made of the collision forces, the acceleration that acted on the person's body as well as the force contacts, which explained the cause of the fatal consequences for the corner marshal. In this work, the obtained results from the simulation were compared with the forensic medical examination of the marshal's injuries.

Reference:

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Accident; Onboard Camera; Vehicle Speed

D13 A Forensic Analysis of Unintended Vehicle Rollaway Incidents

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Learning Objectives: This presentation reviews real-world unintended rollaway incidents that resulted in serious injuries. In each case, the operation of the vehicle and cause of the rollaway is analyzed. In addition, alternative designs to prevent incidents of unintended rollaway will be discussed.

Impact Statement: This presentation will explore unintended vehicle rollaway case studies and present methodologies for analyzing unintended rollaway incidents and alternative designs to prevent them.

Abstract Text: Rollaway events can occur under a vehicle's propulsive force and/or the force of gravity. Three primary factors contribute to unintended rollaway: terrain at the scene, vehicle design and operation, including malfunctions, and driver inputs.

The only regulation intended to address unintended rollaway is Federal Motor Vehicle Safety Standard (FMVSS) 114.[1] This standard was implemented in 1968 and was originally designed to reduce vehicle theft. FMVSS 114 also helped prevent rollaway incidents by requiring a warning to prevent leaving the key in the ignition and interlock mechanisms to prevent vehicle movement without the key. FMVSS 114 was amended over the years to provide additional safeguards against rollaway incidents, but slow and limited improvements have left rollaway risks unaddressed. In 1992, the National Highway Traffic Safety Administration (NHTSA) agreed to define an electronic code stored in a vehicle as a "key" and so-called "keyless" ignition systems were introduced by the 2000s.[2] These systems use a fob to transmit an electronic code to the vehicle, effectively bypassing the safeguards that mechanical keys provided against rollaway under FMVSS 114.

In each of the following case studies, the three factors discussed above were analyzed in order to determine the cause of the rollaway. Alternative designs that could have prevented each incident will be presented.

Case #1: A 2010s SUV with keyless ignition stopped, and the driver exited to close a gate. The vehicle engaged powered reverse and backed over the driver, causing fatal injuries. The subject vehicle utilized two spring and detent mechanisms to control the movement of the gear selector lever. The gear selector lever was susceptible to being placed in a false park position between "P" and "R." The gear selector was observed to spontaneously move to both the "P" and "R" positions when placed in false park. It was determined the gear selector must have been in the false park position to allow the driver to walk behind the vehicle, and the system spontaneously shifted into the "R" position.

Case #2: A 2010s SUV with a mechanical key ignition pulled into a sloped driveway. A dashcam captured the event. The driver exited and the vehicle rolled rearward after about 30 seconds. The driver attempted to stop the vehicle as it exited the driveway, sustaining serious injuries. The slope of the driveway was measured, and an exemplar vehicle was evaluated on road surfaces of varying slopes. It was determined the driver exited the vehicle with the engine idling and the transmission in "D" at a location where the gravitational force from the slope was equal and opposite to the propulsive force of the vehicle at idle. The slope of the driveway increased moving downhill, causing the vehicle speed to increase as the event progressed.

Case #3: A 2010s SUV with keyless ignition pulled into a parking space on a slope. Surveillance cameras captured the event. The driver exited the vehicle, unsuccessfully attempted to open the tailgate, and opened a door on the passenger's side of the vehicle. The driver eventually reached into the open driver's door. The vehicle began to roll rearward, partially rolling over the driver. An exemplar vehicle was evaluated to determine how the accident could have occurred. It was determined the driver exited the vehicle with the engine running and the transmission in "D" in a state where the propulsive force of the vehicle was balanced by the force of gravity acting on the slope. The rollaway began when the driver reached into the vehicle and shut off the engine, removing the propulsive force and effectively putting the vehicle in neutral. This incident likely would not have occurred with a mechanical key ignition system.

References:

1. Docket No. 1-21, Part 255 – Initial Federal Motor Vehicle Safety Standards, *Motor Vehicle Safety Standard No 114, Theft Protection, Passenger Cars,* F.R. Doc. 68-5095, Ar. 26, 1968.
2. Paul Rice to Stephen Selander, NHTSA Interpretation Letter, 5/22/1992.

Crash; Forensic Analysis; Engineering

D14 The Role of Early Assumptions in Driver Determination for an Unwitnessed Motor Vehicle Crash

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Learning Objectives: After attending this presentation, attendees will be reminded how early assumptions can lead a case astray when determining driver versus passenger in a Motor Vehicle Crash (MVC).

Impact Statement: This presentation will impact the forensic community by providing important information that may assist accident and injury reconstructionists with the determination of driver versus passenger when there is seemingly conflicting evidence.

Abstract Text: Two intoxicated men suffered a crash in an early model SUV that was not equipped with a Crash Data Recorder or side airbags. An after-market Global Positioning System (GPS) system provided location and speed estimates. The vehicle went off the left side of a roadway at 61mph, rotated counterclockwise such that the passenger side slammed into a tree. The impact resulted in significant cabin intrusion by the door, passenger B-pillar, and roof. There were no witnesses. One occupant suffered a fatal head injury while the other was relatively unscathed. The survivor claimed he was restrained in the passenger seat during the wreck. Offered as proof of this were the facts that he suffered a linear bruise along the right side of his neck purported to be from his shoulder belt and the location of his hat pinched in the crumpled ceiling on the passenger side. The deceased man was found laying on the ground outside the driver's door. The survivor brought suspicion upon himself by delaying how long he took to seek help and calling his father to the accident scene before police were notified. A forensic pathologist initially indicated that the decedent's head injury would have been immediately incapacitating, so police were suspicious of the survivor's story that the other man was found outside the vehicle. It was decided to charge the survivor for his friend's death after determining that he was the driver.

Police reconstructionists inspected the SUV and initially determined the driver restraint system showed signs of use, while the passenger belt was locked in a stowed position by the pretensioner. The theory was that the deceased man was unrestrained in the passenger seat and suffered his head injury when the roof and B-pillar intruded. The survivor was actually the restrained driver, and he positioned his friend's body outside the driver's door in order to support his story. During discovery, several questions arose, so an outside expert was requested.

A review of the restraint system for this vehicle showed that seatbelt pretensioners are found on the buckles, not in the B-pillar. Neither pretensioner had been triggered in this side impact crash. A search of National Automotive Sampling System data on real-world crashes showed this was common in this model SUV. The dangling passenger belt was actually locked in an extended position by the damaged B-pillar and the length was sufficient to restrain an occupant. Examination of latch plate plastic was consistent with the passenger being restrained while the driver was not. Damage to the center console and passenger seatback were most likely due to interaction with the driver's body as it moved toward the initial impact. The autopsy data showed torso injury consistent with this interaction and the survivor suffered a left clavicle fracture, likely due to his friend's torso as it moved toward the passenger side. The driver's head likely contacted the intruding roof line for the fatal injury. The pathologist eventually agreed that the head injury may not have been immediately incapacitating and thus the man could have exited the SUV before succumbing to his injuries. The survivor's bruised neck was very much in line with the passenger shoulder restraint and the narrow width of the bruise was likely due to folding of the webbing.

In conclusion, the early assumption that the survivor was the driver skewed how thoroughly the evidence was investigated and ultimately how it was interpreted. Although there was no Crash Date Retrieval (CDR), and DNA evidence of blood spatter was inconclusive, a focus on the facts of the accident, the autopsy, and the vehicle inspection led to the withdrawal of charges in this case.

Load Marks; Seat Belt; Pretensioner

D15 Roof System Failure: Causes and Misconceptions

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Learning Objectives: Attendees of this presentation will be presented with methods of analysis and investigation techniques to accurately determine the cause of structural failures during a high wind event.

Impact Statement: This presentation will impact the forensic community by demonstrating a case where an innocent party was improperly blamed for a structural failure and the benefits of performing a thorough forensic engineering investigation.

Abstract Text: This case involves an investigation of a significant structural failure at a pre-owned auto mall in southern West Virginia. In April 2020, a severe wind event affected this area. During this storm, a large portion of the roof and front façade collapsed. The property was renovated approximately eight years prior to the loss. These renovations involved the construction of an aluminum-cladded façade and parapet extension, a garage addition, and a new Ethylene Propylene Diene Terpolymer (EPDM) roof. The roof membrane and insulation boards were installed by our client overtop the original Built-Up Roof (BUR).

Following the collapse, subrogation attorneys attempted to place the blame solely on the roofing contractor. Expert reports commissioned by these attorneys did not identify any actions or omissions on the part of the architect or general contractor involved in the renovations. The property owner and general contractor were all insured by the same company that hired the subrogation attorneys. Despite the apparent bias, the roofing contractor and their insurance carrier decided to commission an independent forensic investigation.

The inspection of the property was performed one month after the loss date. The investigation involved a determination of anchorage of the new façade to the front wall, as well as the lack of anchorage of the original roof beams to the masonry columns. The collapse of columns corresponded with lateral movement of the front façade and parapet. A review of the architectural drawings associated with the renovations involved no detail of the anchorage of the original structure.

The evaluation of the roof membrane and insulation boards revealed no installation defects that would have contributed to this loss. The opposing experts identified the absence of a secondary cleat as the cause of failure. However, this construction detail was not included in the architectural drawings. More importantly, the opposing experts failed to provide a structural analysis to support their conclusions.

Despite having extensive records and news reports indicating that high winds affected this property on the loss date, the opposing parties alleged that the winds were only moderate in severity. An analysis was performed for various wind loading conditions to determine the root cause of failure. This analysis determined that structural failures were likely to occur at wind speeds of 75mph or greater. Lateral loads applied through the façade and parapet would have exceeded the frictional resistance at the beam seats across the front of the building. Security camera footage from an adjoining property provided clear evidence that the winds were severe, as well as supporting our conclusions as to the mode of failure.

The analysis showed that the mode of failure was not consistent with uplift of the membrane or the lack of a cleats at the eaves of the building. Once these facts became indisputable, the opposing parties attempted to place blame on the roofing contractor for failing to evaluate anchorage of the original steel beams to the masonry structure. As these experts represented the general contractor, who oversaw all aspects of the building renovation, their final attempt to shift blame was unsuccessful.

Anchorage; Parapet; Wind Loads

D16 The Analysis of Vehicle Dynamics From Monitor Playback Recordings

Michael Callahan, BSME, Collision and Injury Dynamics, Torrance, CA; Sean Snyder, BS*, Collision and Injury Dynamics, Torrance, CA*

Learning Objectives: Attendees will learn how to identify, process, and reconstruct collisions captured on video and recorded by first responders using mobile devices (derivative video evidence).

Impact Statement: Derivative video evidence, or "video of a video: is an analytical roadblock to collision reconstruction professionals across the globe. The research, testing, and validation presented will establish a methodology to approach this type of evidence, determine its reliability, and solve for vehicle dynamics captured within using reverse-projection photogrammetry.

Abstract Text: The analysis of digital media evidence in collision reconstruction has grown exponentially in the past two decades. Conventional reconstruction methods such as momentum and energy analyses are now frequently supplemented with pre-collision data from vehicle Event Data Recorders, telemetry information from vehicle systems, and video footage from dash cameras and third-party systems.

The increase in video coverage and capture of vehicle collisions creates a paradigm for first responders, who often have little or no training in the recovery of forensically sound digital video evidence. Instead of obtaining true copies of accident-related footage, first responders are relying largely on capturing playback of collision footage with mobile devices and body-worn cameras. The resulting derivative video evidence, or "video of a video," is foundationally challenging for a collision analyst: can the video be relied upon in a reconstruction, and, if so, what are the best methods for processing, analyzing, and quantifying the uncertainty in the video?

To research and develop methodology around this type of analysis, the authors obtained a stand-alone consumer grade Digital Video Recorder (DVR), along with DVR cameras and a playback monitor. The DVR system was used to record an instrumented test vehicle during a variety of maneuvers—braking, acceleration, and lane changes. The DVR playback monitor was then recorded using a cell phone, creating specific evidence of interest for this study.

The derivative video evidence from the cell phone was digitally stabilized, cropped, and rectified to its original proportions and perspective and decomposed into source images. Reverse projection photogrammetry and 3D camera matching were deployed to solve for target vehicle positions or keyframes, with corresponding target times. Using the target positions and times, a dynamic simulation was used to reconstruct the vehicle dynamics and solve the required driver inputs. Finally, the reconstructed motion solved in the simulation was compared to the data from the instrumented test vehicle for uncertainty analysis.

The research performed and findings provide a valuable insight into the proper handling of less-than-ideal video evidence of a collision and how to leverage the appropriate spatial and temporal information within.

Photogrammetry; Video Analysis; Simulation

D17 Forensic Mapping With a Real-Time Kinematics-Equipped Drone (UAS/UAV), With or Without Ground Control?

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Learning Objectives: Attendees will learn about advances in forensic mapping technologies and limitations. Topics include: Real Time Kinematics (RTK), Real Time Network (RTN), and Global Navigational Satellite System (GNSS) vs. Global Positioning System (GPS). Also included are photography aspects: Complementary Metal-Oxide-Semiconductor (CMOS) Sensors, focal length, field of view, Ground Sample Distance (GSD), and global vs. rolling shutters. Attendees will be informed of a case study of 3D terrestrial scan data, photography, photogrammetry, with aerial photos and 3D point clouds (from both scanner and drone).

Impact Statement: This presentation showcases issues with forensic mapping products (orthomosaics and point clouds) when not using proper methodology, as advertised or promoted by new technologies in taking shortcuts from established methodology.

Abstract Text: This study investigates the application of drones, or Uncrewed Aircraft System (UAS) ("Unmanned" is progressing to "Uncrewed" in upcoming gender-neutral language by a Federal Aviation Administration [FAA] committee), equipped with RTK) technology in forensic mapping, focusing on the uncertainty associated with photogrammetry products, including point clouds and orthomosaic images. The integration of RTK in drones enhances the accuracy of geospatial data collection by providing real-time corrections during flight, thereby potentially eliminating the need for extensive Ground Control Points (GCPs) and Post-Processed Kinematic (PPK) corrections.

The research evaluates the precision and reliability of RTK-enabled drone photogrammetry products by conducting a series of comparative analyses. The study examines point clouds and orthomosaic images generated under different scenarios, both actual field scenes, and organized test sites.

The accuracy assessment involves detailed statistical analyses of spatial errors and the consistency of geospatial data across various terrain types and environmental conditions.

The real-world forensic scenarios revealed significant limitations of RTK-equipped drones than pre-planned missions. Initial results indicate that RTK-equipped drones vary based on environment setting, including factors such as terrain, canopy, interference, and network availability.

Current marketing and theoretical RTK technology results potentially can eliminate the use of standardized GCPs. However, the GCPs are established procedures among surveyors and forensic mappers for integrating electronic total stations, three-dimensional terrestrial scanners, and Global Navigational Satellite System (GNSS) mapping instruments with aerial photography.

The findings show that the real-time corrections provided by RTK technology can achieve centimeter-level accuracy with the use of GCPs, which is crucial for forensic applications requiring high precision, such as collision or accident reconstruction, crime scene documentation, and disaster response.

In conclusion, the study highlights the transformative impact of RTK-equipped drones on forensic mapping practices. It recommends best practices for optimizing the use of RTK technology, such as combining RTK with a number of strategically placed GCPs to enhance data reliability and addressing the potential limitations through advanced processing techniques. This research underscores the importance of adopting GCPs with RTK technology to improve the efficiency, accuracy, and reliability of forensic mapping, ultimately contributing to more effective and timely forensic investigations.

Photogrammetry; Reconstruction; Drone

D18 Accidental or Deliberate? Can a Safety Razor Inflict Injuries to a Baby in a Bathtub?

Sarah V. Hainsworth, PhD, University of Bath, Bath, England, United Kingdom*

Learning Objectives: Following this presentation, attendees will understand how to assess accidental or deliberate razor-inflicted injuries to a baby, with particular application to injuries sustained in a bath.

Impact Statement: This presentation will impact the forensic science community by contributing to the understanding of how a baby could sustain injury from a standard consumer safety razor and specifically how this could occur in a bath. The work has been supported by experimentation to allow practitioners to have high confidence in the results.

Abstract Text: A 4-month-old baby was left unattended for a short period of time in a standard white acrylic adult bath with a bathmat covering the length of the base. A baby bath bubble was used and the bath was filled with approximately 2" of water. When the adult returned to the bathroom, the baby was making a distressed noise and there was blood in the bathwater. The adult found a razor under the bubbles by the plughole that had apparently fallen from the side of the bath. Injuries, which were quite deep, were found on both heels.

Modern safety razors consist of a steel blade mounted in a plastic shell attached to a handle. The razor in this case was a cartridge-style razor with five blades and skin guards. Additionally, the razor had a hydrating gel reservoir to hydrate the skin while shaving.

Video analysis was used to determine the typical ways in which a baby moved its feet while kicking. It was noted that in all movements, the legs typically move as a pair, doing the same movement. This is typical for all babies of this age or younger. The legs never become completely straight at this age, remaining slightly bow-legged. The feet do not come down at the same point, they have a small spacing between them as they come down.

An acrylic bath was purchased in order to perform testing under realistic conditions. The original bathmat was placed in the bath, and the bath was filled to a depth of 4.5cm with water. Baby bubble bath was used in the water and thus the conditions for testing were as close as possible to those at the time of the accident. In order to see what happened when a razor was knocked off the side of the bath, a few tests were made to see how the razor landed. The razor tended to land with the blades upward by the side of the bath.

A wooden dummy leg was created to allow some tests to show how a razor loose or secured in the bath would create damage to a child's foot. In order to test against skin, pork skin was obtained from the local butcher and cut to strips. The pork was then stretched over the wooden foot and attached to the heel by staples. A series of tests were then performed to see what damage was created by testing the leg against the razor. The pork skin was replaced between tests.

From the testing conducted, it was only possible to create superficial damage to the skin from the different tests conducted with the razor in the bath. None of the damage created was of the depth found on the injuries to the baby. The testing showed that it would be possible to create superficial damage to one heel, but all the tests showed that while the razor could accidentally be in the position to create damage to one heel, the razor moved as a result of the contact and would not be in a position for damage to be created on the second foot.

A test where the razor was deliberately used to shave the heel showed that it was possible to create the type of deeper injury found in the case. The testing demonstrated that the injuries found could not have been caused accidentally by a razor falling into the bath.

Razor; Bathtub; Injury

D19 An Analysis of Injuries and Injury Mechanisms of Male PMHS in High-Speed, Rear-Facing Frontal Impact Scenarios

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Learning Objectives: After attending this presentation, attendees will have an appreciation for interdisciplinary research in skeletal trauma analysis, specifically focused on injuries to rearward-facing occupants involved in a frontal crash. 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 rearward facing occupants in high-speed frontal crashes. Increased understanding of the mechanisms of trauma will lead to better safety for these occupants.

Abstract Text: Future vehicles equipped with Automated Driving Systems (ADS) may feature unconventional seating configurations in their interior cabin designs. Various configurations, including rear-facing seats or "zero-gravity" seats, have been suggested and reported as desirable in consumer surveys, especially for long-distance travel with children and family members.[1-3] However, there is limited information in literature about occupant safety or injury risk in rear-facing seating configurations during high-speed frontal impacts. Consequently, assessing human injury risks in rear-facing seats under such conditions is essential for developing effective safety and restraint systems for these occupants.

A series of Postmortem Human Subject (PMHS) sled tests were conducted to explore biomechanical responses and injuries in a high-speed rear-facing frontal impact with a velocity change of 56km/h. This study examined the effects of two seatback recline angles (25 and 45 degrees), two Original Equipment Manufacturer (OEM) seats, and two seat belt conditions: an All Belts to Seat (ABTS) condition and a standard Fixed D-Ring (FDR) condition. Six PMHS were tested in the ABTS condition, while eight PMHS were tested in the FDR condition. The ABTS condition features a retractor assembly on the left side of the seatback, whereas the FDR condition has a more distributed and symmetric seatback support frame with a thin seatback suspension in the middle. At least three PMHS were tested in each condition, with a new seat and seat belt used for each test. Reinforced frames were added behind the seat backs, as current OEM seats were not designed for durability and repeatability in high-speed rear-facing frontal impacts.

For PMHS instrumentation, chest deflection was measured using a chest band installed at the mid-sternum level. Uni-axial strain gauges were attached to the periosteal surface of the cutaneous cortex of both the anterior (ribs 3 through 9) and posterior (ribs 3 through 10) aspects of the ribs. PMHS positioning targets were determined using a regression model from a previous volunteer study.[4] The initial tensions for the shoulder and lap belts were set to 18 N and 27 N, respectively. The PMHS used in this study were approximately 50th percentile male and ranged in age from 53 to 71 years. The PMHS had an average height of 176.0 ± 4.8cm and weight of 80.0 ± 12.7kg.

PMHS injuries included minor cervical spine injuries and upper extremity injuries, such as scapula fractures from the retractor structure on the seatback frame, clavicle fractures from excessive ramping up motion in the 45-degree condition, and ulna fractures from forearm flailing. Lower extremity injuries included fibula and distal tibia fractures due to interaction with the front portion of the seat pan. AIS3+ injuries sustained by the PMHS included rib and pelvis fractures caused by interaction with the seatback, with rib fractures being the most common injury.

In both ABTS and FDR conditions, PMHS sustained a higher Number of Rib Fractures (NRF) with the 45-degree recline seatback angle compared to the 25-degree angle. This is likely due to excessive ramping of the PMHS up the reclined seatback and the upward movement of abdominal contents, which may generate combined loading. Most rib fractures occurred after peak Anterior-Posterior (AP) chest compression and peak seatback force, while the PMHS ramped up along the seatback. A complex combination of AP chest deflection with upward deflection was identified as a possible mechanism for rib fractures in PMHS subjected to rear-facing frontal impacts in this study.

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Reclined Occupants; Injury Biomechanics; Injury Mechanisms

D20 Empirical Testing of a Trip-Fall Incident

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Learning Objectives: Attendees of this presentation will gain knowledge of empirical and theoretical methods to determine forces associated with a trip-fall incident.

Impact Statement: This presentation will impact the forensic community by demonstrating the importance of building codes for means of egress facilities.

Abstract Text: This case study involves an employee of a food delivery service who tripped and fell while negotiating a ramp at a restaurant in northern West Virginia. The client was pulling a dolly weighing nearly 300 pounds up a recently constructed ramp. The ramp provided access to the elevated landing at the rear side of the building. This egress ramp was primarily used by restaurant staff and delivery service employees. While pulling the dolly up the ramp, the client lost balance and fell, resulting in bodily injuries.

Initially, our involvement was limited to measuring and documenting the construction of the ramp to compare it to current building codes. This review indicated that the ramp was constructed too steeply, which violated basic provisions of the building code with respect to means of egress facilities. Additionally, the builder of the ramp had installed strips of lumber in order to increase the frictional resistance of the walking surface. It was suspected that these strips would increase the pull force required to move the dolly up the ramp.

We were subsequently commissioned to quantify the pull force required to negotiate the loaded dolly along the ramp. In addition to providing theoretical calculations, empirical tests were devised to quantify the pull force based on several conditions. A ramp was constructed for various slope conditions. An exemplar dolly was procured and loaded with food items that were present on the date of the incident. An S-beam load cell was mounted to the dolly to quantify the pull force for the various slope conditions.

Once these tests were completed, wooden strips were installed to determine the effect this construction had on pull force. The loaded dolly was then pulled up the ramp with the wooden strips for the various slope conditions. The addition of the strips increased the pull force by a factor of two. Moreover, the added resistance resulting from the installation of the wooden strips required that the operator of the dolly overcome this pull force at each strip. The results of the testing confirmed the theoretical model and established that the construction of the ramp contributed to the trip-fall incident.

Load Cell; Empirical Testing; Pull Force

D21 A Windshield Fracture Incident: Injury-Producing Event or Just Cracked Glass?

Michelle Hoffman, MS; Kurt Weiss, MS*

Learning Objectives: This presentation will demonstrate with a case study how objects that directly impact and fracture windshield glass, but do not penetrate through the laminate and into the occupant compartment, produce extremely low forces on the vehicle and its occupants.

Impact Statement: This presentation will assist the forensic community by demonstrating how vehicle windshield impacts that break the windshield glass, but that don't penetrate into the occupant compartment produce extremely low forces on the vehicle and its occupants.

Abstract Text: A 30-year-old, 5'9", 150-pound man claimed concussion and lumbar disc herniation from an incident that occurred when the windshield of the box truck he was driving was struck by an exercise mat that blew out of a stake bed truck in front. A collision reconstruction and biomechanical analysis was conducted, which included impact testing of a similar exercise mat into the windshield of a test vehicle at speeds from 22 to 53 mph, with restrained human volunteers in the driver and front passenger seats. It was concluded that the forces experienced by the incident driver were not greater than those experienced during braking or driving over rough road. This case study demonstrates how human volunteers testing may be used to measure forces when such exposures are clearly below expected injury tolerance thresholds to demonstrate collision reconstruction and biomechanics principles.

The incident involved an import box truck with two male occupants transporting household appliances, traveling at approximately 65 mph. The box truck and cargo weighed approximately 16,500 pounds. A domestic stake bed truck transporting six exercise mats was in the process of passing the box truck when a gust of wind lifted one mat out of the bed striking the box truck's windshield. The exercise mat weighed approximately 40 pounds (Figure 1).

Figure 1

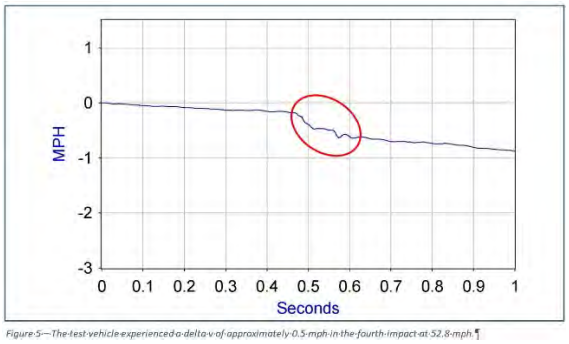
The windshield was fractured on the driver's side (Figure 2). The left side mirror of the box truck was bent rearward when the exercise mat wrapped around the A-pillar. After the windshield impact, the box truck was safely maneuvered to the center median, and the stake bed driver circled back to collect the lost mat.

Figure 2

Figure 3

Impact testing was conducted using a domestic SUV weighing approximately 3,450 pounds, and an exercise mat suspended with ropes (Figure 3). The SUV was driven into the mat such that the driver's side windshield impacted the end of the folded mat. Four impact tests were conducted, with vehicle impact speeds increasing for each subsequent test: 22.8 mph, 29.6 mph, 37.8 mph, and 52.8 mph, respectively. The SUV was instrumented with a tri-axial accelerometer at the approximate static cg location, a portable data logger, and video cameras. The restrained driver (5'9", 160 pounds) had accelerometers on his head, chest, and lumbar spine. An uninstrumented female rode as the restrained right front passenger. Both occupants wore safety goggles. The windshield did not fracture in the first three tests, however the driver's side of the windshield glass shattered, but the windshield laminate remained intact in the last test at the highest impact speed (Figure 4).

Figure 4



Figure·5—The·test·vehicle·experienced·a·delta·v·of·approximately·0.5·mph·in·the·fourth·impact·at·52.8·mph.¶

Figure 5

The delta V experienced by the SUV was calculated from the accelerometer data, and was less than 0.2 mph for the first and second test, less than 0.3 mph for the third test, and less than 0.5 mph in fourth test with the highest impact speed (Figure 5).

The incident box truck weighed over 4.5 times that of the test vehicle; thus, the delta V of the box truck would be expected to have been much less than 0.5 mph. Likewise, it was concluded the forces and accelerations experienced by the incident driver were low, and commensurate with braking or driving over rough road, and concussion or lumbar disc herniation would not be expected outcomes.

Windshield; Fracture; Injury Potential

D22 The Impact of Artificial Intelligence in Clinical Risk Management: A Focus on Fall Prevention

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Learning Objectives: Attendees will : (1) understand the integration of AI in medicine and grasp the universal applications of Artificial Intelligence (AI) in medical practice, particularly in clinical risk management and forensic medicine; (2) recognize the importance of AI in clinical risk management by comprehending how AI can significantly reduce adverse events in hospital settings, leading to improved patient safety and reduced health care costs; (3) explore AI applications in fall prevention by investigating the specific use of AI in predicting and preventing patient falls in hospital wards, thus enhancing the accuracy of risk assessments and minimizing incidents; (4) analyze case study results by reviewing the outcomes of a case study conducted in the neurology wards of the "Policlinico" hospital in Bari, focusing on the effectiveness of AI in profiling patient fall risks; and (5) assess the broader implications of AI by discussing the potential for large-scale AI applications in clinical risk management to improve patient care and reduce legal disputes in health care settings.

Impact Statement: The integration of AI into clinical risk management holds transformative potential for health care. By enhancing the accuracy of risk assessments and predicting adverse events such as patient falls, AI can significantly improve patient safety, reduce the duration and cost of hospital stays, and minimize the frequency of legal disputes involving health care facilities. The promising results from the "Policlinico" hospital case study underscore the practical benefits of AI in real-world settings, suggesting that broader implementation could lead to substantial improvements in patient care and operational efficiency across the health care industry.

Abstract Text: AI is increasingly becoming an integral part of medicine and its various fields, including forensic medicine and clinical risk management. In medical practice, AI applications are diverse, extending from radiological analyses to the diagnosis and treatment of various diseases. In forensic medicine, AI capabilities are being experimented with in autopsy analysis, age and sex estimation, facial recognition, forensic toxicology, DNA analysis and data mining, and firearm injury analysis.[1-7] These applications demonstrate the wide utility of AI in improving the accuracy and efficiency of forensic investigations.

Clinical risk management, particularly in hospital settings, represents a critical area where AI can significantly reduce the occurrence of adverse events during patient stays. Such events are not only harmful to patient safety but also contribute to longer hospital stays, increased costs, and legal disputes involving health care facilities and their staff. Evidence suggests that in low- and middle-income countries, adverse events during hospitalizations contribute to approximately 134 million incidents annually, resulting in about 2.6 million deaths.[8] Similarly, in high-income countries, about 10% of hospitalized patients suffer harm.[9]

The framework of clinical risk management typically aligns with standards recognized as International Organization for Standardization (ISO) 31000:2018 and includes goal definition, risk identification from various sources, risk analysis, and evaluation. It also encompasses treatment strategies such as process standardization and reduction of medical errors, along with risk monitoring with indicators that can measure the effectiveness of preventive strategies against Hospital-Acquired Adverse Events (HA-AE).[10]

A particular focus of AI application in clinical risk management is mitigating the incidence of patient falls within hospital wards. These falls significantly increase the length of stay and costs and can lead to severe injuries or deaths, affecting the reliability of hospital wards and increasing the rates of medical-legal disputes involving hospital facilities. Despite the availability of tools like the Conley scale for fall risk assessment, which shows a sensitivity of 69% but a low specificity of 41%, there remains a lack of predictive accuracy in current methodologies.[11]

The application of AI to the prevention of falls in hospitals represents a vital innovation, offering the potential for accurate prediction of patient fall risk at the time of admission and throughout hospitalization. By profiling each patient based on clinical conditions, comorbidities, and mobility levels, AI can significantly improve the accuracy of fall risk assessments.

Results: In a case study conducted in the neurology wards of the "Policlinico" hospital in Bari during the last quarter of 2023, the use of AI to analyze incident reporting forms revealed significant results. By simultaneously analyzing multiple patient variables—from demographic data to admission diagnoses, from pre-existing conditions to laboratory investigations conducted during hospitalization—AI demonstrated exceptional results in profiling patient fall risk. Although these results are based on a limited sample of patients, they offer promising implications for large-scale application, potentially minimizing fall risks in hospital wards, improving patient care, and reducing the number of legal disputes involving the hospital.

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Artificial Intelligence; Clinical Risk Management; Falls

D23 The Role of Engineering in Forensic Science: Physics Applied in a Case of Hemorrhagic Shock From Central Venous Catheter Blood Loss

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Learning Objectives: Hemorrhagic shock is a type of hypovolemic shock in which severe blood loss results in an insufficient oxygen supply to the cells, ultimately leading to death. The purpose of this work, which concerns the case of a serious error committed by a nurse who failed to close the Central Venous Catheter (CVC) after discontinuing therapy, causing a significant blood loss, is to highlight the importance of integrating medical and engineering competencies in order to calculate the extent of the hemorrhage.

Impact Statement: This work underlines the importance that the engineering consultancy has had in determining the exact extent of the patient's bleeding and, therefore, in supporting the cause of death as a hemorrhagic shock. After this case, we suggest always performing an engineering consultancy in all deaths related to errors in the management of CVC, especially in those cases where health care personnel might try to conceal the evidence.

Abstract Text: Nursing activity in Italy is made extremely complex by the fact that, due to a shortage of nursing staff, the patient load on a single health care worker can often be very high. This can lead to serious errors which, as in the case under consideration, can have dramatic consequences, including the death of the patient. In particular, in the case we present, the failure to close a CVC at the end of a drug infusion resulted in hemorrhagic shock that led to death.

The combined effects of intravascular volume depletion, loss of red-cell mass and procoagulant elements, simultaneous activation of the hemostatic and fibrinolytic systems, compensatory mechanisms, and iatrogenic factors contribute to the development of coagulopathy, hypothermia, and progressive acidosis.[1,2] These factors result in further derangements, ultimately leading to death.[3,4] As patients age, physiological reserves decrease, the likelihood of anticoagulant use increases, and the number of comorbidities grows. Due to this, elderly patients are less likely to handle the physiological stresses of hemorrhagic shock and may decompensate more quickly.[1]

This work presents the case of an 84-year-old man who died during hospitalization at an intermediate care facility. During the evening check, the health care staff found him pale and with poor vital signs. Once uncovered from the blanket, the nurse noted a large blood stain on the bed coming from the CVC for the dialysis, in use for the infusion of drugs. The CVC had two access routes: one for the infusion of saline and glucose solution, and the other for the administration of piperacillin/tazobactam and calcium gluconate, which was no longer in use. In particular, the blood leak came from the unused route, which appeared unclamped. The nurse immediately clamped it and informed the doctor. The medical team promptly administrated oxygen, liquids, and drugs and called the emergency services but, despite the resuscitation efforts and the administration of adrenaline, the patient was declared dead. In this case, the help of an engineer, along with careful medical-legal advice, was decisive in understanding the extent of the bleeding in order to determine if this hemorrhage was indeed decisive for the death.

From the medical records, we were able to find the method and timing of the CVC use. This made it possible to determine when the error of failing to close the catheter occurred and how long the blood flowed out through it. On the other hand, the engineering consultancy enabled an accurate assessment of the blood loss by the analysis of the catheter (length and diameter), integrating the characteristics of the blood (density, dynamic viscosity, and kinematic viscosity), and evaluating the energies applied to both the catheter and the blood (pressure jump between right atrium and catheter, kinetic energy of blood flow exiting the catheter, gravitational potential energy, pressure drop distributed along the catheter, pressure drop concentrated at the catheter entrance).

The results estimated a blood loss of 1.79 liters in 2 hours and 15 minutes, assuming the catheter exit hole was at the same height as the right atrium. The value could vary by up to 20% due to minor fluctuations in height and pressure jump between the right atrium and the catheter exit.

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Engineer Consultancy; Central Venous Catheter; Hemorrhagic Shock

D24 The Use of Stationary Phases Designed for Supercritical Fluid Chromatography for the Reversed-Phase Liquid Chromatographic Separation of Nine Isomers of Δ9-Tetrahydrocannabinol

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Learning Objectives: The purpose of this presentation is to inform attendees on the use of stationary phases normally implemented for drug analysis using Supercritical Fluid Chromatography (SFC) to separate positional isomers of Δ9-Tetrahydrocannabinol (Δ9-THC) using a reversed-phase Ultra High-Performance Liquid Chromatographic (UHPLC) system.

Impact Statement: This presentation will impact the forensic science community by presenting, for the first time, a chromatographic method that implements SFC stationary phases using reversed-phase UHPLC for the separation of forensically relevant phytocannabinoids on a Liquid Chromatographic system. The use of these phases shows potential for difficult separations.

Abstract Text: Phytocannabinoids are a class of naturally occurring compounds that act on the CB1 and CB2 cannabinoid receptors resulting in physiological and psychological effects such as euphoria, increased appetite, cognitive impairment, and sedation, the most notable of which is Δ9-THC.[1] Δ9-THC is classified as a Schedule I substance under the 1971 Controlled Substances Act and is federally outlawed in the United States. However, under the 2018 Farm Bill, no provisions are included for other compounds of this class that are present in hemp, including isomers of Δ9-THC.[2] This, along with variations in the legality of these substances between states, has culminated in an increase in their presence in commercial products, thus rendering the ability to confidently identify them all the more imperative. For the separation of Δ9-THC isomers, liquid chromatography is favored over gas chromatography due to the decarboxylation of acids related to the target compounds by the latter technique.

The primary focus of this study was to investigate the utility of stationary phases normally employed in SFC for the separation of nine positional isomers of Δ9-THC under Reversed-Phase Liquid Chromatographic (RPLC) conditions while also comparing these results to those obtained from more traditional method optimization practices.

Nine THC isomers were selected for examination: Δ9-THC, Δ8-THC, *exo*-THC, (6aR, 9S/R)-Δ10-THC, (9R/S)-Δ6a·10a-THC, (9R)-Δ7-THC, and CBD with another isomer, Δ9-THCP, being used as a reference compound. Relative retention data was collected for all compounds under varying chromatographic conditions, including acetonitrile- vs. methanol-modified mobile phases as employing tandem-column systems comprised of various classical silica-based stationary phases as well as silica hydride columns to which the data produced using the SFC stationary phases could be compared. For these chromatographic conditions, the best separation was obtained using a RP C18-RP PFP coupled-column combination for which four out the nine target compounds were satisfactorily resolved. However, employing the chiral SFC stationary phase CEL1 using a combination, both acetonitrile- and methanol-modified mobile phases satisfactorily resolved all nine isomers.

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Phytocannabinoids; Separation; Δ9-THC Isomers

D25 Augmented Reality Visualization for Postmortem Analysis of a Traffic Accident: Clarification of Injury Mechanism Through PMCT-Based Antemortem Posture Reconstruction

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Learning Objectives: Attendees will learn how to utilize Augmented Reality (AR) in conjunction with Postmortem Computed Tomography (PMCT) for forensic investigations. They will gain an understanding of the process of PMCT-based antemortem posture reconstruction and its application in clarifying injury mechanisms in traffic accidents. Furthermore, participants will learn to create and analyze 3D models of injury patterns, enhancing their ability to present complex forensic findings in a clear and understandable manner.

Impact Statement: This presentation will significantly impact the forensic science community by introducing an innovative approach that combines AR with PMCT for detailed injury analysis and scene reconstruction. By demonstrating the practical application of these technologies, the presentation will provide forensic professionals with advanced tools to improve accuracy in determining injury mechanisms and effectively communicate findings to non-medical stakeholders, including in legal contexts. This will enhance the overall quality and clarity of forensic investigations and reports.

Background: In forensic medicine, PMCT has proven to be a very helpful tool that complements conventional dissection. PMCT combined with AR offers a novel approach to analyzing injury mechanisms and reconstructing crime scenes with precision. Here we report a case in which AR visualization using PMCT-based antemortem posture recreation was useful for demonstrating the mechanism of injury and recreating the scene.

Case Presentation: A man in his 80s was found lying at the roadside and was later confirmed dead at a hospital. Subsequent investigation suggested that the deceased might have been struck by a car. Inspection of the suspect vehicle revealed a bent at the left front bumper, and the lower part of the left front light was found to be covered with the deceased's hair and sebum. PMCT and autopsy findings suggested that the deceased may have been struck by the vehicle from the right side first, given the severe damage to the right side of the body, including abrasions, the right chest bruising, and multiple fractures of the right ribs.

To clarify the mechanism of injury, a 3D model of the bones arranged in three postures, including standing, prone and crouching patterns created from PMCT data, was fitted to a car of the same model as the suspect vehicle using AR. This revealed that the lower part of the left frontal light, where the victim's hair and sebum had been found, matched the location of the skull fracture if the victim had been in a crouching posture. Moreover, in this posture, the location of the right rib fracture coincided with the left front part of the car. Therefore, the deceased was most likely initially struck by the car from the right side when in a crouching position.

Discussion and Conclusion: Sometimes, the positional relationship between the deceased and the wounding instrument can be explained by 2D information such as drawings or sketches, which are often difficult for non-medical scientists to understand. In the present case, AR visualization using PMCT-based antemortem posture reconstruction was able to reveal the mechanism of injury more accurately than drawings or a person of similar physique because it was difficult to reproduce the antemortem posture, including joint movements, in this way. This method has considerable potential for conveying information to non-medical personnel, such as in court cases, so that they can share the same visualization or view, leading to common recognition of circumstances related to an accident among forensic pathologists and non-medical individuals. Thus, AR visualization using PMCT-based antemortem posture reconstruction may facilitate detailed simulation of the injury mechanism and reconstruction of the scene.

Forensic Imaging; 3D Technology; Virtual Reality

D26 Environmental Signature Extraction for Forensic Analysis of Audio Recordings Using AI/LLM Models

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Learning Objectives: After attending this presentation, attendees will understand the key principles of environmental signature extraction from audio recordings, the necessary elements for implementing the EnviroSound framework, characteristic patterns of environmental noise in various contexts, and practical applications of this technology in forensic investigations. This presentation will impact the forensic science community by providing a systematic approach to analyzing background noise, enhancing traditional forensic methods through advanced audio processing techniques and interdisciplinary collaboration.

The accuracy and scope of forensic audio analysis can be significantly improved by incorporating background noise analysis. This approach allows for a more comprehensive understanding of the audio environment, including details such as location, integrity, speaker count, and distance. By evaluating these elements, investigators can gain valuable insights into the context of audio recordings, which may be critical in legal and investigative settings.

The term "EnviroSound" is proposed as a description of an advanced audio analysis framework that utilizes deep learning and Large Language Models (LLMs) to distinguish and map environmental contexts from background noise. This technique serves as an adjunct to traditional forensic methods, offering a more detailed and nuanced understanding of the audio evidence.

This presentation will include a case study demonstrating the practical application of EnviroSound, illustrating its potential benefits and use cases. It is recommended that forensic investigators and professionals in related fields become familiar with the principles of EnviroSound to enhance their analytical capabilities and improve the accuracy of their investigations.

Impact Statement: This presentation will significantly impact the forensic science community by introducing EnviroSound, a framework for environmental signature extraction from audio recordings. By effectively isolating and analyzing background noise, EnviroSound offers a powerful tool for forensic investigations, enabling a more detailed understanding of the circumstances surrounding recorded events. The framework's advanced capabilities in distinguishing environmental contexts, such as location and speaker count, provide crucial insights that can aid in the reconstruction of crime scenes and the verification of alibis.

The introduction of EnviroSound augments traditional forensic methodologies by incorporating state-of-the-art deep learning models and LLMs, which enhance the accuracy and depth of audio analysis. This approach opens new avenues for interpreting complex auditory data, making it an asset in the investigation of crimes where audio evidence plays a critical role. As a result, EnviroSound has the potential to become a key component in the toolkit of forensic scientists, offering a systematic and scalable solution for audio-based forensic analysis.

Abstract Text: The extraction of environmental signatures from speech for contextual inference has garnered significant interest in recent years, contributing to various applications, from smart assistants to surveillance systems and forensic analysis. In contrast to speech processing, environmental sounds are often disorderly and noisy and despite technological advancement, current methodologies often encounter challenges in accurately extracting significant environmental data from background noise, thus limiting their efficacy.

Our research presents EnviroSound, a two-phase framework used to separate background noise from speech and uses this data to know the location, integrity, speaker count, and distance. Our framework integrates deep learning techniques and LLMs for micro and macro signal mapping. Given an audio signal artifact $X \in Rn$, where n is the length of the audio signal trace, the objective is to design an algorithm that outputs a classification C. This problem is defined as: $X = S + N$, where S is the speech component and N is the background noise. The goal is to isolate N and map it to specific Environmental contexts, E. We also aim to map a function to determine the Location (L), Integrity (I), number of speakers (K), and Distance (D) from the extracted background noise, $\{E,L,I,K,D\} = f(N)$.

Previous research has explored different techniques for audio signal processing, such as Denoising Autoencoders (DAE) and U-Net architectures. However, these approaches struggle to comprehend the complexities of environmental sounds. Recent advancements in AI and LLMs like Audio Spectrogram Transformers (AST) exhibit great potential but need further refinement for practical implementation. EnviroSound uses self-supervised learning techniques to minimize the need for labeled datasets and also allows the model to generalize across diverse environments and scenarios. The innovation of EnviroSound lies in its end-to-end processing pipeline, which not only isolates specific noises (micro signals) but also places them within broader environmental contexts (macro signals). This dual capability facilitates the precise identification of environmental factors.

We conducted evaluations using the UrbanSound8K and ESC50 datasets as well as custom-collected audio samples representing various environments and scenarios. Our approach showed high accuracy in distinguishing between background noises and accurately mapping environmental contexts. Initial observations suggest that EnviroSound excels compared to conventional techniques in both noise separation accuracy and environmental context inference. Our findings indicate significant improvements in the functionality of smart assistants, surveillance systems, and forensic analysis, making them more context-aware and effective.

Preliminary results have shown the potential of EnviroSound to advance the field of intelligent audio processing; future research could explore the integration of additional contextual data, such as metadata from recording devices or geographical information Systems (GIS), to improve accuracy in environmental mapping. Additionally, incorporating advanced techniques in unsupervised and semi-supervised learning could further reduce the need for labeled datasets, making the model more adaptable and scalable.[1-5]

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Audio Analysis; Machine Learning; Forensic Science

D27 Quantitative Similarity Assessments of Forensic Images

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Learning Objectives: This presentation will describe the methods that underpin ShoeComp, a shoeprint comparison tool developed by CSAFE following a similarity assessment workflow for shoeprint images. We will focus on techniques from graph theory and image processing that have been used to quantify similarity between different kinds of images and explain how these were applied to shoeprint comparisons at relevant steps in ShoeComp.

Impact Statement: This presentation will have an impact on the community of forensic footwear examiners by exposing them to the techniques explored in CSAFE for obtaining quantitative assessments of similarity between two shoeprint images. The new methods will empower examiners to carry out quantitative evaluation of footwear evidence to confirm (or raise questions in) their categorical conclusions.

Abstract Text: Algorithms for quantifying shoeprint similarity use techniques from image processing to obtain a score, and their performance can be evaluated on a benchmark dataset of known ground-truth pairs. These automated comparison methods calculate a similarity score between the images by estimating properties the images may have in common. For example, the score could be calculated using common configurations of interest points, like corners or centers of circles, patches of the image that output similar values when processed by neural networks, or a process combining the two steps as well.[1-4] Benchmark datasets for evaluating the methods include the CSFID-300 database, and more recently, ShoeCase, a collection of mock crime scene impressions, and a collection released as part of a study of the accuracy and repeatability of footwear examiners' decisions.[5-7]

However, shoeprint images recorded at a crime scene may have effects that were ot part of the benchmark dataset. Crime scene prints may reflect a small portion of the outsole, lack of the "L" scale, suffer from stretching, shearing, and perspective distortions, and be contaminated by noise from the substrate on which the impression was made. Such effects complicate the similarity assessment. Automated methods to compare images need to account for these factors by providing the examiner with a quantitative and/or visual indicator of the comparison process.

At CSAFE, we have developed ShoeComp, a semi-automatic shoeprint comparison tool that is meant to be a component in a workflow for calculating the similarity between shoeprint images. ShoeComp implements an algorithm based on maximum cliques to align and compare shoeprint images using interest points that are marked by the user. After the alignment is complete, ShoeComp displays similarity scores calculated with the provided images along with a visual overlay so the user can evaluate how the quantitative assessment agrees with their comparison process.

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Footwear Evidence; Software; Statistics

D28 *Kumho*, Engineering, and the Linguistics of Definitions

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Learning Objectives: Attendees will learn basic definitions of terms such as science, technology, expertise, experience, methodology, and reliability that are crucial for forensic evidence. Further, attendees will learn how the *Kumho* decision defines terms relevant to forensic engineering methodology.[1] Attendees will see how the language of definitions affected the lower court's decision that led to the United States Supreme Court ruling.

Impact Statement: This presentation will impact both forensic engineers and the legal community, as well as other forensic methodologies, by demonstrating the precise and imprecise definitions that are used in discussions of forensic evidence.

Abstract Text: Definitions are crucial. In the *Kumho* decision, the definitions of terms such as scientific and technical are the crucial elements for understanding the Supreme Court's reversal of the Eleventh Circuit Court of Appeals and the exclusion of the testimony of a tire expert presented as a forensic engineer.[1] This talk presents standard definitions of terms such as science, technology, expertise, experience, methodology, and reliability, in the context of how definitions are created from the perspective of linguistics. The linguistics of definitions include context, collocation, syntagmatic and paradigmatic relations. The *Kumho* majority, concurring, and dissenting opinions, and the lower court decisions are examined for evidence of these definitional strategies. The linguistics of interpreting definitions include structural and lexical ambiguity, scope of quantifiers, and other adjectives and conjunctions.

The linguistic analysis shows that for the Supreme Court opinion, knowledge subsumes paradigmatically both scientific and technical, and scientific subsumes technical. This aligns with common knowledge that there is no "non-technical" science. Further, the linguistic analysis shows that for the Supreme Court opinion, experience is not a defining feature of reliability whereas validation testing and publications are defining features of reliable methods. Experience is to the non-scientist what experiment is to the scientist (i.e., experience is what non-scientists want to rely on while true scientists rely on experimental results to demonstrate the reliability of a method).

Current confusions about these terms are discussed, including the idea of a "non-technical science," "experience-based science," "experiential discipline like engineering"—none of which occur in the Supreme Court opinion. Especially dangerous is the notion that engineering is an "experiential discipline," apparently based on the false idea that the witness engineer in *Kumho* was actually practicing engineering when actually he was relying on his experience as an employee of Michelin and using visual inspection rather than experimentation. The expert's testimony in *Kuhmo* should not be in any way considered representative of forensic engineering; the Supreme Court's holdings and dicta do not make the faulty assumption that *Kumho's* expert actually is representative of forensic engineering. These and similar phrases indicate that a lack of understanding of science and technical knowledge is still rampant. The talk concludes with operational definitions that can be used by both engineers and attorneys to evaluate the scientific or technical status of methods that provide admissible or inadmissible evidence.

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Engineering; Evidence Evaluation; Kumho

D29 A Tool to Quickly Estimate Uncertainty of a Forensic Test

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Learning Objectives: After attending this presentation, attendees will gain experience on how to identify the major sources of uncertainty, estimate them, and combine them with the aid of an open-source software tool, developed in a free programming language that allows multi-platform implementation. By using this tool, a quick, preliminary estimate of measurement uncertainty is possible.

Impact Statement: This presentation will impact the forensic science community by showing how uncertainty associated to the result of a forensic test can be quickly estimated by means of a software tool that guides the operator in identifying and quantifying the different contributions to uncertainty and combine them to obtain the desired uncertainty value.

Abstract Text: According to the best practice in measurement, any measurement result, that is any value obtained from experimental activities, shall include an uncertainty statement.[1-3] More recently, in an attempt to provide a scientifically sound answer to the questions raised by the 2009 National Research Council (NRC) document and the 2016 President's Council of Advisors on Science and Technology (PCAST) Report about the validity of the forensic activity, the application of metrology was proposed.[4-7]

It was proved that the evaluation of measurement uncertainty associated with a measurement result allows the technical expert to express the measurement result as an interval of values that can be attributed to the measurand with a give probability.[2,7] Consequently, whenever the measured quantity has to be compared with a threshold or a limit set by the law, as is the case, for instance, in Blood Alcohol Concentration *or* Content (BAC) tests, it is possible to submit a probability of making a wrong decision to the trier of fact, who can then decide with full awareness[7].

While clear indications are given by Standards on how to evaluate uncertainty, the identification of all contributions to uncertainty, from the definitional uncertainty contribution to the instrumental uncertainty contribution due to previous calibration, possible drift and the operator, is not straightforward and "calls for insight based on experience and general knowledge, and is a skill that can be learned with practice."[2]

The availability of open-source platforms, such as Python, to develop efficient software tools is of great help to the practitioners who need to quickly assess measurement uncertainty and, in the case the measured value shows that a limit has been exceeded, also the probability that, on the contrary, the limit has not been exceeded.

Using such platforms, it is possible to develop, in a relatively simple way, Graphic Users Interfaces (GUIs) that can guide the user to estimate the different contributions to uncertainty and input them in the generated graphic interface, so that the software tool can combine all contributions, evaluate the final measurement uncertainty and, given a threshold, evaluate the probability that the actual value of the measured quantity is above or below the threshold.

The presentation deals with an example of such software tool developed by the authors, shows how a well-designed GUI can guide the user in identifying the required input data, that is the uncertainty contributions, how they are combined, and how the result is presented to the user in a graphic, self-explaining way. An example on how this tool can be employed to assess the probability that a BAC value provide by a breathalyzer is above a given threshold is also provided.

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Forensic Metrology; Uncertainty; Probability of Wrong Decision

D30 Artificial Intelligence Tools Applied to Forensic Engineering Cases: Overcoming This Challenge Through an Academic Cooperation Program

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Learning Objectives: Participants will learn how the authors, as forensic engineering experts, are overcoming the challenge of developing AI tools to improve their activities thanks to an academic cooperation agreement established between a forensic laboratory and universities.

Impact Statement: We believe that the forensic science community will be inspired by the example of academic cooperation established between a forensic laboratory and universities, with the aim of solving complex problems together and advancing research in the field of forensic sciences.

Abstract Text: Forensic examination in engineering contracts requires a thorough analysis of bureaucratic processes, making it indispensable for professionals to have access to all documentation.[1] Currently, Brazilian Federal Police cases of Forensic Engineering involve the analysis of voluminous digital files. This task requires a true "excavation" among thousands of pages of documents, making it an activity susceptible to human error, and it also consumes a lot of time for criminal experts.

Given this context, one of the alternatives envisioned to optimize this work is the design and implementation of tools that use Machine Learning (ML) techniques. In recent years, ML has revolutionized Natural Language Processing (NLP) by enabling the development of models that can learn complex representations of unstructured data, such as texts written in human language.[2,3].

To classify documents in a way that identifies and distinguishes them automatically, a computational tool was developed, titled Aavia (an acronym for *Feasibility Analysis Document Assistant* in Portuguese). Computer and data scientists from the Federal University of Paraíba collaborated with criminal experts from the Brazilian Federal Police Forensic Laboratory in Paraíba to develop the prototype of Aavia. It is now being deployed to the institution's Technical-Scientific Directorate for use by Brazilian federal forensic engineers and accountants nationwide. The software's widespread adoption will lead to substantial time savings for forensic investigations, enabling professionals to focus on qualitative and more objective analyses of organized reports produced by the tool.

Overcoming this challenge of implementing AI in Forensic Engineering in a non-predatory manner—that is, without affecting the users' ability to think, but merely facilitating the automation of non-intellectual stages of forensic work—was only possible thanks to the Academic Cooperation Program in Public Safety and Forensic Sciences (PROCAD/CAPES). This program has been developed since 2021, encouraging university research on real problems presented by forensic laboratories in more than one area of forensic sciences—in this case, in Engineering and Computer Science.

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Artificial Intelligence; Forensic Engineering; Research

D31 Falsely Accused: Generative AI and the Allegation of Academic Plagiarism

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Learning Objectives: Attendees will learn how ChatGPT and other generative AI writing systems are affecting higher education with regard to allegations of plagiarism. Attendees will learn how to test the allegation and, if needed, to defend against the allegation of generative AI-aided plagiarism.

Impact Statement: Academics within the forensic science community will be helped by learning about a case of being falsely accused of generative AI-aided plagiarism, and how one university handled the investigation.

Abstract Text: In a health sciences department of a highly ranked, prestigious university in the southern United States, a graduate student completing a Master's degree was accused of using generative AI to compose a final paper. As a violation of the university's Honor Code, plagiarism is treated very seriously at this institution. The graduate student was facing being expelled from the program, losing all of her graduate credits, and having her reputation and future career plans ruined. The graduate student was advised by one of her professors to fight the charge by contacting a forensic linguist. This talk presents the forensic linguistic analysis.

For AI detection, the question is: is the disputed essay more like machine-generated or human-generated text? This line of research produces tools called AI Detectors, at least one of which was apparently used to support the allegation that the disputed essay was generated by machine rather than a human. The currently available tools have either high false positive rates or unpublished false positive rates.[1] For this reason alone, an allegation about machine-generated authorship should not rely solely on AI Detectors, because there is such a high chance of a false allegation based on the high chance of false positive classification.

For Authorship Identification, the question is: is the disputed essay more like the known authorship of the graduate student or more like the authorship of machine-generated text? This line of research has three classes of methods: (1) stylistics; (2) stylometry; and (3) computational linguistics.[2,3]

The graduate student provided, as data for analysis, the questioned document (the disputed final paper) and known documents whose authorship was not disputed from her completed classes at the university, contemporaneous classes, and publications before she attended the university. The issue in this case was two-fold: AI Detection and Authorship identification. Further, data was collected using Mcirosoft CoPilot to get AI-generated text on the subtopics within the disputed final paper. Micropsoft CoPilot generated text on most but not all of the subtopics. For these subtopics of the final paper, generative AI could not have been deployed because it would not generate text on the subtopic.

Using the ALIAS AI-Detector, the known texts, known Co-Pilot results, and the disputed paper's segments were tested. The results confirmed that the disputed paper was not machine generated. Using ALIAS SynAID for Authorship Identification, the known Co-Pilot text was tested against the known graduate student texts and differentiated with 100% cross-validated accuracy. This model of authorship then classified the segments of the disputed paper, all to the known graduate student class and none to the machine-generated class.

The student won her case in the Honor Board hearing.

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Artificial Intelligence; Plagiarism; Accuracy

D32 Weaknesses of Artificial Intelligence Proxies of Topic for Forensic Linguistic Applications

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Learning Objectives: After attending this presentation, attendees will better understand two different notions of the term "topic" in linguistics, the proxies that are used for them in many artificial intelligence systems, and the consequences these could have for forensic linguistic applications, particularly with respect to authorship analysis.

Impact Statement: This presentation will impact the forensic science community by clarifying crucial underlying assumptions of "topic control" in various types of Machine Learning (ML) applications and exposing some of their weaknesses from a linguistic perspective, calling attention to an important potential area of bias if such technology were to be applied to the forensic examination of, for example, authorship analysis.

Abstract Text: In the domain of computational authorship analysis, we find different kinds of systems. Some are rule-based and their developers know how they operate, such as ALIAS SynAID. These best match the criteria for the admissibility of scientific evidence in court as they are explainable and reproducible.[1,2] However, a majority of authorship analysis systems are now unexplainable ML systems, as neural models in artificial intelligence have gained favor outside of the forensic community. Such systems are shown in peer-reviewed publications to have very low error rates in some settings, despite being so-called "black boxes."[3] There is a worry, then, that they might be used in analyses that would be presented before a court. This presentation argues that if discourse topic were properly controlled, the error rates of such systems would likely be much higher.

Many ML systems use a rough notion of semantic similarity (related to discourse topic) to make distinctions to a certain degree. For example, say you train a system to distinguish Author A and Author B, but all of the texts from A are about the weather and all the texts from B are about food; if, during evaluation, you give the system a text about pizza, it's not difficult for it to attribute it to B. For this reason, there is much interest in so-called "topic control"; that is, trying to make sure that both A and B are talking about the same topics so that the system cannot rely on "topic" as a basis for its discrimination. The ways in which topic is understood and controlled, however, are not always well informed by a linguistic understanding of these terms. In this talk, we:

- define and distinguish sentence topic and discourse topic in linguistics,
- discuss the difference between topic in conversational v. non-interactive texts,
- describe proxies used for topic control in artificial intelligence, including, for example, using different Reddit forums as different topics,
- expose weaknesses in these proxies with examples,
- present experimental evidence showing that linguistic topic control is superior to the traditional ML proxies and that it leads to higher error rates for ML systems, and
- explain how language models like the popular BERT model are biased to rely on semantic similarity such that they are disproportionately affected by topic control, unlike more syntactically oriented models.[4]

Our experimental evidence comes from tests run using as data the Fisher corpus where people were asked to have phone conversations with a stranger on one of a range of provided topics, such as "hobbies."[5] The calls were then transcribed and verified by humans. We compare three conditions on several off-the-shelf ML authorship models, including BERT, as follows. In each case, authorship verification trials matched two transcripts from either the same speaker or different speakers. In the first condition, there was no topic control and lower error rates. In the second condition, we used a traditional proxy for topic, the provided conversation topic, such that people in the "different speaker" pair had been assigned the same general subject to discuss. This showed slightly higher error rates. Finally, we used a linguistically controlled topic in which the "different speaker" pairs were the two sides of the same conversation; in other words, the people who were speaking to one another. In this third condition, the systems were reduced to chance from ~80% success rates in the first condition, showing that this technology is not currently appropriate for forensic contexts.

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Artificial Intelligence; Authorship Analysis; Linguistics

D33 A Computational Method for Detecting Sophisticated Plagiarism

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Learning Objectives: Attendees will learn the three types of plagiarism, how the three types of plagiarism differ, and how one type is more sophisticated and harder to detect than the other types. Attendees will understand a method for detecting sophisticated plagiarism that includes an error rate.

Impact Statement: This presentation will impact the forensic linguistic and legal communities by demonstrating a method for detecting plagiarism which can be litigation support in several types of cases.

Abstract Text: In the United States, unlike other jurisdictions, plagiarism is not a crime, but it can play a role in civil suits. In copyright infringement cases, evidence of plagiarism supports the claim of violating copyright. In employment cases involving issues in academia, charges of plagiarism must be substantiated as part of the decisions about tenure and appointments. In both criminal and civil investigations, "copycat" communications can be approached with plagiarism detection.

There are three types of plagiarism: (1) copy-paste; (2) mosaic, and (3) conceptual or semantic (for forensic linguistic descriptions, see Reference 1 below). Copy-paste plagiarism is, as it sounds, a direct copying from an unacknowledged document; this unsophisticated kind of plagiarism has been aided and abetted by electronic word processing where it is so easy to copy and paste text. Copy-past plagiarism is detected by a straightforward comparison of documents with exact matches of words in a row. It is generally accepted that plagiarism occurs when there are at least six words in a row that match, although some forensic linguistics have argued for common authorship on the basis of even three matching words in a row.[1-4] Mosaic plagiarism occurs when text from an unacknowledged source is rearranged.[1] From a syntactic perspective, mosaic plagiarism uses the four methods of transformation used in early transformational grammar to create the paraphrase.[5] Again, this kind of plagiarism is fairly easy to detect using matching algorithms because the matches are very close to each other, even though it is clever as a plagiarism strategy.

The most sophisticated and difficult to detect plagiarism is conceptual or semantic.[5] In this type of plagiarism, synonymy often replaces the original source words, but for copyright infringement cases, the exact words must be found in both source and derivative texts. The semantic plagiarist, however, can use the same words as the source text so that there is lexical overlap between the text, but the plagiarist rearranges from afar, so that matching of sentences is nigh impossible. This talk presents a method for detecting semantic plagiarism with lexical overlaps. Important features of this method are that it provides a base rate for lexical overlaps from comparable texts; this base rate is used to measure the level of similarity between the source text, the suspect text and the base rate comparison texts, the level of similarity is statistically computed using two different statistical methods, the binomial probability and cosine similarity. Finally, this method can detect all three types of plagiarism.

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Computational Linguistics; Plagiarism; Copyright Infringement

D34 Analyzing Generative AI's Truth-Bias in Deceit Detection: An Empirical Experiment in English and Spanish

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Learning Objectives: In this presentation, attendees will learn about the automated behavior of generative AI models in linguistic deception detection tasks as compared to human judges, testing Truth-Default Theory (TDT) on two datasets of native speakers of English and Spanish, in order to test the reliability and error rate criteria for scientific evidence.

Impact Statement: This presentation will impact the forensic science community by contributing to the main question framed by AAFS 2025: Is technology a tool for transformation or tyranny? Specifically, the contribution of this presentation will highlight the importance of not relying simply on AI models for linguistic tasks in the forensic setting.

Abstract Text: The empirical study of deception in language dates at least from Undeutsch's research, who firmly believed in the existence of linguistic cues to deceit, a potentially relevant issue in the forensic setting.[1] In the past two decades, much research has been devoted to investigating deceit detection, and researchers have become increasingly interested in the development and evaluation of automated tools for identifying lies, giving rise to automated and computer-aided deceit detection.[2-4] The potential applications of automated and computer-aided deceit detection cover the areas of law enforcement, computer-mediated communication, and national security, to name but a few. In parallel, studies have explored the human ability to detect deceit across different levels of expertise, generally showing that even expert judges rarely get better than 50% accuracy.[5-7]

From that evidence, the TDT was formulated, claiming that humans have a truth-default, that is to say, a cognitive default state set to believing others.[8] This theory has recently started being evaluated on generative AI models too, specifically on ChatGPT-4 by OpenAI.[9] When prompted for a veracity judgment in English, the tool assumed that the user was honest (truth-bias), and upon some guidance by the researchers, the model slightly improved its performance.

In the first experiment presented in this talk, ground-truth writing of 20 authors who were native speakers of English is judged for veracity, both by college students and by the latest, highest intelligence models of ChatGPT and Microsoft Copilot. As for the second experiment, it is conducted on a Spanish dataset, as it is the most common non-English language spoken in the United States and the second most widely spoken language globally in terms of native speakers. The same procedure is adopted on ground-truth writing of 20 native speakers of Spanish for comparison sake—it is worth noting that the difference from the study presented by Markowitz et al. lies both in the languages explored and in the AI models used.[9] By virtue of the previous annotation of the ground-truth datasets, both tests results with error rates and accuracy rates can be reliably calculated and reported.

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Linguistics; Generative AI; Veracity

E1 Transforming Forensic Nursing Documentation and Enhancing Patient Outcomes Through Electronic Health Record Technology

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Learning Objectives: Attendees will: (1) comprehend the need for enhanced forensic nursing documentation strategies of Electronic Health Record (HER) implementation adhering to regulatory expectations, best practices for accuracy, clarity, and legal considerations as well as creating effective and legally defensible forensic patient records; (2) examine the intricacies of EHR implementation in forensic nursing by sharing challenges and successes, clinical workflow redesign, and data management; and (3) analyze how to leverage EHR technology utilization as a tool for transformation through enhanced patient safety, elevated care quality, upholding professional standards, and enriching the overall forensic patient experience.

Impact Statement: This presentation will provide the forensic science community, regardless of the role, a blueprint for leveraging technology to streamline forensic nursing documentation. It will demonstrate how to permanently document medical forensic assessments, ensuring their availability for continuity of care and future investigative and judicial processes.

Abstract Text: Violence is an urgent public health problem pervasive through each society.[1] Patients who are victims of violent crimes require trauma-informed, compassionate Forensic Nurses with competency in comprehensive assessment, evidence preservation, and detailed documentation. Regardless of the practice setting, Forensic Nurses will always be expected to complete thorough documentation for a variety of purposes.[2] Currently, some facilities use handwritten paper documentation, while others use electronic medical record documentation or a hybrid of written and electronic documentation.[3] Health care organizations that have transitioned to an EHR are complying with the legislative mandate of 2009.

The Health Information Technology for Economic and Clinical Health (HITECH) Act of 2009 is legislation that was created to stimulate the adoption of EHRs and the supporting technology in the United States signed into law by President Barack Obama.[4] HITECH stipulated that, beginning in 2011, health care providers would be offered financial incentives for demonstrating meaningful use of EHRs until 2015, after which time penalties would be levied for failing to demonstrate such use.[4]

When incorporating forensic patient interactions and subsequent documentation into the EHR, the six domains of health care quality serve as foundational building blocks for process improvement and redesign.

The Institute of Medicine (IOM) has identified these crucial six domains as patient safety, effectiveness, patient-centeredness, timeliness, efficiency, and equity.[5] Strategies to transform forensic nursing documentation from handwritten paper records to legible EHR documentation engage all six health care quality domains

Patients who have experienced sexual or domestic violence often have very complex documentation, including consent of care options, history of events, injury diagrams with measurements and descriptors, strangulation-specific assessments, drug-induced symptom reviews, photographs, details of evidence items collected, and chain of custody release. Forensic nursing programs adopting EHR implementation have opportunities to optimize data analytics and provide legible documentation accessible for continuity of care as well as investigative and judicial processes indefinitely.[6]

While clinical responsibilities center on competency for quality patient outcomes, the application of technology involving electronic documentation and digital imaging storage advances clinical outcomes and operational efficiency. Over the past few years, our large not-for-profit health system has undergone a comprehensive EHR transition, which included forensic nursing, providing first-hand experience and valuable insights to share with the broader forensic science community.

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Operationalize; Forensic Nursing Documentation; Electronic Health Record Technology

E2 Mitigating the Risk: Navigating the Legal and Ethical Challenges of Forensic Nursing Photography

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Learning Objectives: The goals of this presentation are to: (1) describe the purpose and value of forensic photography in patients who have experienced interpersonal violence; (2) recognize the legal and ethical challenges related to forensic photography in the health care setting; and (3) identify solutions to mitigate risk and reduce the revictimization of forensic patients within the forensic photography process.

Impact Statement: Forensic photography has been well-established as a standard of care in the health care response to interpersonal violence. It is well established that photographing injuries sustained in an assault holds great evidentiary value in the investigatory and judicial process. However, there are many benefits of forensic photography aside from evidence. The technology to capture images that illustrate the physical assessment of a forensic patient has introduced legal and ethical challenges. This presentation will open the discussion to mitigating these risks and implementing a trauma-informed approach to forensic photography.

Abstract Text: Forensic nurses are responsible for the care of victims of interpersonal violence, including sexual assault/abuse, intimate partner violence, and child or elder abuse, among others. An important part of the medical forensic examination is the assessment for physical and anogenital injuries, which are found in a substantial number of forensic patients.[1-4] Photo documentation is the standard of care in the documentation of findings, particularly in cases of child sexual abuse.[5]

From an evidentiary standpoint, these images function to corroborate the history provided, are an enduring record of the physical findings, contribute to the investigation of crimes, and are powerful visual aids to present in legal proceedings. These images hold value for the forensic health professional as well as for the victim of violence. Access to photo documentation from a forensic examination allows for peer and expert review, presents learning opportunities for forensic clinicians and other professionals, and contributes to research that serves to improve patient care. Perhaps an underappreciated value of forensic photography is for the patient. Capturing physical findings through photography may validate the victim's experience and provide teaching opportunities, particularly for children and families impacted by child sexual abuse.

Advancements in the technology to capture and maintain photographic evidence of the physical examination of a victim of violence or abuse have created many challenges. The predominant issues are the maintenance of the security of these images as well as the potential for revictimization of the patient. Secure data storage and controlling access to these sensitive images is paramount. The patient has the right to informed decision-making regarding the release of these images. The failure to incorporate a trauma-informed approach to forensic photography or disregarding the impact of imaging used as part of an assault or abuse will refute any benefits of this form of evidence. Forensic health care professionals and their multidisciplinary partners must work together to mitigate the risks to the safety and security of this evidence while ensuring that the victim's well-being is the highest priority. The goal of this presentation is to signal a call to action within organizations and communities to establish policies and procedures related to the storage, security, and release of this sensitive data.[6]

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Forensic Nursing; Photography; Digital Evidence

E3 Variability in Uploaded CODIS Profiles From Sexual Assault Kits

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Learning Objectives: After attending this presentation, attendees will: (1) understand the variability found in uploaded Combined DNA Index System (CODIS) profiles of foreign contributors from sexual assault kits as reported in the literature and in recent research, and (2) assess the multiple factors contributing to this variability.[1]

Impact Statement: This presentation will impact the forensic science community by stimulating discourse and evaluation on the factors contributing to the CODIS profile upload variability, especially for forensic nurses/examiners and forensic scientists. Additionally, this presentation will highlight the need for collaboration between forensic nurses/examiners, forensic scientists, and researchers.

Abstract Text: As reported in the literature and in recent research findings from three publicly funded crime laboratories, the percentage of uploaded CODIS profiles of suspected assailants from sexual assault kits varies from 25% to 57%.[1-6] Prior studies reporting the percentage of uploaded CODIS profiles from sexual assault kits have not delved into the variability between crime laboratories. The presenters will share some of the potential factors contributing to the uploaded CODIS profile variability range between crime laboratories. Factors may include different evidence collection practices and policies by forensic nurses/examiners to swab selection for testing and the interpretation of Federal Bureau of Investigation (FBI) CODIS guidelines. These factors and others will be explored in the presentation through PowerPoint presentation and discussion.

National guidelines for sexual assault kit evidence collection, time frames for collection, and practice/policy implications will be reviewed.[7,8] FBI CODIS guidelines for uploading CODIS entries of foreign contributors will be discussed. Suggestions for improving communication and collaboration between forensic nurses/examiners and forensic scientists in publicly funded laboratories will be shared. A case study with group discussion and response will highlight the challenges in addressing the variability of sexual assault kit-uploaded CODIS profiles and offer potential solutions to decrease variability and standardize uploaded CODIS profile from sexual assault kits across crime laboratories and jurisdictions.

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Sexual Crimes; CODIS; Forensic DNA

E4 The Development of Serial Gaming for Forensic Nurses to Advance Disaster Training

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Learning Objectives: After attending this presentation, forensic nurses will be able to better understand the usefulness of the serial game as an innovative and problematized strategy for training in disaster contexts.

Impact Statement: This presentation seeks to demonstrate the process of developing a technology for simulated and problematized training of forensic nurses in chaotic disaster contexts.

Abstract Text: **Introduction:** Disasters are complex phenomenon that generate physical, emotional, social, and economic impacts for affected individuals, whether directly or indirectly.[1-3] In these contexts, the work of forensic nurses is essential for the care of victims of violence, trauma, and human trafficking.[1-4] Therefore, it is necessary to develop immersive simulations that more effectively prepare these professionals to work in disaster scenarios.

Objective: Develop a serial game for the simulated and problematized training of forensic professionals, aiming to improve their performance in disaster contexts.

Method: This methodological study based on Pressman's Software Engineering involved seven nurses and two software engineers.[5] The development of the serial game occurred in five prototyping stages: (1) problematization, literature review, and preparation of the Game Design Document (GDD): definition of game mechanics, characters, levels, and User Interface (UI); (2) prototyping of the serial game: development of the operating mechanics and sketch of the icons; (3) development with Unity Engine 5: use of C Sharp as the main programming language; (4) arts development: creation of arts using Adobe Photoshop; and (5) final construction of the game: polishing to improve performance, improving mechanics and adding sounds, with sound effects and themes created by a sound designer.

Results: The serial game presents a narrative to address the context of a hydrological disaster. The scenario is presented through a journalistic plot, and the gamer is directed to a menu of options with different cases: (1) management of bodies in a gym—the player will come across the scene of a gym with more than 50 bodies of victims dead from the disaster and will be asked to take care to identify the bodies and collect evidence; (2) assistance to adolescent victims of sexual violence during the disaster—the player will be directed to assist a teenager who suffered sexual abuse after the disaster and will be directed to communication with the victim, forensic interview, and collection and preservation of evidence; (3) caring for a child with autism spectrum disorder, alone and without identification—the player will be asked to provide care to contribute to managing the victim's identification; (4) address a situation of negligence involving a pregnant woman and a family with several children and the need to manage a body—the player will be directed to indicate care for identifying the body and forensic care for victims with post-traumatic stress disorder; (5) assistance to elderly people who suffered physical and psychological violence during the disaster—the player will be directed to reflect on how to carry out forensic recording and documentation in the context of disasters; and (6) assistance to young people who use a wheelchair and have a dog that requires screening care—will be aimed at providing forensic care for screening, identification, and prevention of violence.

Conclusion: The development of a serial game to prepare forensic nurses in disaster contexts represents an innovative and significant approach in the education and training of these professionals. This methodological study integrates elements of storytelling and immersive simulation. Serial games provide a means of improving critical skills and understanding the complexities involved in acting in disaster scenarios, based on problematized and interactive learning through the intersection between education, technology, and forensic health practices. As a future development, the serial game will be validated by professional forensic experts and will be launched on a game hosting website.

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Forensic Nursing; Disaster; Game

E5 The Importance of Selection Areas for the Collection of Sexual Assault Evidence From the Oral Cavity

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Learning Objectives: Attendees will learn about new areas of the oral cavity that can successfully yield DNA profiles especially when used with a modification to modern solid phase DNA extraction commercial kits commonly used in forensic science laboratories.

Impact Statement: Oral cavity samples are thought to contain minimal quantities of DNA likely resulting in non-usable DNA profiles; however, the results of this study indicate that full Y-chromosomal Short Tandem Repeat (Y-STR) profiles can be obtained up to 24 hours post-fellatio.

Abstract Text: The presented research proposes methods to maximize results of sexual assault collection kits by determining optimal methods for collection and analysis of samples collected from mock sexual assault samples within 24 hours of oral sex. Fellatio, commonly performed during sexual assault, was investigated to determine if male DNA is prevalent enough in a female's mouth to result in a male DNA profile.

For this study, previous work provided guidance for the areas in the oral cavity best suitable for the collection of samples.[1] These collection areas in the oral cavity differed from published recommended guidelines.[2] This study examined cotton and nylon flocked swabs to determine which may yield better profiles for collection of samples after fellatio. Swabs were taken of the lips with a moist swab, followed by a dry swab, where each swab was analyzed separately. Another swab was used to collect inside the mouth, which consisted of swabbing behind the molars, along the gumline, the back of the cheek, and under the tongue on both sides as far back as possible. In addition, different time intervals were tested including 1, 6, 12, and 24 hours. Participants in this study were asked to record activities that included eating, drinking, and oral hygiene to help gauge the effects those activities have on the amount of DNA profile obtained.

Samples underwent traditional DNA extraction with the addition of Dithiothreitol (DTT), followed by Polymerase Chain Reaction (PCR) amplification with Y-STR primers. Full male Y-STR DNA profiles were found at each time interval and swabs of the lips yielded the best results with cotton and nylon being moistened with molecular grade water. At least once at each time interval, full male DNA profiles were obtained. An increase in observed alleles was obtained by increasing the electrophoresis injection to 15 and 20 seconds. When looking at the 144 swabs after post-PCR purification was performed, 57% of the swabs had a full profile and when entered in Y Chromosome Haplotype Reference Database (YHRD), 75% of the DNA profiles had no matches. The identification of successful sample collection methods, including swab type and sampling areas, could improve sexual assault investigation. This research can be integral in standardizing sample collection by forensic nurse examiners and improving analysis by forensic scientists to enable the best chance of obtaining a DNA profile.

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Sexual Assault; Y-STR; Oral Swabs

E6 An Analysis of Police-Led Jail Diversion Programs in Massachusetts: Evaluating Efficacy and Proposing Enhanced Crisis Response Models

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Learning Objectives: After attending this presentation, attendees will understand the various models of police-led pre-arrest Jail Diversion Programs (JDPs) in Massachusetts, the predictors of successful diversions, and the potential for innovative crisis response models that extend beyond traditional frameworks.

Impact Statement: This presentation will impact the forensic science community by providing a comprehensive evaluation of diversion strategies, proposing ethical decision-making enhancements, and suggesting new models for crisis response that prioritize individual welfare and public safety.

Abstract Text: The interface between mental health and criminal justice presents a stark reality: individuals with mental illness are vastly overrepresented within the justice system, often not due to criminal actions but due to factors beyond their control.[1] Law enforcement is increasingly responding to behavioral crises involving serious mental illness, acute distress, substance use, and behaviors stemming from personality disorders by leading or partnering with JDPs. This underscores the need to further evaluate the impact of these programs.[2]

Insights from behavioral science and police and forensic psychology are critical in these efforts. JDPs, including joint response models (i.e., dual response of officer and clinician) and Crisis Intervention Teams (CIT), have been established to redirect individuals experiencing a behavioral crisis away from incarceration toward mental health treatment and recovery.[3] Police involvement in these situations has led to significant strides in training and protocol development, reflecting increased awareness and sensitivity to the complex needs of vulnerable populations.[4,5]

This study provides a comprehensive analysis of police-led pre-arrest JDPs in Massachusetts. It describes the various models within the Massachusetts Department of Mental Health (DMH) JDPs division, including CIT, Co-Response programs, and Component programs that integrate elements of CIT, co-response, and other crisis response strategies. Using uniformly collected data from 165 JDPs across the state, covering May 2023 to December 2023, the study utilized diversion records to conduct a descriptive overview of the different program types. Additionally, machine learning and AI techniques were employed to develop a predictive model for incident diversion outcomes based on factors such as charge severity, mental health status, substance use history, and incident specifics.

Outcome measures focused on diversion decisions. The data revealed 21,964 police encounters, among which 3,324 cases involved criminal offenses and were considered for potential diversion. The results indicate that among the cases in which diversion was an option, 86% were diverted, with less severe charges and first-time encounters predicting appropriate diversions, favoring rehabilitation. Effective de-escalation techniques and on-scene supports significantly influenced jail diversion, while encounters during off-hours were linked to increased arrests.

The findings underscore the complexity of implementing ethically aligned JDPs, emphasizing the need for nuanced approaches that consider individual and situational factors. Enhanced training and resources are essential to support ethical decision-making in police-led JDPs, ensuring that diversion decisions balance both individual welfare and public safety.[6,7] This study contributes to the discourse on reforming police practices to better address mental health crises, suggesting pathways for policy enhancements. Additionally, the study proposes the potential for new models of crisis response that extend beyond traditional frameworks, incorporating multidisciplinary approaches from behavioral science, police and forensic psychology, and forensic nursing to effectively address behavioral and mental health emergencies, medical crises, and drug-related incidents. The integration of forensic nursing, in particular, offers a holistic perspective and emphasizes the decriminalization of mental illness.[8]

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Police and Forensic Psychology; Forensic Nursing; Behavioral Science

E7 Responding to Sexual Violence in the LGBTQIA+ Community

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Learning Objectives: This presentation will: (1) help attendees understand the dynamics of sexual violence in the LGBTQ Community and effective responses of forensic nurses and law enforcement.[1,3] Participants will explore the unique factors contributing to sexual violence in the LGBTQ community, including societal stigmas, barriers to reporting to law enforcement, and cultural sensitivities. They will also learn strategies for providing informed and compassionate support in the context of the objective medical/forensic examination; (2) describe the difference between sexual orientation and gender identity.[2] Attendees will gain a clear understanding of the distinction between sexual orientation and gender identity, including how these concepts shape individual experiences and identities within the LGBTQ community; and (3) provide an LGBTQ-competent medical/forensic examination.[4] Professionals will be trained to perform medical and forensic examinations that are sensitive to the needs of LGBTQ individuals, ensuring the process is inclusive, respectful, and free of bias, while adhering to best practices for trauma-informed care.

Impact Statement: The presentation will highlight the necessity for specialized training, inclusive practices, and comprehensive understanding. Such a presentation will underscore the unique challenges and types of violence faced by LGBTQ individuals, encouraging forensic professionals to approach the medical/forensic exam with heightened sensitivity and awareness. It will promote the adoption of best practices for examination and evidence collection, ensuring that bias and discrimination do not compromise the integrity of forensic investigations. Additionally, this presentation will foster collaboration between forensic nurses/scientists, law enforcement, and advocacy groups, ultimately contributing to more effective and just outcomes for LGBTQ victims of violence. By educating and raising awareness within the forensic community, the presentation will play a crucial role in advancing both the scientific and ethical standards of the field.

Abstract Text: Sexual violence is a pervasive and deeply troubling issue that affects individuals across all demographics, but the LGBTQIA+ community faces unique and heightened vulnerabilities.[1] This presentation aims to address the multifaceted nature of sexual violence against LGBTQIA+ individuals, providing a comprehensive framework for understanding and responding to these challenges.

The presentation will include an analysis of current statistics, highlighting the alarming prevalence of sexual violence within the LGBTQIA+ community. Studies indicate that LGBTQIA+ individuals experience sexual violence at significantly higher rates compared to their heterosexual and cisgender counterparts. This disparity is often rooted in societal stigma, discrimination, and systemic barriers that exacerbate vulnerability and hinder access to resources.

A critical component will be the exploration of intersectional factors that contribute to the heightened risk of sexual violence for LGBTQIA+ individuals. We will delve into how factors such as race, socioeconomic status, disability, and immigration status intersect with sexual orientation and gender identity to compound experiences of violence and marginalization. Case studies and survivor testimonies will be used to illustrate these complex dynamics, providing a humanizing and relatable context to the statistical data.

The presentation will also address the significant barriers that LGBTQIA+ survivors face in reporting sexual violence and seeking support. These barriers include fear of discrimination, lack of culturally competent services, and mistrust of law enforcement and judicial systems. We will discuss the importance of creating inclusive and affirming environments in health care, legal, and support services to encourage reporting and facilitate recovery.

Best practices for responding to sexual violence in the LGBTQIA+ community will be a focal point of the presentation. Attendees will learn about culturally competent medical/forensic examinations, evidence collection, and the provision of holistic support services that address the unique needs of LGBTQIA+ survivors. Emphasis will be placed on the need for interdisciplinary collaboration and the development of policies and protocols that prioritize the safety and dignity of survivors.

Finally, the presentation will highlight existing legal protections and identify gaps that need to be addressed to ensure comprehensive support for LGBTQIA+ survivors. Attendees will gain practical knowledge and tools to advocate for and implement inclusive strategies within their respective fields, whether in health care, law enforcement, social work, or advocacy. By the end of the presentation, participants will have a deeper understanding of the complexities involved in responding to sexual violence in the LGBTQIA+ community. They will be equipped with the knowledge and skills necessary to provide empathetic, effective, and equitable responses, ultimately contributing to the creation of safer and more inclusive communities for all individuals.

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Human Rights; Sexual Crimes; Diversity

E8 Alternate Light Sources in Subclinical Bruising: A Systematic Review

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Learning Objectives: Attendees will be able to discuss the current literature of alternate light sources and its role and accuracy in identification of subclinical bruising, a common forensic technique used in investigations of domestic violence and strangulation They will also be able to describe the methodological approaches and validation techniques used to date.

Impact Statement: This presentation will impact the forensic science community by serving as a comprehensive summary of the current literature on the accuracy and reliability of alternate light sources.

Abstract Text: Alternate light sources is a technology that uses light emitted at a controlled wavelength (typically 400 to 700 nanometers) to identify and collect evidence for a variety of forensic investigations. In 2013, a study by Holbrook and Jackson postulated that alternate light sources can reveal soft tissue injuries that are not visible under white light in patients who report strangulation.[1] Since then, there has been a significant rise in the interest and utilization of alternate light sources to identify subclinical bruises. Despite this rise in use for both patient care and forensic casework, to date, no systematic review has been conducted. As forensic nurses and the forensic science community evaluate the benefits and next steps for alternative light sources, a systematic evaluation of the research literature is needed.

The authors conducted a systematic review (PROSPERO registration: CRD42024571124) to summarize the current body of literature on alternate light sources' role and accuracy in identifying subclinical bruises. One hundred fifty-three titles and abstracts were screened for inclusion and 26 full-text articles from 2013 onward were ultimately reviewed. To capture as many relevant citations as possible, a wide range of medical and scientific databases were searched to identify primary studies of alternate light sources and its role in any post-traumatic evaluation. The review was completed by two independent emergency medicine physicians who work at a Level 1 trauma center with long-standing experience caring for post-assault patients. Studied populations included live human, cadaver (embalmed and non-embalmed), and animal models as well as post-assault patients and those who had their injuries induced for the purpose of the study. The majority of study designs were cohort studies (retrospective and prospective) with few randomized control trials. The authors will further summarize each article's methodological approach, validation technique (if any), and reported sensitivity and specificity of alternate light sources to identify subclinical bruises. Avenues of future research are identified that could improve methodological approaches and validation techniques for alternate light sources.

Reference:

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Forensic Investigation; Strangulation; Bruising

E9 Revolutionizing Forensic Nursing Education: The Impact of Virtual Reality, Artificial Intelligence, and Performance Data Analytics

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Learning Objectives: After attending this presentation, participants will understand the methodology, implementation, and potential outcomes of using Virtual Reality (VR), Artificial Intelligence (AI), and performance data analytics in forensic nursing education and identify broader application potential to continue to shape the future of education and practice.

Impact Statement: This presentation explores implementation of VR, AI, and performance data analytics in forensic nursing education with the potential to enhance the competence, efficiency, and quality of forensic practices, ultimately benefiting the entire forensic science community by promoting continuous improvement, adaptation, and innovation in the field.

Abstract Text: The nursing workforce shortage continues to be a global problem directly impacting recruitment, education, and retention for the forensic nursing specialty. In the evolving landscape of forensic nursing, the demand for advanced, scalable, and effective training methods has never been more critical. Leveraging an AI-enhanced VR simulation platform to revolutionize the education of forensic nurses will offer immersive, facilitator-free simulations that replicate real-world scenarios with high fidelity. The platform can be tailored to include many clinical scenarios targeted at caring for victims of violence, enabling forensic nurses and front-line staff to hone their skills in a controlled, risk-free environment. Clinical virtual simulation in nursing education has the potential to improve knowledge retention and clinical reasoning.[1]

AI-driven feedback and advanced gaming technologies enhance the learning experience of forensic nurses by providing personalized, adaptive training paths tailored to individual learning needs and specific patient scenarios that incorporate appropriate evidence identification and preservation. Compared to conventional teaching methods, VR has been shown to be more superior in advancing nursing students' theoretical knowledge, practice proficiencies, and overall satisfaction.[2]

Performance data analytics play a crucial role in this training paradigm. By continuously monitoring and analyzing clinical performance metrics, the platform identifies strengths and areas for improvement, ensuring that training is both comprehensive and targeted. This data-driven approach not only standardizes the quality of education but also assures clinical competence, enhances care quality, and improves patient safety by reducing variability in care practices. VR simulation, as a supplemental tool for teaching, may also play a pivotal role in acquiring the critical steps necessary to perform clinical skills.[3]

The goal is significant improvements in clinical competence and training efficiencies while meeting the complex needs of forensic nursing and the varied patient population cared for. The implementation of VR and AI in training offers a scalable solution that can be accessed anytime, anywhere, facilitating continuous professional development and readiness. Immersive VR training offers promise and demonstrates its potential in the future of nursing education.[4] This will provide significant benefit for the forensic nursing community.

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Forensic Nursing Education; Virtual Reality; Artificial Intelligence

E10 Beyond the Numbers: Investigating Homicide Risk Factors for Indigenous Women and Girls

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Learning Objectives: After attending this presentation, participants will have greater insight into the current needs and trends concerning violent crimes against Indigenous women and girls. Additionally, this presentation will examine the historical, present, and emerging knowledge related to the influence of social and structural determinants on the issue. Further, attendees will identify opportunities to improve interventions that address modifiable violence risk factors and enhance culturally competent forensic practice.

Impact Statement: This presentation aims to impact the forensic science community by presenting research that highlights the complex and intersecting factors contributing to the crisis of Missing and Murdered Indigenous Women and Girls (MMIWG). Relevant variables, including methods of murder, history of domestic violence, sexual assault, and on- or off-reservation crimes, call attention to the role of forensic professionals in promoting effective interdisciplinary responses to cases of violence.

Abstract Text: **Introduction:** Violence against Indigenous women and girls is a crisis with national and international implications, disproportionately affecting females in First Nations, Inuit, and Métis communities in Canada, and Native American communities in the United States.[1] Homicide is the third leading cause of death for Indigenous women and girls aged 10 to 24 and the fifth leading cause of death between 25 and 34 years of age.[2] Members of Indigenous communities have long fought to bring awareness to the issue of MMIWG, but disparities persist. This study aims to gain a better understanding of cases involving the homicide of Indigenous women and girls and to identify patterns associated with the violence committed against them.

Method: This study used a descriptive, correlational design of public access data from the Justice for Native People database. A detailed analysis was conducted for 115 randomly selected cases of MMIWG from the United States and Canada, with victims ranging in age from 14 months to 55 years. Cases were coded and analyzed for information pertaining to victim, perpetrator, and crime characteristics. Descriptive analysis and tests of association were performed.

Results: Relationship dynamics between murdered Indigenous women and perpetrators were found to be statistically significant ($p < .05$). Age of victim was related to perpetrator sex with girls less than 5 years old were more likely to be murdered by a female perpetrator who had a familial or caretaker relationship to the child whereas 100% of Indigenous girls and women older than 11 years of age were murdered by males ($p < .002$). In the cases of adult victims, nearly 73% of cases in which the victims had experienced a prior history of Interpersonal Violence (IPV), the homicide was perpetrated by a romantic or intimate partner ($p < .001$). These findings show specific patterns that warrant further examination and analysis, potentially identifying predictors that may enhance the practice of forensic professionals.

Discussion: The number of Indigenous girls and women exposed to violence and murder is unacceptably high. Coordinated prevention and response efforts are urgently needed from professionals across disciplines. Interdisciplinary and culturally competent collaboration among professionals within the forensic science community is essential to promote effective action at the intersection of health, culture, and justice. Study findings support several areas where forensic professionals can intervene with regard to violence against Indigenous women. Violence risk assessments and screening for IPV are primary areas of concern to clinical forensic practice. Importantly, validated tools that are currently available to assess for risk of lethal or near-lethal outcomes in IPV, such as the Danger Assessment, could be adapted and validated for use in the Indigenous population.[3] Finally, forensic professionals may play a key role in the investigative processes, supporting the protection of Indigenous women while striving for healing and justice.

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Violence Against Women; Indigenous; Forensic Nursing

E11 Changes in Patient Pain Post-Medical Forensic Examination

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Learning Objectives: This presentation will review data from medical forensic examination reports provided by a free-standing clinic in a large metropolitan area of Texas that provides post-sexual assault services. This presentation will discuss the relationship between medical forensic examination processes and assault characteristics and their impact on changes in patient pain before and after a medical forensic examination. The attendees will gain a better understanding of how examination processes and assault characteristics have the potential to elicit changes in pain and how this information can improve trauma-informed care that could influence the quality of forensic evidence collection.

Impact Statement: Investigating how patients experience pain changes after a medical forensic examination can help forensic nurses and other medical personnel conduct more effective trauma-informed care and evidence retrieval during an examination. Minimizing and managing pain experienced during a medical forensic examination can lead to patients being more receptive to evidence collection procedures and decrease procedure declination that can positively impact the forensic significance of these examinations. Providing patient-centered and trauma-informed care can yield higher quality forensic evidence and better forensic outcomes.

Abstract Text: **Background:** Medical forensic examination purposes are to provide health care and evidence collection for victims of abuse and assault. Trained medical professionals provide these exams and complete a medical history, clothing collection, physical exam, biological and physical evidence collection from the body, photography, injury documentation, treatment of injuries, and medication administration. Due to the nature of sexual assaults, these examination processes can be invasive for the patients and may elicit memories of the assault. This can contribute to more physical and emotional distress or pain.

In existing literature, even when patients found the medical forensic examination empowering, they viewed the examination as unpleasant, triggering, and intrusive.[1] Common evidence collection includes breast swabs, penile swabs, vulva swabs, vaginal swabs, and anal swabs. These collections can be physically and emotionally triggering to patients who have been sexually assaulted. For medical forensic examinations, patients can decline or consent to each procedure and evidence collection before actions are taken. If someone experiences or anticipates pain related to a procedure, then they may be more likely to decline the procedure. Our goal is to investigate what medical forensic examination processes and assault characteristics relate to an increase in pain post examination. Pain should be assessed, treated, and reassessed during a medical forensic examination. However, little is known regarding how pain ratings change over the course of an exam and what patient, assault, and examination characteristics are associated with pain scores.

Methods: We extracted data from 969 medical forensic examination reports from January 1, 2020, to December 31, 2023. Patients provided pain reports on a 0–10 scale at the start and end of each exam. We examined changes in pain scores and associated patient, assault, and examination characteristics using regression analysis. Assault characteristics data includes anogenital injury, strangulation, physical assault, and whether fear, coercion, and threats were used during the assault. Examination data includes penis/vulva swabs, breast swabs, anal swabs, and speculum use. Additionally, pain medications and comfort care such as hot packs, blankets, and showers are factored in as influencing pain scores, too.

Moving Forward: The data shows that 526 examinations resulted in no pain score change. Additionally, 356 examinations resulted in a decreased pain score. No pain score change or decreased pain score is a desired outcome of a medical forensic examination. However, 87 examinations resulted in an increase in pain score. If the desired outcome is a decreased or no pain score changes, then what factors contributed to those results? Similarly, if the undesired outcome is an increase in pain score, then what factors are contributing? We are expecting our analysis to show the more invasive examination processes, speculum use, and more severe assault characteristics, anogenital injury, to be related to an increase in pain reports. With this information, we can anticipate exam processes that are more likely to be declined due to pain or what assault characteristics are linked to increased pain to better treat patient pain during examinations and educate our patients on the importance of evidence collection and best practices that potentially yield higher quality evidence.

Reference:

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Forensic Nursing; Examination; Pain

E12 Forensic Nursing Science: The Practice of Virginity Testing

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Learning Objectives: This presentation will: (1) state three reasons why virginity testing is becoming an abandoned practice in the Balkan countries; and (2) list organizations or groups of professionals who condemn virginity testing based on scientific, ethical, moral, and legal principles.

Impact Statement: At the conclusion of this presentation, participants will understand the changing perceptions of virginity testing and will be prepared to defend why such practices are futile, violate human rights, and negatively impact women's mental and emotional health.

Abstract Text: This presentation outlines why virginity testing should not be performed for any reason by health care, legal, or forensic professionals. These examinations are now considered unethical, medically imprecise, and traumatizing, and cannot confirm whether someone has had penetrative vaginal sex. These revelations have not completely halted the practice of virginity testing in the Balkan region. Now, however, enlightened countries are becoming aware that virginity testing is a violation of human rights and are taking action to stop the practice.

Virginity testing is an invasive inspection of the female genitalia that has been believed to determine whether a woman or girl has had penetrating vaginal intercourse. However, such testing has been found to have no merit and is now considered to be an act of sexual and emotional abuse as well as a violation of human rights. The concept of virginity is a social, cultural, political, and religious construct with neither a medical nor scientific rationale. There is no examination that can reliably prove a history of vaginal penetration. The World Health Organization recommends that this testing should not be performed under any circumstance.[1]

Virginity testing was formerly thought to be a reliable method for determining whether a female had been penetrated during sexual intercourse, typically accomplished by performing a pelvic examination involving digital exploration to confirm the presence or absence of an intact hymen. This examination has been done under the erroneous belief that you can confirm an act of penetrative vaginal sex by inspecting genital anatomy. It has been a longstanding practice in Kosova to examine females for medicolegal purposes in sexual offenses allegedly perpetrated against them or to determine whether she is a "virgin" prior to or immediately after marriage.[2] This practice has now ended with the inclusion of forensic nurse examiners in sexual violence cases. Forensic nurses do not perform virginity checks regardless of the reason, location, or who has requested the testing.

It is important to raise awareness of changing times and evolving laws in the Republic of Kosova in regard to virginity testing. The new legal framework is now consistent with international human rights standards that prohibit forced virginity checks. Revisions have been made to supplement or amend the Criminal Code. Virginity testing is now considered to be a criminal offense and perpetrators will be subject to punishment. In 2002, Turkey also issued a decree banning forced virginity testing. The United Nations and the World Health Organization support the rights of all women by prohibiting virginity testing.[3]

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Forensic Nursing; Human Rights; Virginity Testing

E13 Enhancing Equitable Access to Quality Forensic Sexual Assault Care: The SAFE-T System Telehealth Model

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Learning Objectives: After attending this presentation, attendees will gain a clear understanding of how the Sexual Assault Forensic Examination Telehealth (SAFE-T) System provides real-time expert guidance to health care providers via secure telehealth technology to enhance the quality of sexual assault forensic examinations. This presentation will impact the forensic science community by demonstrating how the SAFE-T System aims to improve the accuracy and accessibility of forensic nursing, particularly in underserved areas, with hopes of aiding in better legal and health care outcomes for Sexual Assault (SA) victims.

Impact Statement: Victims of SA in rural areas often encounter significant gaps in care compared to those in urban settings. Sexual Assault Nurse Examiners (SANEs) are essential for providing comprehensive exams, yet many communities lack access to these vital services. The SAFE-T System model can bridge this divide by offering 24/7 live expert teleSANE consultation and high-quality cybersecure technology to ensure all victims receive equitable care.

Abstract Text: More than 50% of women (over 84 million) and 30% of men (over 49 million) living in the United States will experience sexual violence in their lifetime. Survivor/Victims (SV) require timely, skilled, trauma-informed health care to address injuries, risk of infection/pregnancy, forensic evidence collection to aid justice outcomes, and to be connected to psychological support essential to healing.[1,2] When SV receive forensically defensible, trauma-informed care from skilled professionals, such as Sexual Assault Nurse Examiners (SANEs), they experience improved physical health, mental health, and judicial outcomes relative to care provided by untrained providers.[3] Utilizing secure, high-quality evidence collection technology, the SAFE-T System aims to improve health outcomes and patient experience for SV by providing 24/7 live expert teleSANE (IAFN-certified) consultation to hospitals in underserved communities. Additionally, the easy to use SAFE-T System technology allows providers to capture hands-free magnified (up to 10x) images that are instantly uploaded to a secure cloud to eliminate data collection loss, ensure patient privacy, and aid in better judicial outcomes. Since 2018, quality assurance measures, judicial interview data, and patient experience surveys have been gathered. Eligible patients (aged 12 and over, English-speaking, consenting to medical forensic exam) were asked to rate their level of agreement with pre- and post-examination worry items using a 5-point Likert scale and data are presented as percentages of combined "strongly agree" and "agree" responses. To examine whether the individual perceived that the examination itself had a healing effect and if telehealth consultation was perceived as beneficial, an agreement-based 5-point Likert scale was used, and data are presented as percentage of combined 4 and 5 scores. SAFE-T has been implemented in twelve underserved hospitals, providing telehealth consultation to 399 SA victims. Many patient participants had at least one pre-examination worry (76%), yet the majority stated their fears were alleviated during the examination with a teleSANE present. Most patients endorsed that they felt in control (81%), believed (87%), not blamed (88%), and not judged (90%). Participants stated the exam helped them feel better (94%). And telehealth improved their care (94%). The SAFE-T System telehealth model and technology empowers nurses to securely collect the high-quality evidence needed to complete a quality forensic SA examination while ensuring patient comfort. The SAFE-T System telehealth model and technology empowers nurses to securely collect the high-quality evidence needed to complete a quality forensic SA examination while ensuring patient comfort. The SAFE-T System is a viable option to enhance forensic nursing workforce issues in underserved communities, thereby promoting equitable access to SA quality care for all SV.

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Forensic Nursing; Sexual Crimes; Forensic Imaging

E14 Mitigating Bias in Sexual Assault Evidence Collection Kit Analysis

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Learning Objectives: After attending this presentation, attendees will understand cognitive bias, the sources of potential bias in Sexual Assault Evidence Kit (SAEK) analysis, the types of bias, and how they impact the outcome of kit analysis.

Impact Statement: In forensic science, the possibility of bias during an investigation compromises the integrity of evidence, inadvertently affecting the justice system. This presentation will impact the forensic science community by continuing the conversation on the impact of cognitive bias in evidence analysis and decision making.[1] The results of this research will help improve the efficiency of the kit analysis process and may potentially reduce the occurrence of wrongful convictions.

Abstract Text: In forensic science, the possibility of bias during an investigation compromises the integrity of evidence, inadvertently affecting the justice system. This presentation will discuss the development of a standardized SAEK purposefully designed to reduce bias originating from overload of irrelevant information, differential sequencing, and human cognitive processes.

The absence of a standard SAEK kit leaves room for inconsistencies during analysis. Analysts may reach different conclusions depending on the order with which they process a SAEK.[2] A standardized SAEK with a predetermined sequence of analysis will help mitigate discrepancies that may arise from differential sequencing and increase the reliability of forensic analysis. This is paramount in maintaining the integrity of our justice system, as forensic results should not depend on who examines the evidence.

Some information included in SAEK documentation, such as race, may be deemed irrelevant to analyses. An overload of such information may impair the efficiency of the analysis process and create internal bias in the minds of analysts. To mitigate this, we propose the differentiation of medical forensic exam documentation into three categories—Forensic Analysis, Law Enforcement, and Medical—to ensure the provision of relevant information to the appropriate party. By controlling the flow of information through the incorporation of linear sequential unmasking, we may also minimize the amount of biasing information made available to analysts.[3]

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Cognitive; Bias; Sexual Assault

E15 The Reality of Intimate Partner Violence in Piedmont, Italy: The Challenges Posed by the Frequency and Severity of Violence and the Proposals to Combat It

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Learning Objectives: With this presentation, participants will acquire knowledge about the best practices used in health care facilities in Piedmont (Northwest Italy) to intercept cases of Gender-Based Violence (GBV) when accessing these facilities.

Impact Statement: The forensic science community will be informed on:

- The findings of a series of studies regarding cases of GBV recorded in health care facilities in Piedmont (Northwest Italy) and on cases of femicide.
- The measures implemented in Piedmont and coordinated by the "Health Network for the Care of Victims of Sexual and Domestic violence": specific bills, shared protocols, basic and advanced training courses for health care professionals.
- The development of the project PAUSE (Prevention of Assault Under Scientific Evidence), an interdisciplinary network of professionals who collaborate to raise awareness and promote specialized initiatives to prevent GBV. In order to achieve this, an artificial intelligence system for tracking recurring patterns in violence cases has been implemented beginning with the analysis of clinical records in Emergency Departments (ED).

Abstract Text: Intimate Partner Violence (IPV) refers to any violent behavior perpetrated by partners or former partners. IPV not only has a significant negative impact on the health and quality of life of victims and their family members but can also lead to femicide. According to the World Health Organization (WHO), one in three women worldwide have experienced some form of GBV in their lifetime. A study conducted by the European Union Agency for Fundamental Rights in 2014 found that 22% of women surveyed had suffered physical and/or sexual violence at the hands of their partner, 43% had been victims of psychological violence, and 18% had suffered economic violence. In Italy, a telephone survey conducted in the same period by the National Institute of Statistics (ISTAT) revealed a similar situation: 22.4% of women had been psychologically abused by their current partner, 13.6% had been victims of physical and/or sexual violence, and 11.8% had experienced some form of violence during pregnancy. This survey also found that partners or former partners often perpetrate the most severe forms of violence, including femicide, which is consistent with recent scientific evidence.

We will provide an overview of a series of cross-sectional studies on violence against women intercepted in the ED of different health care facilities in Piedmont and the results of a study on femicide that occurred specifically in Turin (Piedmont) from 1970 to 2020.[1-4] In addition, the results of the PAUSE project coordinated by the University of Turin and the actions of the "Health Network for the Care of Victims of Sexual and Domestic Violence" will be discussed. Specific regional bills and common protocols adopted by all public health institutions in Piedmont will be presented, as well as the large-scale basic and advanced training for health care professionals working with survivors of GBV.

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Gender-Based Violence; Intimate Partner Violence; Femicide

E16 What About the Swabs? A Retrospective Review of DNA Analysis Findings From Sexual Assault Kit Evidence

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Learning Objectives: After attending this presentation, attendees will: (1) evaluate factors associated with DNA analysis findings from collected swabs contained in Sexual Assault Kits (SAKs), and (2) explore practice implications for forensic nurses and forensic scientists.

Impact Statement: This presentation will impact the forensic science community by contributing to the growth of evidence-based practice guidelines within forensic nursing in SAK evidence collection.

Abstract Text: The findings shared in this presentation were obtained from a National Institute of Justice grant (2019-NE-BS-0001) entitled "Assessment of Sexual Assault Kit (SAK) Evidence Selection Leading to the Development of SAK Evidence Machine Learning Model (SAK-ML Model)."[1] The first research questions outlined in this grant report relate to SAK swab selection for DNA analysis and the percentage of uploaded Combined DNA Index System (CODIS) profiles per swab site (i.e., vaginal, rectal, oral, breasts). This information is highly relevant to forensic nursing practice in informing decision-making regarding swab collection. Additional research findings on patient and assault factors that were significant in machine learning models on predicting the development of full or partial Short Tandem Repeat (STR) DNA of foreign contributors will be outlined and discussed. A review of machine learning principles as applied in this study will be presented. Findings will be shared through PowerPoint presentation. A case study will be provided to stimulate discussion and application of the findings.

Forensic nursing national best practice guidelines will be reviewed as informing forensic nursing practice within the three states that were represented in this study.[2,3] Strategies to enhance collaboration between forensic nurses and forensic scientists to achieve optimal outcomes from DNA analysis of SAKs will be presented. The impact of evolving forensic science technologies on SAK evidence collection and DNA analysis findings will be discussed in terms of how these emerging technologies may or may not alter SAK evidence collection.

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Sexual Crimes; Forensic Nursing; Forensic Analysis

E17 The Experiences of Child Advocacy Center Staff on Child Abuse and Neglect: A Qualitative Study

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WITHDRAWN

E18 Rewrite the Law? Everyone Is (Not) Equal Under the Law

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Learning Objectives: Attendees will increase their understanding of the Outreaching Service of Forensic Nurses (ADFN) and its societal significance. {articipants will be able to (1) describe the structure and function of the ADFN in the Canton of Zurich (Switzerland), (2) explain the importance of low-threshold access to forensic examinations and their contribution to violence prevention and victim support, (3) analyze the legal and societal implications of ADFN Services, (4) explain the legal framework and the possibilities of evidence collection by forensic nurses without immediate criminal charges, and (5) identify the challenges and (potential) areas of conflict from the perspective of the allegedly violent person to ensure a holistic view of forensic nursing practice.

Impact Statement: This presentation will significantly impact the forensic science community by showcasing the innovative approach of the ADFN in the Canton of Zurich in Switzerland. By providing low-threshold access to forensic examinations for victims of sexual and domestic violence without the immediate need for a police report, the ADFN model facilitates comprehensive evidence collection and documentation. This service ensures that victims receive essential medical and forensic care while preserving their autonomy and decision-making power regarding legal action.

The presentation will highlight the societal and legal advantages of this approach, emphasizing its contribution to violence prevention and victim support. It will also address the practical benefits for health care providers, allowing medical staff to focus on core medical duties while specialized forensic nurses handle forensic procedures. Furthermore, by exploring the potential challenges from the perspective of the alleged perpetrators, the presentation aims to foster a holistic understanding of the forensic nursing practice, ensuring balanced and comprehensive care and legal processes. This discussion will encourage the forensic (nursing) science community to consider broader implications and potential areas for improvement in forensic nursing services, ultimately enhancing the quality and effectiveness of victim support and forensic investigations.

Abstract Text: The ADFN was established in the Canton of Zurich (Switzerland) to support victims of sexual and domestic violence.[1] The ADFN operates in all hospitals with emergency departments in Zurich and is staffed by specially trained forensic nurses from the Institute of Forensic Medicine at the University of Zurich (IRM-UZH). This initiative provides a low-threshold service that allows victims to receive forensic examinations without having to file a police report. This approach ensures that victims do not have to make an immediate decision about legal action, thus preserving their autonomy.

Forensic nurses collect relevant information, photograph injuries, preserve biological evidence, and write brief reports. They also inform victims of further support options, such as counseling services, and coordinate additional steps with the victim's consent. The Zurich model guarantees free and accessible medical and forensic care for victims, regardless of police involvement. Other cantons in Switzerland have similar models. For example, the canton of Graubunden offers a forensic nursing consultation, the canton of Vaud has a clinic for victims of violence (UMV), and the Berne model provides comprehensive examination and counseling services for victims of sexual violence.[2-4] Ongoing developments in various cantons aim to further improve these services. At the political level, initiatives are being taken to establish crisis centers for victims of violence.[5]

There are many personal and situational reasons why victims may not feel able or safe to contact the police. The ADFN allows victims to secure comprehensive and legally admissible forensic evidence, giving them the option to pursue legal action at a later date. The IRM-UZH stores data and evidence for one year, allowing authorities to request full documentation and expert reports if the victim decides to file a complaint or lawsuit at a later date. This process ensures that perpetrators can be held accountable. From a societal perspective, ADFN represents a promising approach to support victims and prevent violence in accordance with the National Action Plan for the Implementation of the Istanbul Convention.[6] It emphasizes that domestic and sexual violence is unacceptable. In addition, from an institutional perspective, ADFN allows medical staff to focus on core duties while forensic nurses handle forensic tasks, highlighting the operational benefits for hospitals.

While ADFN appears to be a win-win situation for all involved, especially the victims, it is also important to consider the perspective of the alleged perpetrators. This presentation will explore these aspects through a realistic example, identifying potential gaps and discussing the societal and legal implications. Understanding these dynamics is essential for forensic nurses to adopt a comprehensive and balanced approach to their practice, ultimately improving the effectiveness of forensic investigations and victim support.

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Forensic Nursing; Istanbul Convention; Comprehensive Forensic Care

F1 A Small Molecule Chemical Analysis of Residual Odor From Odor Recognition Tests by Human Remains Canine Teams

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Learning Objectives: Residual odor is caused by lingering Volatile Organic Compounds (VOCs) in the absence of its target. This study will aid viewers in the understanding of the chemical analysis performed on residual odor of live and deceased humans in combination with recognition tests by Human Remains Detection (HRD) canine teams, specifically, an analytical method that can differentiate between living and deceased humans, determine the differences in their respective VOC profiles, and profile the metabolic signatures that may be responsible for residual odor will be highlighted.

Impact Statement: Limited studies profiling the molecular milieu that composes residual odor have been conducted for a myriad of reasons, one of which is due to the instability and volatility of VOCs . This study will address this knowledge gap by providing techniques for sampling, Gas Chromatography/Mass Spectrometry (GC/MS) metabolite profiling, and statistical analyses of the data; all of which are required so that the composition of residual odors can be determined.

Abstract Text: Some studies have focused on the chemical analysis of human bodily fluids (blood or decomposition fluid), but few have examined residual odor of deceased individuals.[1,2] In this study, residual odor from previously cleaned gauze placed on three separate deceased individuals for ten minutes were analyzed as the target group. The same procedure was conducted using three live humans as the distractor group. Chemical analyses were facilitated through a headspace Solid-Phase Microextraction (SPME) sampling module that is specifically designed to capture and concentrate VOCs. Compounds extracted via SPME were analyzed via Gas Chromatography coupled with High Resolution Mass Spectrometry (GC-HRMS) to provide data optimal for the analysis of the complex VOCs. Simultaneously, the experiment utilized 35 certified HRD teams to assess responses by the canines to those same samples. Each canine team was administered a double-blind Odor Recognition Test (ORT), and the gauzes used to provide the VOCs were also profiled by GC-HRMS. Handlers reported their canine responses, if any, to residual odor from the decedents, the live donors, or blank samples during those double-blind ORTs.

By coupling analytical analyses with behavioral analyses of the HRD teams, this study aimed to determine the reliability of HRD teams, establish a small molecule profile of living cohorts, and assess potential VOC signatures of deceased residual odors. Chemical mixture analysis by MS often provides information on both molecules of known and unknown chemical structure. In this work, the unidentified VOCs composed ~95% of the molecules detected and were statistically of most importance in differentiating the experimental groups. Approximately 1,800 unidentified spectral were detected in the analysis, underscoring the versatility of the headspace SPME extraction. Small molecule profiles of each group were compared by Partial Least Squares Discriminant Analysis (PLSDA) resulting in three unique profiles corresponding to the two experimental groups, deceased and live human residual odors, and the gauze blanks. These data suggests that there is a small molecule profile difference between groups and that the HRD teams have the potential to be trained to distinguish the groups' residual odors more efficiently. Deceased samples yielded less statistically significant VOCs than the living human group in comparison to the blank gauze. This may indicate that residual odor from human decedents is of lower complexity and may explain HRD inconsistencies in the odor recognition test. Further research will focus on identifying specific VOCs that contribute to the potential lower complexity of decedent residual odor.

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Mass Spectrometry; Residual Odor; Chemical Analysis

F2 An Analysis of Hidden Childhood Exposure to Xenobiotics in Cases of Child Abuse: A Decade-Long Study at the Meyer Children's Hospital IRCCS of Florence. Italy

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Learning Objectives: After attending this presentation, participants will gain an analytical perspective on the relationship between child abuse and parental exposure to xenobiotics, based on an Italian case series from a national pediatric reference center.

Impact Statement: This presentation is intended to stimulate a debate within the forensic scientific community on the need for a thorough specialist evaluation in cases of child abuse, with particular attention to potential intoxication by legal or illegal substances.

Abstract Text: As evidenced by the literature, the childhood exposure to drugs of abuse in the domestic environment is an emerging public health and social problem with multiple potential effects on children's health and may be a factor in abuse situations.[1,2] Given the significant association between parental drug use and child maltreatment, substance abuse exposure has been recognized as an important factor in the assessment of children suspected of being victims of abuse or neglect.[3,4] From the 2016-2023 database, 764 children aged <14 years suspected of physical or sexual abuse who underwent urine toxicology testing during hospital admission were selected from a local service at IRCCS ("Istituti di Ricovero e Cura a Carattere Scientifico" which means "Scientific facilities for hospitalization and treatment") Meyer Pediatric Hospital of Florence, called "GAIA" ("Gruppo abusi infanzia e adolescenza" which means "Childhood and Adolescent Abuse Group").[5] Cases were categorized according to age, sex, type of substance detected, social living conditions, reason for emergency department presentation, possible source of exposure, and actions taken by health care providers to protect the child. Of the 764 cases, 124 tested positive for a xenobiotic substance; the most detected substances were sedative-hypnotics (29.4%, of which 11.1% were benzodiazepines), cannabinoids (16.7%), cocaine (8.7%), and antidepressants (7.1%).

A complete screening for substances could be considered in the initial assessment of a suspected case of child abuse. The occasional finding of xenobiotic substances in biological samples from minors of age, even in cases where intoxication was not suspected, led to the modification of procedures and the development of a standardized protocol at IRCCS Meyer, which now includes routine urine toxicology testing in all suspected or confirmed cases of child abuse. Analysis of the cases demonstrated the importance of a multidisciplinary approach and close collaboration between the judicial authorities and health professionals (doctors, nurses, etc.) who are mandated by law to report these cases because of the potential crimes committed by caregivers and because of the health consequences for the children.

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Child Abuse; Xenobiotics Substances; Hidden Intoxication

F3 An Interdisciplinary Review of Standard Operating Procedures Among United States-Accredited Laboratories

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WITHDRAWN

F4 The Development and Identification of a Thin Layer Chromatography Procedure for the Rapid Identification of Cannabis Plants Through Its Major Cannabinoids

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Learning Objectives: This presentation will showcase a new rapid method to differentiate low and high THC samples

Impact Statement: This presentation will provide a useful method to be applied in forensic sciences and analytical chemistry.

Abstract Text: The use of drugs and narcotics has increased with 296 million people using them. Cannabis is widely used, with 8% of European adults (22.6 million) having used it in the past year and 1.3% (3.7 million) being daily users. In 2021, 2,026 tons of cannabis were seized globally. *Cannabis sativa* contains over 120 cannabinoids, with Delta-9-tetrahydrocannabinol (Δ9-THC) being the primary psychoactive component.

Thin Layer Chromatography (TLC) is a cost-effective technique for detecting and separating compounds, despite newer and more accurate methods such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatograph/Mass Spectrometry (GC/MS). These advanced methods are costly and resource intensive. Forensic medicine uses techniques like GC/MS to detect drug components, but increased drug use strains resources.

This study optimizes TLC protocols to differentiate between legal (low THC) and illegal (high THC) cannabis by identifying the primary components, using accessible equipment and non-specialized personnel. The main methodology used was different plates as TLCsSilica gel, TLC plates cellulose, and TLC aluminum sheets with varying mixtures of solvents such as hexane-diethylether (80:20, v:v), cyclohexane-toluene-diethylether (75:15:10, v:v:v), benzol-diethylether (95:5 ;v:v), and benzol-hexane (90:10 ;v:v). Plate visualization was performed using UV 254-360 and Fast Blue RR (FBRR) salt solution acidified. Cannabinoids extraction was performed by directly grinding the plant sample in methanol and adding 2µl to the plate. The best resolution was achieved using TLC aluminum sheets and benzene-hexane (90:10, v:v) as the mobile phase. This combination enabled rapid TLC performance within 30 minutes. Visualization was possible using UV 360 after drying the TLC plate. To further enhance visualization, the FBRR solution allowed naked-eye observation after drying for one hour. This method permits rapid, easy, low-cost cannabis detection that can be applied in any laboratory. As well, it permits differentiation between low THC samples and high THC samples, opening a possible methodology that can be used by non-scientists, such as police forces involved in anti-drug trafficking tasks.

Cannabinoids; Thin Layer Chromatography; Method Development

F5 Does Technology Weaken the Fight for a Fair Trial? Modern Policing and Challenges of the Criminal Defense Investigation

Briana Verdoorn, MA, Hennepin County Public Defender's Office, Minneapolis, MN*

Learning Objectives: After attending this presentation, attendees will better understand the current technological climate of policing in the United States and the associated challenges observed within a public defender's office.

Impact Statement: This presentation will impact the forensic community by demonstrating and promoting awareness of the genuine lack of technological resources observed in criminal defense, specifically the public defender's office.

Abstract Text: Special care has been taken over the years and by various organizations to develop and implement forensic science standards to achieve justice for all parties in criminal cases. Organizations like the American Academy of Forensic Sciences and the Innocence Project, for example, have aimed to strengthen forensic science through universal methods, education, and scientific examination. However, a great resource disparity is still present in criminal cases that can lead to prejudiced court proceedings or even the misapplication of forensic science methods and knowledge. Emerging technology, complex data, and access to both the experts and the technology needed to analyze that data differs greatly for the defense when compared to that of the prosecutors and police.

Modern technology in policing will be discussed as it relates to both legal and structural barriers experienced by the criminal defense team. Not only do we as the criminal defense team start after the initial investigation, but we are limited by the databases we have access to, the software and electronics available for use, and the lack of collaboration with experts in the field. With the arrival of predictive policing methods, the continued expansion of surveillance technology use, and private companies pushing technology without a thorough understanding of Constitutional rights or the scientific method, more focus needs to be placed on making sure that the same access to resources is there for all parties involved.

Technological investigative resources will be arranged by and discussed within five central categories: databases, software, electronics, knowledge, and communication. The challenges caused by current policing and surveillance methods will be discussed as they are observed by the varied professions that make up the criminal defense team within the scope of a public defender's office. This presentation will provide several strategies that all within the legal system can use to reduce this gap in resources and collectively move toward providing justice for all parties within our court system.

Surveillance; Public Defense; Predictive Policing

F6 Eye in Crime Scene Investigation

Jagmahender Singh Sehrawat, PhD, Panjab University, Chandigarh, Chandigarh, India; Sushil Bhatt, MS, Department of Anthropology, Panjab University Chandigarh, Chandigarh, India; Vishali Gupta, MS, Panjab University, Chandigarh, Chandigarh, India*

Learning Objectives: Participants will gain a comprehensive understanding of forensic techniques involving ocular evidence to aid in criminal investigations. They will analyze ocular biometrics such as iris patterns, sclera characteristics, and retinal biometrics to accurately identify individuals involved in crimes. Additionally, they will conduct tear analysis to obtain biochemical and molecular information that can aid in identification when traditional bodily fluids are unavailable. The examination and interpretation of eyewear evidence found at crime scenes will enable them to connect suspects, victims, and crime scenes based on prescription criteria and unique characteristics. They will also utilize psychological insights derived from observing eye movements, gaze, and expressions during interviews or interrogations to detect deception or psychological conditions. Last, participants will perform postmortem ocular examinations to gather evidence of trauma, toxins, or other relevant information critical for determining the cause and manner of death.

Impact Statement: Integrating ocular evidence into forensic investigations is a major advancement, offering diverse crime-solving methods. Ocular biometrics improve identification accuracy, tear analysis provides biochemical insights when traditional evidence is lacking, and examining eyewear links suspects, victims, and scenes. Observing eye movements and expressions helps detect deception during interrogations. Postmortem ocular exams reveal details about trauma and toxins, aiding in determining the cause of death. These techniques collectively enhance the forensic toolkit, making criminal investigations more effective and accurate.

Abstract Text: In forensic science, the human eye has emerged as a critical tool in Crime Scene Investigation (CSI), offering diverse applications across forensic anthropology and digital forensics. This presentation examines the multifaceted role of the eye in forensic contexts, focusing on its anatomical significance and recent technological advancements. By analyzing ocular evidence, such as retinal patterns, distinctive eye features, and eyewear characteristics, investigators can enhance the identification and connection of individuals involved in criminal activities.[1] It highlights the impact of advancements in digital imaging and ocular biometrics, which afford unparalleled precision in crime scene analysis and reconstruction.

Innovative forensic techniques, including tear analysis, are discussed for their capacity to provide valuable biochemical and molecular insights, particularly when traditional bodily fluids like blood, saliva, semen, vaginal secretions, urine, and tears are not available.[2] The analysis of tears' biochemistry and molecular properties can facilitate the identification of individuals through the acquisition of a thorough DNA profile.[3] Dried tears can be observed on a range of surfaces, including sheets, tissues, and clothing, subsequent to the occurrence of an emotional or painful incident.[4] Furthermore, the process of identifying an individual can involve meticulous observation of distinct eye traits like iris, sclera and retina biometrics, which exhibits a high level of individuality. Examining and interpreting eyewear evidence—based on prescription criteria and unique characteristics—can establish critical links between suspects, victims, and crime scenes. Psychological insights derived from observing eye movements, gaze patterns, and expressions during interviews or interrogations are also addressed, offering valuable information for detecting deception and assessing psychological states.[5] Furthermore, postmortem ocular examinations are examined for their role in gathering essential evidence related to trauma, toxins, or other factors crucial for determining the cause and manner of death.

This comprehensive approach underscores the significant potential of ocular evidence in forensic science, aiming to enhance the accuracy and efficiency of criminal justice processes. By leveraging the diverse roles of the eye in both living and deceased individuals, the presentation contributes to advancing forensic science and improving investigative practices.

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Anthropology; Bodily Fluid; Digital Evidence

F7 Forensic Facial Matching—A Tool for Concealed Identity: A Case Report

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Learning Objectives: After attending the presentation, attendees will understand a facial identification tool.

Impact Statement: This presentation will impact the forensic science community by helping in identification of perpetrators by digital imagery.

Abstract Text: Cameras and photographic imagery have been used in identification, surveillance, and detection of criminals as early as the 19th century. Facial anthropometry standards have been used to depict portraits of regular criminals for investigation agency registries, similar to today's mugshot system. These registries were focused as a means for witnesses and victims to conduct a facial review of potential suspects. However, the lack of standardization in image capture processes made these registries ineffective. The advent of judicial photography, in the late 19th century, incorporated anthropometry and relied on standardized conditions of image capture, featuring the well-known anterior and lateral facial views with neutral expression and stance.[1,2]

Depicting faces, facial anthropometry, medical examiners relied mostly on forensic photography. In the 21st century, technological advancements such as Closed-Circuit Television (CCTV) surveillance systems became increasingly more common and relied upon throughout the world and are in fact considered by many communities the norm in public areas

Forensic facial matching presents a comprehensive morphological analysis of the individuals involved in the investigation. Utilizing new guidelines and methodologies, the analysis aims to determine the likelihood of a match between facial features, helping to establish or refute the identity of the subjects under scrutiny.

The primary objective is to provide an expert assessment that assists in resolving the crucial question of facial similarity, thus aiding in the resolution of the case. By examining the distinct morphological characteristics of the individuals under investigation, we aim to provide an accurate and scientifically sound evaluation, contributing to the pursuit of justice.

The present case report will highlight the analysis of a facial features wherein the prime suspect of a cognizable offence had eloped and was found with a disguised identity in the official documents by the police after 20 years. Therefore, the span of 20 years and age progression had to be taken into consideration while analyzing the morphological facial features to derive identification.

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Identification; Age Progression; Facial Matching

F8 From Flat Photos to 3D Wonders: Cutting-Edge Tech Is Changing Forensic Investigations

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Learning Objectives: This paper aims to highlight the significance of advanced 3D documentation technologies like photogrammetry and Light Detection And Ranging (LiDAR) in forensic medicine, showcasing their application, benefits, and challenges in improving the accuracy and detail of forensic investigations.

Impact Statement: This paper underscores the transformative impact of advanced 3D documentation technologies, such as photogrammetry and LiDAR, on the forensic science community, demonstrating their potential to enhance the precision, detail, and efficiency of forensic investigations.

Abstract Text: Photographic documentation is crucial in forensic medical practice, particularly during autopsies and crime scene investigations. Traditional photography, despite its utility, is limited by its two-dimensional nature, which can lead to misinterpretations of depth and color. This has driven the adoption of advanced technologies like photogrammetry and 3D LiDAR scanning.

Photogrammetry is a passive optical 3D measurement technique that constructs 3D models from multiple photographs taken at different angles. Specialized software analyzes these images to create an accurate 3D model, useful in various forensic applications such as representing bodies during autopsies, measuring anthropometric data, reconstructing bullet trajectories, and analyzing blood patterns. While it is a precise and cost-effective alternative to other 3D technologies, its effectiveness depends on the quality of the initial images and lighting conditions, requiring specific technical skills and methodical acquisition.

Since 2020, Apple, Inc. has integrated LiDAR sensors into high-end devices, such as iPhone Pro or iPad Pro, making 3D laser scanning more accessible and portable. These sensors improve photo and video quality and enhance the augmented reality experience, providing precise focusing in low-light conditions and facilitating detailed environmental maps and 3D models.

Recent studies have explored the feasibility of these technologies in forensics. Some authors have produced realistic and accurate images of bodies, organs, and injuries, with reliable color rendition and precise measurements; others have highlighted the simplicity, low cost, and ease of use of Apple devices for digital anthropometric measurements.[1] Other studies demonstrated the accuracy, speed, and ease of use of the iPhone 13 Pro and the Recon-3D application in simulated crime scenes.[2] Scanning techniques have also been used to compare bullet trajectory documentation using Recon-3D on an iPad Pro with TLS, and some have applied LiDAR technology to bloodstain analysis and promising results.[3,4]

In this paper, we tested these 3D documentation tools in the daily activities of the Institute of Forensic Medicine at the University of Pisa. Using an iPhone Pro with the Polycam PRO application, we documented bodies and organs at autopsy, settings at the site inspection, and anthropological studies of skeletal remains. Despite the lack of specific training for the personnel involved, the tools proved easy to use, with fast scanning and data processing times. The resulting 3D models were realistic and accurate, overcoming the distortions of 2D photography and providing depth information, which was particularly useful for assessing complex traumatic injuries and taking accurate anthropometric measurements.

The ease and speed of use, portability, and affordability of these devices, along with the ability to quickly share and store models, are significant advantages. However, challenges include the lack of specific forensic applications and software, dependence on more complex general-purpose software, and limitations in scanning highly reflective or transparent surfaces. Despite these limitations, the study demonstrates the potential of these tools to improve forensic documentation practices. LiDAR could also be used in conjunction with AI systems, thereby expanding its applications in many forensic fields.

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LiDAR; 3D Models; Forensic Pathology

F9 Long-Term DNA Stability on Buccal Swabs: Findings From Simulated Paternity Cases

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Learning Objectives: Attendees will learn about the long-term stability of DNA collected using buccal 4N6FLOQSwabs in simulated paternity cases. The study evaluates how DNA integrity is maintained over extended periods, specifically after five years of storage at room temperature. Key findings include the successful generation of full DNA profiles from long-stored samples, highlighting the reliability of these swabs for forensic applications and paternity testing in prolonged legal scenarios.

Impact Statement: This study will impact the forensic science community by providing valuable insights into the long-term stability of DNA samples collected using buccal 4N6FLOQSwabs (4N6FS). By demonstrating that DNA profiles can be reliably obtained even after extended storage periods of over five years, the research supports the use of these swabs in forensic investigations where long-term sample preservation is critical. This has implications for improving the accuracy and reliability of paternity testing and other forensic analyses, particularly in cases involving extended legal proceedings and delayed sample analysis.

Abstract Text: To accurately establish paternity, it is crucial to obtain full DNA profiles that can confirm biological relationships among individuals. In forensic applications, Copan buccal 4N6FS are commonly used due to their ease of use and effectiveness in obtaining non-invasive, high-quality samples. This study aimed to assess the long-term stability of DNA collected with buccal 4N6FS and stored at Room Temperature (RT) for over five years. Conducted within the context of six simulated paternity cases, the research evaluated how extended storage might affect DNA integrity and determined if the quality of DNA remains adequate for reliable paternity testing after prolonged periods.

Materials and Methods: Starting from 2018, six simulated paternity cases have been set-up. For all cases, two buccal 4N6FS were collected from each person of interest; one was analyzed shortly after collection (T_0), and the second was analyzed at the Time to Analysis (TTA) interval, as described below:

- Case 1 (T_0: 07/2018 – TTA: 5 years and 8 months): Daughter, Presumptive Mother
- Case 2 (T_0: 11/2019 – TTA: 4 years and 4 months): Son, Mother, Presumptive Father
- Case 3 (T_0: 01/2020 – TTA: 4 years and 2 months): Son, Mother, Presumptive Father
- Case 4 (T_0: 09/2020 – TTA: 3 years and 6 months): Son, Mother, Presumptive Father
- Case 5 (T_0: 07/2021 – TTA: 2 years and 8 months): Daughter, Presumptive Father
- Case 6 (T_0: 10/2021 – TTA: 2 years and 4 months): Son, Presumptive Father

The buccal 4N6FS analyzed at the TTA were processed following the standard workflow, which included: DNA extraction using QIAamp DNA Investigator Kit on QIAcube; DNA quantification by real-time Polymerase Chain Reaction (PCR) using Quantifiler Trio Kit on AB 7500; profiling with GlobalFiler kit on Veriti 96-well thermal cycler; capillary electrophoresis using AB 3500 Genetic Analyzer and GeneMapper ID-X v1.6 software. The profiles obtained at TTA were compared to those obtained at T_0.

Results: Full and concordant DNA profiles were obtained from the same individuals both at T_0 and at TTA. The average human DNA yield recovered from the TTA samples provided adequate input for profiling. Despite a certain degree of variability in terms of average peak heights, peak height ratios, and intralocus balances, obtained profiles were complete and consistent.

Conclusion: Maintaining the stability of DNA on samples stored up to analysis is essential for forensic purposes, as it ensures the validity of genetic testing results, meeting various judicial steps and timeline. This study demonstrates that buccal 4N6FS swabs allows us to successfully generate full DNA profiles even after long-storage of buccal samples at RT for more than five years. This supports their use in forensic scenarios where maintaining DNA stability over extended period is needed, such as paternity determination in prolonged legal proceedings.

Buccal Swab; Backlogs; DNA

F10 Protecting Arson Investigators From Exposure to Toxic and Hazardous Substances in Fire Scene Debris

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Learning Objectives: The attendees after reviewing the poster will know the different health and safety risks faced by arson and forensic investigators, who are exposed to a wider variety of toxic chemicals and hazards than firefighters. They will recognize the difference between acute and chronic toxicities resulting from exposure to irritants, asphyxiants, carcinogens, and other hazardous compounds present at fire scenes. The goal of this presentation is to enhance awareness and improve training on the use of Personal Protective Equipment (PPE) and provide advanced education on reducing risks for these professionals.

Impact Statement: This poster will enhance the forensic science community's competence by equipping arson and forensic investigators with critical knowledge and techniques to abate exposure to risky compounds in fire remains. By applying these practices, arson and forensic investigators will advance their performance in much safer and more effective investigations; this will lead to better safety outcomes and more dependable forensic analyses.

Abstract Text: In recent years, research on arson and forensic investigators' health and safety has not kept up with that of firefighters. It is possible to transfer some information from a firefighter's environment to an arson investigator's environment, but not all of it.[1,2] The primary duties of arson investigators include examining more areas than those that were directly affected by the fire, staying for a longer period of time processing the fire scene, examining the area in detail to look for debris, and packing samples from the burned area. They are consequently exposed to a greater variety of hazards than firefighters.[3-5]

Exposure of arson investigators to combustion products and smoke may cause acute and chronic toxicities. Common toxic chemicals that may occur in fire scenes include irritants, asphyxiants, carcinogens, mutagens and teratogens, and sensitizers; asphyxiants gases such as carbon Monoxide (CO) and Hydrogen Cyanide (HCN), irritant gases such as Hydrogen Chloride (HCl), Oxides of Nitrogen (NOx), acrolein, and phosgene; complex molecules as carcinogens, such as some Volatile and Semi-Volatile Organic Compounds (VOCs/SVOCs) and polycyclic aromatic Hydrocarbons (PAHs), or mutagens and teratogens, such as Polychloro- and Poly-Bromo Dibenzo-P-Dioxins and Dibenzofurans (PCDD/Fs and PBDD/Fs) and Polychlorinated Biphenyls (PCBs), or sensitizers such as isocyanates.[6-10]

Therefore, it is necessary to increase awareness of the risk of exposure to hazardous compounds faced by arson investigators by: (1) training on PPE to reduce exposure to hazardous compounds; (2) the generation and development of new policies and procedures to protect arson investigators from exposure to a variety of risks that may happen at fire scenes, including covering the use of PPE, the decontamination of equipment, and the monitoring of investigators' health; and (3) advanced education to arson investigators on exposure risks, covering the risks of exposure to material at fire scenes and procedures that can be taken to minimize the exposure.[1,2,6,11]

Applying these steps will highly improve the safety and health of arson investigators. Ensuring that they are well taught and 100% ready to deal the many risks involved with the fire scenes will not only safeguard their health but also expand the quality and reliability of forensic investigations. This approach represents an important step toward bridging the gap in occupational health research and practice between firefighting and forensic investigation environments.[1,2,6,8]

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Arson; Hazardous; Chemical Products

F11 Multiple Firearm Wound Suicides in Maryland: A Ten-Year Look Back

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Learning Objectives: The goals of this presentation are to discuss the prevalence of gun violence in Maryland, discuss the prevalence of suicide in Maryland, and explore the incidence of multiple gunshot wound suicides in Maryland.

Impact Statement: By discussing the prevalence and frequency of multiple gunshot wound suicides, we hope to provide a better understanding to an otherwise challenging investigation and autopsy.

Abstract Text: In the Unites States, Maryland is ranked 24th highest for firearm violence. Every year, the rate of firearm suicides steadily increases. Currently, 35% of firearm deaths in Maryland are suicides. Suicide by multiple firearm wounds is a rare event. The presence of more than one gunshot or shotgun wound can be suggestive of homicide, especially when involving different body regions. As such, multiple firearm wound suicides can present a confusing phenomenon for investigators and medical examiners alike.

Study: In this study, we examined 40 multiple firearm wound (more than one gunshot or shotgun wound) cases in the state of Maryland between 2014 and 2024.[1] The current data will be extended through 2024 at the conclusion of the year. Cases were selected by the cause and manner of death written on the death certificate. Location, demographics, and firearm type were obtained through investigation reports.

Results: In this study, all but two of the decedents were male (38/40, 95%). Half of the study population were under the age of 50 years (20/40, 50%) with an age range of 16 to 92 years old. Around two-thirds of the decedents were of the White race (27/40, 67%).The remaining were Black (11/40, 27.5%), Hispanic (1/40, 2.5%), Asian (1/40, 2.5%), and other (1/40, 2.5%). The weapon type primarily used was a handgun (32/40, 80%), followed by shotgun (4/40, 10%), rifle (3/40, 7.5%) and one incident not recording weapon type. The most commonly targeted body regions were the head, followed by the chest. Sixteen cases had firearm wounds to at least two different body regions. Prince George's County and Baltimore City, MD, had the highest number of cases, respectively.

Discussion and Conclusion: Multiple firearm gunshot wound suicides are a rare occurrence but, on average, occur in Maryland approximately two to three times a year. Major commonalities between the 40 cases include: male gender, White race, and handgun usage. The most commonly affected areas of the body were the head and chest, but many cases demonstrated at least two different regions of the body with firearm wounds. This case series highlights the importance of a thorough forensic investigation for the proper determination of manner of death in firearm-related deaths. Additionally, increasing awareness of multiple firearm wound suicides can aid in the public health initiative of investing in mental health services and suicide prevention.

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Handguns; Suicide; Shotgun

F12 Northern Microbes Tell a Story: Human Decomposition Influences on the Soil Microbiome in the Upper Peninsula of Michigan

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Learning Objectives: After attending this presentation, attendees will understand how human decomposition impacts the microbial communities in grave soil in a northern climate.

Impact Statement: This presentation will impact the forensic science community by providing baseline information for soil microbiome successional patterns during human decomposition in a relatively cold climate. This will augment the quickly expanding knowledge of forensic soil microbiology, which can improve Postmortem Interval (PMI) estimations to better inform death investigators and other forensic practitioners.

Abstract Text: Human decomposition is a complex process that is influenced by the interaction of many factors. Despite such complexity, previous research suggests that human decomposition follows basic frameworks that make it relatively predictable. The emerging field of forensic microbiology helps tell the story of decomposition through changes in the microbiome over time. Environmental and human-associated microbiomes both impact and are impacted by decomposition, and successional patterns can be explored. Researching bacterial community structures is advantageous for PMI estimations because bacteria are ubiquitous and available in large quantities.

Soil microbiology is further advantageous for sampling because a body is not necessary for testing the soil where decomposition has occurred and because decompositional impacts on the soil microbiome are evident for over a year. Recent research shows great potential for using grave soil microbiome samples for PMI estimation, and given technological advancements like next-generation sequencing, machine learning, and AI techniques, necrobiome samples are poised to be sources of PMI estimation that are more robust, reliable, and readily accessible. Still, more research is needed to establish baselines across multiple soil types and climates, standardize techniques for collection and analysis, and identify key forensically important bacteria so expensive shotgun sequencing is not required.

This research focuses on forensic soil microbiology and how biodiversity and bacterial abundances change relating to weather data and sample site location in a northern climate. This study examines the grave soil microbiome around four human donors who were placed outside at the Forensic Research Outdoor Station ([FROST], operated by the Center for Forensic Anthropology at Northern Michigan University in Marquette, MI). Sampling dates ranged up to 106 chronological days, from early June through late November in 2021. The average temperature during the summer months (June–August), was 66°F, and the average temperature during the autumn months (September–November) was 47°F. The data from FROST represents a relatively cold climate since this taphonomy research facility is at the northernmost latitude compared to other similar facilities in the United States.

Following extraction and purification of the bacterial DNA, bacteria in the soil samples were identified and quantified using 16S rRNA sequencing and QIIME2. The taxa data, along with weather data, were used to visualize and analyze the soil microbiome changes related to time, temperature, and sample location using R Studio. The analysis of the present research involves Random Forest modeling, a machine-learning algorithm that can illuminate which microbes are the most accurate predictors of the PMI.

Results from the bioinformatic and random forest statistical analyses show that many of the expected phyla of bacteria are present in association with the donor remains at FROST. These include genera known to be associated with the human gut microbiome (*Lactobacillus* and *Carnobacterium*) and phyla significant for human decomposition (e.g., Firmicutes and Proteobacteria). Results also show that some genera of intrinsic soil bacteria that are known to have high tolerance to extreme temperatures, including extreme cold, are also present in the majority of the soil samples collected from FROST (e.g., *Exiguobacterium* and *Variovorax*). In addition to presenting some important climate-specific baseline microbiome data, this study highlights the usefulness as well as the limitations of employing these advanced methods for quantifying and comparing the human decomposition-associated microbiome composition and succession in soil.

Necrobiome; Taphonomy; Soil Microbial Community

F13 Optimizing DNA Retrieval for Insect Identification From Specimens Prepared for Morphological Analysis

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Learning Objectives: Attendees will understand the importance of using complementary techniques of microscopy and DNA barcoding to unlock the knowledge of forensic entomology.

Impact Statement: This research allows investigators trained in standard DNA techniques to access the tools of forensic entomology. Preliminary DNA analysis can later be confirmed by experts through images and preserved specimens.

Abstract Text: Calliphoridae are some of the first insects to arrive at a corpse to lay eggs; however, their larvae can be difficult to identify.[1] Two complementary methods for insect identification are Scanning Electron Microscopy (SEM) and DNA barcoding.[2,3] Morphological identification of insects by microscopy is the gold standard but requires considerable training and expertise, while DNA barcoding relies on standard molecular techniques. In this research, we test the hypothesis that DNA suitable for barcode identification may be obtained from specimens prepared for SEM imaging in a non-destructive manner.

Two different specimen preparation methods for SEM are tested: a dilution series of ethanol and acetone, and a dilution series followed by treatment with Hexamethyldisilazane (HDMS). Three different DNA extraction methods are used following the two specimen preparation methods using the Qiagen DNeasy Kit: soaking intact specimens in lysis buffer, soaking specimens that have been perforated with a fine needle, and specimens that are homogenized in lysis buffer. Total DNA yield from each method is measured by Qubit fluorometry. The suitability of extracted DNA for DNA barcoding is tested by amplification via Polymerase Chain Reaction (PCR) of the cytochrome oxidase I mitochondrial gene. Identity of the PCR products is confirmed by Sanger sequencing and comparison of sequences to known references.

Results of these studies will be presented. This research will inform forensic scientists on best practices for obtaining DNA for preliminary identification of insects while simultaneously obtaining images and maintaining intact specimens for expert identification as needed. The knowledge gained from this research broadens investigative agencies' access to forensic entomology as a tool for determining PMI without sacrificing the rigor of expert morphological analysis.[1,4,5]

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Entomology; DNA; Morphology

F14 Sparks and Burns: A Histological Study on the Effect of Firework Explosions on Pig Skin

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Learning Objectives: After attending this presentation, attendees will be able to identify the principles underlying firework-related injuries, the characteristic injury patterns associated with such injuries, and the histological findings of firework explosions. They will also be able to appreciate the importance of ballistic examination, which can be useful in determining the firing distance of the explosive and reconstructing the crime scene.

Impact Statement: This presentation will significantly influence the forensic science community, offering a comprehensive insight into the pivotal elements of histological firework-related lesions and injury patterns that can be frequently underestimated during such investigations.

Abstract Text: Fireworks are employed globally to commemorate a range of significant and personal occasions. It is regrettable that, along with the festivities, a series of injuries, both fatal and non-fatal, are registered every year. A review of the available data consistently indicates that the areas of the body most frequently injured are the hands, followed by the head and neck, and finally the eyes.[1-3]

This report details the findings of an experimental study in which explosive spheres containing 200 grams of powder were detonated in a controlled environment at varying distances from an oscillating piece of pig meat. The effects of these explosions on pig skin were observed and documented both macroscopically and microscopically.

The lesions observed during the experiment were primarily limited to the dermal tissue. In particular, we identified the presence of both smoking and tattooing effects. Furthermore, in a limited number of samples, we were able to discern the presence of necrosis of the epidermis, which was attributed to the thermal effects. It should be noted that the fragments in question were derived from an animal that had undergone evisceration and exsanguination. Additionally, the animal was in a state of decomposition, rendering it unsuitable for a comprehensive assessment of the lesions observed. This is due to the fact that the gradual increase in pressure was not taken into account, as well as the lack of responsiveness of the tissue, which was non-existent due to the use of tissue immediately postmortem, and that had not undergone any treatment to improve preservation. The damaging effect observed can be attributed to the presence of smoking and the infliction of unexploded gunpowder fragments, which resulted in tattooing of the skin. Histological investigations corroborated this conclusion.

The tattooing effect is produced by the impact and infiltration of unburnt granules of the firing charge into the skin. This marking is not entirely representative of the combustion of the powder grains, as punctiform excoriating effects predominantly characterize it. The combustion of the firing charge, in conjunction with the presence of unburnt gunpowder residue, results in the production of smoke, which is indicative of a smoldering process.[4] Other reliable types of injury typical of fireworks explosions, such as hemorrhages and blast injuries due to the overpressure created by the explosion, were not detected or measured.[5] This is likely due to the type of biological material used in the experiment.[3]

In this experimental series, given the deflagration of a considerable quantity of pyrotechnic product, a considerable thermal effect was observed in the vicinity of the fire. This phenomenon was then subsequently followed by a displacement of air due to a gradual increase in pressure. It is also imperative to highlight the phenomenon of lingering burning propellant powder and the flash fire of the mortar. Those effects are crucial to ballistic experts in providing data concerning the distance and position of a potential victim to the muzzle fire.

This work demonstrates that the deployment of pyrotechnic projectiles, such as those used in this study, possess a significant potential for harm considering the thermal and blast effects they determined and the copious smoking and tattooing effects they produce. Nonetheless, some limits of the study were highlighted, such as the positioning of the sample meat. The oscillation of the pig piece secondary to the high pressure of the blast may be one of the reasons why we did not find pressure lesions on the skin.

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Fireworks; Histological; Firework Injuries

F15 The Detection of Pharmaceutical and Illicit Drugs in Municipal Wastewater Using Gas Chromatography/ Mass Spectrometry

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Learning Objectives: Through this presentation, attendees will gain insight into how Wastewater-Based Epidemiology (WBE) can be leveraged to provide an overview of community consumption of pharmaceutical and illicit drugs. Attendees will learn about the application of a validated Gas Chromatograph/Mass Spectrometer (GC/MS) method for detecting targeted and untargeted drugs in municipal wastewater.

Impact Statement: The presentation will inform forensic scientists about the capability of a GC/MS method to detect drugs in wastewater. The results from WBE analysis often correlate with results collected from population surveys, pharmaceutical purchase records, and law enforcement activity. This information will allow for a better assessment of law enforcement tactics and public health measures, leading to real-time monitoring of community drug usage trends and the earlier detection of novel compounds.

Abstract Text: WBE utilizes wastewater to detect the presence of pharmaceutical and illicit drugs, pathogens, and chemicals within an area served by a Wastewater Reclamation Facility (WWRF).[1,2] Samples collected from multiple WWRFs over several weeks or months yield spatial and temporal datasets mirroring those reported by traditional survey methods such as population surveys, law enforcement seizure records, and public health records.[3] While they can provide demographic information, traditional methods can be time-consuming, under-report the true extent of drug use, and do not reach every individual within a community.[1,4] On the other hand, WBE has the advantage of being faster, unhindered by under-reporting, and ensures a sizeable anonymous sample.[1]

Local WWRFs provided 24-hour composite wastewater influent (untreated) samples. The samples were filtered to remove large particles and were acidified to a pH of 2.5. Solid-Phase Extraction (SPE) cartridges were used to extract the drugs of interest. Several SPE cartridges were evaluated to determine the most suitable for the method. A comparison study between Pentafluoropropionic Anhydride (PFPA) and N,O-Bis(Trimethylsilyl)Trifluoroacetamide (BSTFA) was conducted to determine the most effective derivatizing agent for the analytes. Samples were derivatized and reconstituted in ethyl acetate, then analyzed using a GC/MS run in Selected Ion Monitoring (SIM) mode with splitless injection. Reference standards were purchased from Cerilliant. MassHunter Unknown Analysis was used in conjunction with the National Institute of Standards and Technology (NIST) Library (v20, 2020), the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) library (3.13.L, 2023), and Cayman Library (v20022024, 2023) libraries to identify compounds of interest.

SPE cartridges based on a broad spectrum copolymeric, cation exchange sorbent were the most suitable for the analysis. PFPA was suitable for the targeted analytes, although BSTFA performed better for Gamma-Hydroxybutyric Acid (GHB). The resulting qualitative data from the use of MassHunter Unknowns Analysis identified propofol, methaqualone, bezendrone, flumazenil, various synthetic cannabinoids, delta 8-THC, delta 10-THC, pregabalin, gabapentin, butonitazene, naproxen, methazolamide, and LSD. Prior and preliminary qualitative studies confirmed the presence of acetaminophen, ibuprofen, cocaine, and methamphetamine in municipal wastewater through comparison with reference standards.

This study, as far as the authors are aware, is the first qualitative data reported for the detection of various illicit and pharmaceutical drugs, such as methamphetamine, methaqualone, butonitazine, and ibuprofen, in the greater Birmingham area.[5] Wastewater samples were monitored for targeted and untargeted drugs to determine their presence in the community.[6,7] Along with existing methods, WBE can provide a better picture of the extent of drug use for an area than traditional methods can alone. Qualitative data can identify substances that are new to an area and may be unaccounted for within existing datasets. The current projects in our lab seek quantitative data. By first determining the amount of methamphetamine and cocaine in wastewater, back calculations can be performed to translate this amount to the amounts of drugs consumed within the community.[6,7] Thus, WBE analysis can provide insight into the effectiveness of law enforcement, public health, and legislative efforts to curb drug usage and addiction. The improved data will allow government officials to take steps to mitigate the potential aftermath of a new illicit drug entering the community and adapt strategies for preventing addiction, overdoses, and drug-related violence.

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Wastewater-Based Epidemiology; Chromatography; Drug Analysis

F16 The Development of LAMP-ICS and FastPCR-ICS of Human/Male Multiplex Assay for Rapid On-Site Screening

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Learning Objectives: This study developed a direct multiplex method for simultaneous Human/Male screening based on molecular diagnostic technology. The Loop-Mediated Isothermal Amplification (LAMP) and fastPCR method were combined with Immunochromatographic Strip (ICS) to identify male DNA faster and more conveniently in the field. The aim of this study is to verify the performance of portable equipment and suggest a screening assay for on-site test.

Impact Statement: Mixed-source is a type of sample that is often encountered in forensic cases, especially in sex-related crimes. Therefore, it is important to screen for male DNA in these types of cases. The assay developed in this study can be performed in the field rather than in a sophisticated laboratory setup as it can be amplified directly without DNA purification and its results are easily interpretable. This methodology is expected to contribute to a more efficient forensic workflow.

Abstract Text: Screening valid evidence from a crime scene is a crucial step that can improve the forensic workflow efficiently. Specifically, screening male evidence is necessary as it is used to identify male-specific genes in mixed gender samples, such as sexual assault cases. The purpose of this study is to develop a method for simultaneous Human/Male screening in crime scenes based on molecular diagnostic technology. In this study, the assay was designed for rapid and simple analysis in the field by combining DNA amplification with ICS. Additionally, this assay was developed to be suitable for on-site analysis by applying the direct method, which uses NaOH as a direct lysis buffer without DNA extraction. This assay was built to target the human-specific gene HLA-DRA and the male-specific Y chromosome marker SRY gene.

ICS was designed to be probe-labeled specific by fixing different antibodies into one strip, with each antibody representing its specific line (Control, Human, and Male line). LAMP and fastPCR, a modified version of conventional Polymerase Chain Reaction (PCR), were used for DNA amplification. Characteristics of each method were compared and analyzed, and they were improved to be suitable for on-site screening. The LAMP method does not require high-cost equipment, as it can be amplified in a short time with a single temperature. These aspects make it easier for it to be used in the field.

Three pairs of primers, FIP/BIP, F3/B3, and LF/LB, were designed, with a probe at the 5' end of FIP and biotin labeled at the 5' end of LF to confirm the results by ICS. LAMP was tested at various times from 20 to 40 minutes to optimize the visibility of the band formed on the ICS. LAMP has many advantages, as previously described, but has limitations such as non-specific amplification or carryover contamination. To eliminate false positives, appropriate concentrations of Uracil DNA Glycosylase(UDG) and Deoxyuridine Triphosphate(dUTP) were added to the LAMP reaction composition. On the other hand, PCR's primer design is more convenient and presents a lower risk of contamination than LAMP; however, it is time consuming. Therefore, the rapid PCR protocol, which shortened the time to about 30 minutes, was applied to optimize the study.

Various types of master mixes containing Taq DNA Polymerase, as well as multiple reaction volumes of PCR were compared to select the most suitable condition for fast PCR. In addition, time of denaturation, annealing, and extension was adjusted for optimization. Finally, the performance of the miniPCR (miniPCR bio) was validated to demonstrate the suitability of portable equipment for on-site screening. As a result, this study developed LAMP-ICS and fastPCR-ICS methods based on molecular diagnostics. This method offers quick detection of the male sample in the field. Three lines is observed if a male contributor is included and two lines if only a female contributor is present. The specificity for human and male such as sex-typing was confirmed and its limit of detection was verified.

Forensic DNA; Molecular Testing; Rapid DNA

F17 The Impact of Sound Suppressors on Gunshot Residue Deposition

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Learning Objectives: Attendees will learn about suppressors and how gunshot residue patterns change when a suppressor is used compared to a gunshot residue pattern created without a suppressor. They will also learn about the results of the Modified Griess Test and the Sodium Rhodizonate Test and their uses in distance determination.

Impact Statement: This research answered a question that had been posed by a medical examiner. This research could potentially be added to the standard operating procedures and aid medical examiners in their analysis should they encounter a suppressor in casework.

Abstract Text: Inorganic gunshot residue found on targets is composed of burned or partially burned gunpowder particles, nitrites, and vaporous lead.[1] Sound suppressors, commonly referred to as silencers, work by using internal dividers, and sometimes an additional physical barrier, to trap and cool gases created from igniting gunpowder.[2] We hypothesized that, firing with the suppressor, the amount of gunshot residue would be reduced, and the resulting pattern would be more concentrated while still containing less residue than a firearm without a suppressor at the same distance.

A Cobray 9mm Luger sub-machine pistol and a Cobray 45 Auto sub-machine pistol, with commercially available detachable suppressors, were fired at cloth witness panels to create a pattern of gunshot residues at distances of 0, 3, 6, 9, and 12 inches (or 0, 7, 15, 22, and 30cm), both with and without the suppressors. To test for a different pattern between skin and cloth, as inspired by previous research, additional witness panels were created using pig skin and shot under the same conditions using the Cobray 45 Auto sub-machine pistol and the 9mm Luger sub-machine pistol, at distances of 0, 3, 6, and 12 inches with and without the suppressor.[3] Visual examination and pattern measurements were performed using the Leeds Spectral Vision System (LSV2), and chemical testing was performed using the Modified Griess Test and the Sodium Rhodizonate Test. A second set of witness panels were created for the 45 Auto pistol without the suppressor and the 9mm Luger pistol, with and without the suppressor due to debris from the suppressor being deposited on the witness panels and a potential contamination issue when no suppressor was used. The visual and chemical examinations showed that patterns created using a suppressor are more concentrated and contain less gunshot residue than patterns created without a suppressor.

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Firearms; Gunshot Residue; Research

F18 The Impact of Storage and Container Options for Human Remains Detection Training Aids

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Learning Objectives: This poster presentation will offer insight on the impact that different storage and conservation approaches have on Human Remains Detection (HRD) training and their Volatile Organic Compounds (VOCs) profiles. The significance and magnitude of common containment and storage practices to the training aids' odor profile will be discussed.

Impact Statement: The results presented will underline which storage options and environments carry the most impact on the integrity and longevity of the training aids' VOCs while also suggesting an optimal regimen to adopt when storing human remains training aids to minimize alterations and contamination.

Abstract Text: HRD dogs can track and locate a wide range of odor sources (such as whole bodies, tissues, bones, or blood/fluids), including a varying degree of aging and decomposition, and across an extensive set of environments. During their training period, these working dogs are exposed to and familiarized with the suite of VOCs emanating from decaying samples, which they will then associate with their target. As such, it is imperative to control and maintain the preservation of the training aids' odor profile and to avoid alterations or contaminations that could inadvertently imprint on the dogs and affect their future field performances.

Despite the crucial role played by the training aids and the notable effect of environment on remains VOCs, no widespread or codified protocols for storage and containment exist, giving rise to a large degree of dissimilar methods across agencies and laboratories.[1] This knowledge and regulation vacuum introduces unaccounted, and possibly significant, variations in the training aids' distinctive scent.[2,3] As such, this research focuses on recording the nature and severity that the most common storing and containment options for human remains training aids have on the samples' VOCs profile, and on establishing a hierarchy of beneficial and harmful practices. For these purposes, excised portions of porcine abdominal tissue were stored and aged in containers and conditions mimicking those commonly adopted by HRD dogs handlers and agencies. The experimental variables include the container's material, the storage conditions, and the frequency and duration of usage. To faithfully characterize the VOC complexity of decomposing flesh and to account for the possible identifying scents recognized by the dogs during the olfactory process, known decomposition markers have been selected and detected from the following chemical functional groups: alcohols, aldehydes, alkanes, amines, ketones, organic acids, and sulfides. The choice and presence of these compounds is consistent with the existing literature on the subject.[4,5]

Regularly scheduled headspace collection was achieved by way of Solid-Phase Microextraction (SPME), while analysis was performed via Gas Chromatography/Mass Spectrometry (GC/MS). The project is ongoing as it aims to collect data for the aforementioned variables over an extended period of time to accurately represent all the stages of decomposition and the lifespan of HR training aids. However, preliminary results for the experimental proposition, with factors such the container's material altering the background and the sample's profile, with glass and metal being less pervasive than plastic, while oxygen availability greatly correlates to the sample's rate of decay. Once all the factors are analyzed and compared, a data-supported HRD training aid storage protocol will be drafted and submitted for consideration for the benefit of forensic canine handlers and researchers.

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Human Remains; Volatiles; Cadaver Dog

F19 The Popularity of Biometrics in India, Along With the Challenges

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Learning Objectives: The current status and growing importance of biometrics and the role of biometrics in the Indian criminal justice system is addressed. This presentation identifies key challenges and opportunities facing the field of biometrics and the impact of recent legislation on biometrics in India.

Impact Statement: The increasing integration of biometrics in India's criminal system, driven by technological advancement, and supportive legislation has the potential to significantly enhance investigation efficiency, improving conviction rate and ensure justice for victims. By addressing challenges in the system and the sample collection, India can fully harness the power of biometrics to create a safer and more equitable society.

Abstract Text: Biometrics is composed of two Greek words, bio that means life and metrics that means measurement. Biometrics play a crucial role in forensic science, providing methods for identifying individuals based on unique physiological and behavioral characteristics. This intersection has evolved significantly as multiple developments have taken place in the evolution of biometrics, with advancements in technology enhancing the accuracy and efficiency of forensic investigations. There are three major types of biometrics. One is physical, which includes bodily measurement such as iris identification, face recognition, fingerprint identification, hand recognition, and many more. The second type is behavioral biometrics, including gait pattern, the signature recognition system, and the keystroke recognition system. The third is physical and behavioral biometrics, which includes voice recognition. Biometric is operated with multimodal system. Design faces multiple issues so manifold things need to be considered to overcome the problems.[1-3]

There are seven basic criteria to get a better biometric system: uniqueness, universality, permanence, collectability, performance, circumvention, and acceptability. The digital signal processing system involves two types of signals. First is the analog system, which is uninterrupted waves in a continuous way. Second are digital signals; the waves are converted into 0 and 1. Digital signals need to acquire, edit, store, and processe. Digitalization is done. In digitization, sampling and quantization in done. However, several challenges persist in the integration of biometrics within forensic science. Data quality gets altered due to uncontrolled environmental and external factors. Biometrics also faces issues in the court of law as it raises privacy issues and concerns regarding consent. A new law, Bhartiya Sakshya Adhiniyam (BSA), 2023, has replaced the Indian evidence act so as to allow digital evidence to be permissible in the justice system but still a fundamental right, that is, the right to privacy is under question.

Multiple cases are filed every day in India regarding the unsecured system of biometrics as due to False Acceptance rate by the multimodal system. According to the National Crime Records Bureau, the overall crime rate in India has seen a decline, from 445.9 crimes per lakh population in 2021 to 422.2 in 2022. This trend suggests that the introduction of advanced technologies, including biometrics, may be contributing to improved crime management and reduction.

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Forensic Biometrics; Accuracy; Law Enforcement

F20 The Prevalence of DNA on Car Vehicles for Guidance in Collecting Samples

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Learning Objectives: From this presentation, attendees will understand the optimal sampling sites on vehicles for DNA collection and understand the timeline for effective sample collection.

Impact Statement: This research will impact the forensic community by streamlining vehicle processing times for crime scene investigators and reducing the number of swabs submitted for DNA analysis.

Abstract Text: Crime Scene Investigators (CSI) collect many different sample types for analysis at crime laboratories, including DNA swabs for "touch" or "trace" DNA. Unlike identifiable blood or seminal stains, trace DNA is difficult to pinpoint a certain area. There are additional challenges when it comes to vehicle processing. The first challenge is the time it takes to have the vehicle processed for evidence, as DNA degrades over time. The second challenge is the different sampling strategies utilized by CSI personnel. While all follow the same procedure for prepping the swab, individuals will differ in how the sample is collected. One person may focus on the outer portion of a handle while another will swab the whole handle, both outer and inner portions.

This study investigated the persistence of DNA on different vehicle areas of a vehicle that has been waiting for processing for a length of time. In addition, the study examined which areas of exterior and interior door handles provide the best DNA results to guide in sample collection.

Vehicles (n=17) were selected from the Kansas City Vehicle Tow Lot. These vehicles had already been processed by CSI and had been in the lot for at least a year. DNA was deposited by six donors, each acting as the sole depositor, on the following sample areas of each vehicle: exterior door handles, interior armrest, interior door handles, steering wheel, and gear shift. The deposited DNA remained on the vehicles for up to 28 days before collection. At the time of sampling, the weather conditions and time were recorded, and temperatures of the sample areas and the interior of the vehicle were measured before swabbing. DNA extraction was performed using the QIAGEN EZ1 XL Large Volume Protocol. Quantification was performed with the Quantifiler Trio DNA Quantification Kit on a QuantStudio5 using a half volume reaction. A threshold of 0.05ng was used as the minimum amplification template amount and helped guide potential sampling area quality. Selected samples were amplified using GlobalFiler and Polymerase Chain Reaction (PCR) products were separated and detected on the Applied Biosystems 3500 Genetic Analyzer.

DNA on the exterior door handles remained above 0.05ng threshold for approximately 4 days post-deposition, with the inner portion retaining DNA slightly longer than the outer. Interior samples showed DNA presence throughout the study, but the interior handle and armrest samples fell below the threshold at days 14 and 21, respectively. When the samples were combined, the DNA remained above threshold at those time points. Steering wheel and gear shift samples had high initial DNA recovery, declining by day 14 and nearing the threshold by day 21.

Overall, these findings suggest exterior handles should be swabbed within the first week, focusing on the inner portion of the exterior handle. DNA persisted on all interior portions, making them all viable swabbing options. Both the interior handle and armrest followed similar trends throughout, although the DNA concentrations still decreased over time, approaching the threshold by day 14. Last, if swabbing occurs two or more weeks after seizure, then the armrest and interior handle should be collected on a single swab.

Touch DNA; Vehicle Processing; Crime Scene Investigation

F21 A Highly Portable and Ultra-Sensitive Platform for Rapid Forensic Identification of Wildlife Species

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Learning Objectives: The goals of this presentation are to help attendees: (1) understand the principles of forensic identification of wildlife species which are protected by law by learning the fundamental concepts and techniques used in forensic science for identifying wildlife species and understanding the role of DNA analysis and molecular biology in wildlife forensics; (2) obtain insights into sample collection and handling by gaining proficiency in the proper methods for collecting, preserving, and handling biological samples from wildlife to ensure accuracy and prevent contamination and to learn to identify and use appropriate tools and equipment for sample collection in various field conditions; (3) become familiar with DNA extraction and analysis Techniques by learning the procedures for extracting DNA from wildlife samples, including tissue, blood, and hair, and understanding and applying various DNA analysis techniques, such as Polymerase Chain Reaction (PCR), sequencing, and genotyping; (4) understand legal and ethical considerations of wildlife forensic identification and the importance of maintaining chain-of-custody and documentation in forensic investigations; (5) explore applications of wildlife forensics by understanding the diverse applications of wildlife forensics, including conservation efforts, anti-poaching measures, and wildlife trafficking investigations and learning how forensic identification can aid in the enforcement of wildlife protection laws and regulations; and (6) promote Conservation Awareness by understanding the impact of wildlife crime on biodiversity and ecosystems and raising awareness about the importance of wildlife conservation and the role of forensic science in protecting endangered species. Participants will have a comprehensive understanding of wildlife species identification techniques and appreciate the significance of wildlife forensics in conservation and law enforcement. [1]

Impact Statement: Our wildlife species identification project significantly enhances conservation efforts by providing a rapid, ultra-sensitive platform for forensic analysis. This technology aids in accurately identifying species involved in wildlife crimes, thereby supporting anti-poaching initiatives and combating illegal wildlife trade. It promotes biodiversity protection and informs effective wildlife management policies. Additionally, the project fosters collaboration between scientists, law enforcement, and conservationists, ensuring comprehensive and coordinated efforts. By advancing forensic capabilities, this project contributes to preserving endangered species and maintaining ecological balance, highlighting the critical role of science in safeguarding our planet's natural heritage.

Abstract Text: Wildlife preservation is paramount for maintaining ecological balance, regulating climate, and supporting sustainable livelihoods crucial for human well-being. However, the illegal trafficking of endangered animals and their products remains a formidable global challenge, recognized under the United Nations Sustainable Development Goals. Wildlife trafficking, with its intricate networks spanning international borders, poses significant hurdles for law enforcement due to differing legal systems and capacities among nations. Despite these challenges, the lucrative nature of the trade, driven by demand for rare species and their products, continues to incentivize traffickers. Insufficient resources for enforcement and conservation efforts further hinder effective protection of habitats and patrols.

A holistic approach involving collaboration among governments, law enforcement agencies, conservation groups, communities, and international stakeholders is imperative. This approach should prioritize enhancing enforcement capacity, reducing demand through education and sustainable alternatives, strengthening legal frameworks, and addressing underlying issues such as poverty and corruption.

At SERATEC, we are developing novel and highly sensitive diagnostic kits for animal species identification in close collaboration with government authorities specialized in combating wildlife crimes. Our project aims to significantly reduce the cost of identifying endangered species by offering a solution that demands reduced skilled labor and provides a cost-effective equipment-consumable kit, up to 5-6 times cheaper than current market alternatives.

Our efforts focus on pioneering a groundbreaking platform that integrates features of digital-PCR and isothermal amplification of DNA for wildlife species identification. The device is highly portable and can operate on USB power. Additionally, it is equipped with digital data transfer features for appropriate data handling. This advancement holds immense potential for conservationists, researchers, and wildlife authorities, facilitating informed decision-making and effective management strategies against wildlife crimes. Our primary objective is to significantly contribute to the protection and sustainable management of wildlife populations globally while fostering a novel market in this field. A presentation at AAFS will provide invaluable exposure to our project, showcasing its innovative approach to wildlife species identification and highlighting its potential impact on combating illegal wildlife trafficking. This platform will facilitate networking opportunities, collaboration, and potential partnerships with experts and stakeholders in the field, accelerating project progress and adoption.

Reference:

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Wildlife Forensics; Identification; Point-of-Need

F22 A High-Throughput, Multi-Biochemical Analysis of Pollen Grains for Their Identification in a Forensic Context

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Learning Objectives: Attendees will learn about the importance of pollen as trace evidence and gain insight into the niche field of forensic palynology. This presentation will discuss an alternative method for plant species identification based on the unique chemical "fingerprints" of pollen. The use of lipid profiling and DNA barcoding as tools for pollen characterization will be demonstrated.

Impact Statement: The results of this study will improve palynology as a forensic tool in the modern crime laboratory. The proposed method increases the accessibility of pollen analysis to forensic technicians by utilizing common instrumentation that requires minimal training. Additionally, the method can be optimized using automated instruments and is compatible with high-throughput analysis.

Abstract Text: The accurate identification and quantitation of pollen grains is crucial for a variety of palynological applications, including forensics, fossil dating, food and agricultural safety (melissopalynology), and aeroallergen monitoring. As a forensic tool, pollen is often used as a proxy to connect individuals to an object or place related to a crime. Traditionally, taxonomic resolution is achieved using microscopy to characterize morphological features of the grain—size, shape, aperture type, and granularity—which vary between pollen types. While identification at the species level is desired, manual classification using microscopy is often limited only to the genus or family level. Additionally, this specialized task is time-consuming and heavily subject to human bias as it relies on visual identification (while some studies have begun looking at machine learning and image matching as possible routes for pollen identification, the methods are still in early development and the necessary instrumentation is often expensive or not readily available to most crime labs).[1,2] Thus, the development of a more accessible, objective, and rapid pollen identification method is necessary to integrate forensic palynology in the modern crime lab.

To address this, our approach relies on an orthogonal biochemical analysis of pollen utilizing lipid profiling and DNA barcoding strategies to create unique chemical profiles capable of species differentiation. Several studies have shown the composition of the lipid-rich exterior of pollen grains varies between species; however, none have sought to use this as a tool for species identification.[3-5]

A single "one-pot" non-destructive extraction is performed to isolate pollen coat lipids and DNA contained within the pollen, while leaving the cleaned grain intact. Lipid profiles are generated using Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS) and Triple quadrupole (QqQ) tandem Mass Spectrometry (MS/MS). Three genetic markers with high variability between species, *rbcL*, *matK*, and *ITS2*, are targeted and amplified using Polymerase Chain Reaction (PCR) with species-specific primers and visualized using agarose gel electrophoresis. Quantification of extracted DNA is achieved using quantitative Polymerase Chain Reaction (qPCR).

Using this method, the chemical profiles of 12 species have been generated from bulk pollen samples. Each species analyzed had a unique lipid profile with partial overlap occurring primarily between plants within the same genus, suggesting taxonomic resolution to the species level is achievable using lipid profiling. The *rbcL*, *matK*, and *ITS2* markers for five species were successfully targeted from the extracted DNA. Amplicon sizes were compared to those found in the literature for confirmation. Our results indicate that chemical profiling of pollen can address the issues associated with current palynological techniques. The non-destructive nature of our method allows for complementary physical characterization, and its orthogonality reduces the potential for errors that may arise from using either technique separately. Furthermore, the method is compatible with high-throughput analysis and shows promising implications for single pollen grain analysis.

References:

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Palynology; DNA; Lipid Profiling

F23 A Universal Method for Biological Stain Identification Using Raman Spectroscopy: An Investigation of Potential False Positives

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Learning Objectives: This presentation will inform attendees of the limitations of current methods for biological stain analysis during forensic investigation, the significance of a universal, non-destructive method for confirmatory identification of body fluid traces, and advantages of Raman spectroscopy combined with machine learning in forensic investigation, including that for the identification of body fluid traces.

Impact Statement: When fully developed and implemented, the proposed method will significantly improve the detection and characterization of biological stains discovered at the crime scene.

Abstract Text: After attending this presentation, attendees will understand the principles of a novel, universal method for the identification of all main body fluids based on Raman spectroscopy combined with machine learning. In addition, the attendees will learn why this method can be considered confirmatory.

There is a substantial need for a rapid and on-site method for detecting and identifying Body Fluids (BFs) at crime scenes. This is a crucial step since traces of BFs are typically the primary source of DNA evidence in a criminal investigation. However, current BF detection methods are complex, involve several steps, are costly, and can provide false positive results, especially in the case of presumptive tests. We have recently developed a universal and non-destructive test for confirmatory identification of all main body fluids based on Raman spectroscopy.

This study evaluated potential false positives of the method due to Environmental Interference (EI) substances. Forty-nine substances were analyzed that might be misclassified as body fluid stains (blood, semen, urine, vaginal fluid, sweat, and saliva). The EI substances were either because they cause false positives in the current presumptive tests or show a similar appearance to body fluid stains. A machine learning algorithm, Random Forest (RF), allowed for the identification of all six body fluids and the discrimination against the EI substances with 100% accuracy at the sample level during external validation. These results demonstrate that Raman spectroscopy combined with machine learning has excellent potential as a universal, non-destructive, confirmatory, and rapid method for the detection of body fluid traces for forensic purposes.

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Bodily Fluid; Raman Spectroscopy; Machine Learning

F24 Considerations for Fieldable Drug Detection and Identification by High Resolution Mass Spectrometry

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Learning Objectives: Attendees will gain an understanding of the role certain parametric- and application-based considerations play in the chromatography-free detection and identification of illicit drugs using a fieldable high-resolution time-of-flight mass spectrometer. Attendees will be presented with investigations into a number of hardware configurations, ion sources, and analysis avenues.

Impact Statement: This presentation will impact the forensic science community by introducing the characterization and analysis of a rugged time-of-flight mass spectrometer for fieldable applications in drug detection and identification. Hardware parameters critical for chemical detection and data analysis methods critical for compound identification will be discussed, including considerations impacting on-site chemical analysis. This work seeks to lower implementation barriers and improve understanding of critical aspects for on-site drug identification.

Abstract Text: Chemical analysis targeting the accurate identification and classification of illicit drug compositions remains critical to the forensic science, public health, and law enforcement communities. Pushing technologies out of the traditional laboratory and into the field can aid in obtaining near real-time information for these communities. In conjunction with advancing these technologies, algorithms and novel data analysis methods can improve detection and identification of known and unknown compounds, as well as aid in data accessibility and transferability. Many of these needs were highlighted in the recent TRANQ Research Act (H.R. 1734) of 2023 and by the Office of National Drug Control Policy's 2023 declaration.[1,2]

We have initiated the characterization of multiple sample introduction, ionization, and chemical analysis schemes for the fieldable identification of illicit drugs by high resolution mass spectrometry. A rugged transportable time-of-flight mass spectrometer (Vocus S, TOFWERK AG, Thun, Switzerland/TOFWERK USA, Boulder, CO, USA) primarily used for on-site volatile organic and inorganic compound detection was employed. The chromatography-free capabilities of ambient and atmospheric pressure ionization mass spectrometry enabled rapid and sensitive chemical analysis. Cause-and-effect diagrams were employed to gain a high-level look at parameters impacting measurement variability and identification confidence. Characterization and optimization of a series of hardware and ionization parameters were completed to enhance mass spectrometric detection. The response of the time-of-flight mass analyzer was optimized across these parameters using sample introduction avenues of both wipe-based samples (i.e., from swipe collection) and melting point capillary samples (i.e., dipped in a liquid extraction). Similarly, both in-line Dielectric Barrier Discharge Ionization (DBDI: Soft Ionization by Chemical Reaction In Transfer [SICRIT], Plasmion GmbH, Augsburg, Germany) and acetone-assisted Vacuum Ultraviolet (VUV) ionization (Aim reactor, TOFWERK AG) were characterized. The mass spectrometer generally demonstrated protonation in positive ion mode and sub-nanogram sensitivities of a range of illicit narcotics across sample introduction and ionization schemes, including within mixtures.

Characterization of the platform performance was supported by investigating parametric effects and avenues for data analysis critical to compound identification. These analyses considered isomeric interferences, mixture analysis, spectra calibration, and library matching. Avenues for improving identification confidence, such as incorporating in-source Collision-Induced Dissociation (isCID) for compound fragmentation, were also investigated. Ongoing work is expanding the overall platform configurations to include additional sample introduction and ionization schemes, as well as alternative workflows and advanced library matching algorithms. The presented analysis will aid in the development of a framework used for on-site detection and identification of drugs that will seek to include standard workflows, documented analysis protocols, and implementation packages for end-users.

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Mass Spectrometry; Drug Analysis; Field Deployable

F25 Investigating Odor Signatures of Electronic Storage Devices

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Learning Objectives: After attending this presentation, attendees will learn how different methods of sampling for the analysis of volatile organic components of electronic storage devices by Gas Chromatography/Mass Spectrometry (GC/MS) affects the resulting odor signature, which has implications for the development of training aids and protocols for canine detection.

Impact Statement: This presentation will impact the forensic science community by examining the odor profiles produced when electronic storage devices are sampled in different ways, which has meaningful implications for understanding the science behind canine odor detection as well as for the development of canine training aids.

Abstract Text: Electronics are integral tools for many aspects of our everyday lives, and thus may contain valuable evidence for a criminal investigation. In 2015, when former Subway restaurant spokesman Jared Fogle's house was raided in a child sex tourism and child pornography investigation, a hidden USB drive investigators found in his residence was originally overlooked and only recovered thanks to the detection capabilities of a Federal Bureau of Investigation (FBI) - trained canine. Recent developments in canine detection have shown that dogs are capable of distinguishing characteristic odor signatures (the odor profile) of electronic devices. These dogs have demonstrated the ability to sniff out micro cell phones, USB drives, and Secure Digital (SD) cards with levels of accuracy identical to when they detect drugs, explosives, and people. While it is known that canines can detect these electronic Mass Storage Devices (MSDs), the specific chemistry of said detection is unknown, since the sensitivity of a dog's nose far exceeds that of modern instrumentation.

Current research on electronic device odors is both minimal and contradictory. Foundational but unpublished research from 2012 by the Connecticut State Police, Emergency Services Unit - K9 Division, in collaboration with a Connecticut state forensic chemist, used direct thermal analysis GC/MS to analyze various MSDs. Triphenylphosphine Oxide (TPPO) was identified as a target odor for training canines. TPPO is a flame retardant that coats all electronic printed circuit boards to prevent their overheating. TPPO has since been used successfully as a target odor training aid for the detection of MSDs, with canines successfully uncovering hundreds of devices hidden in recycling bins, vents, radiators, and more for a variety of case types including child pornography, homicide, and organized crime. The dogs are initially trained on TPPO; however, it must be recognized that this is only the first stage of training since odor signatures of MSDs are complex. As training continues, a selection of real samples of MSDs are used so that the canines can recognize the full odor signatures for each type of device.

In 2017, room temperature headspace analysis via Solid Phase Microextraction (SPME) with GC/MS of electronic devices found several volatile compounds common to SIM and SD cards, as well as USB drives.[1] The authors concluded that MSDs do have characteristic odor profiles, making detection with minimal false alerts feasible for trained canines. However, no TPPO was detected or identified in this study, contradicting prior research and the demonstrated TPPO in canine detection training. The lack of TPPO detection in room temperature SPME/GC/MS analysis is feasible due to TPPO's low volatility. However, because dogs have detection capabilities at least 10^3-10^5 times greater than our chemical instrumentation, the canines may be able to detect and use TPPO. This research aims to resolve this question through an investigation of different GC/MS sampling methods of MSDs.

In the first part of this study, GC/MS sampling and method optimization for TPPO detection and identification was performed. Different GC/MS sampling methods were investigated, including room and high temperature headspace SPME with different fiber phases, direct heated headspace analysis, and direct thermal analysis, to evaluate their capabilities and limitations with respect to the detection and identification of TPPO. In part 2, various electronic storage devices (e.g., RAM drives, SIM cards, USB flash drives) were analyzed using the optimized methods from Part 1 to investigate their odor signatures. The results of this research demonstrated that MSDs do have a characteristic odor profile; however, the specific profile detected depends on the method of sampling the volatile organic components. This information is important for consideration when determining the components of training aids for the canine detection of MSDs.

Reference:

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Canine Detection; Electronic Storage Devices; Odor Signature

F26 Sustainable Wood Sourcing and Species Identification Through ATR/FTIR Spectroscopy and Machine Learning in Forensic Applications

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WITHDRAWN

F27 Finding the Ignition Source in Burned Bones: A Forensic Research Investigation

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Learning Objectives: The goals of this presentation are to understand the surface layer changes porcine bone can experience through varying intense temperatures, such as changes in size and color; analyze microscopic changes to the porcine bone (i.e., changes to hydration, porosity, and collagen levels and crystallinity size), and use multiple analysis techniques, such as Scanning Electron Microscopy (SEM), X-Ray Fluorescence (XRF) and Fourier Transform Infrared (FTIR) to compare the results pre- and post-heating (i.e., alterations to mineral concentrations).

Impact Statement: The focus of this research is to understand the changes that can occur to the bones through intense heat and fire with the idea of effectively "reverse engineering" what we have found to be able to decipher what may have happened at the scene. This would therefore allow us to apply said findings to forensics investigations of a medicolegal nature.

Abstract Text: With an increase of around 10% in fire-related fatalities in the year ending June 2022 (ONS Statistics), forensic investigations have begun to focus on thermal alterations to skeletal remains and the various techniques required to analyze them.[1] The focus of this research is to understand the changes that can occur to the bones though intense heat and fire, with the idea of effectively "reverse engineering" what is found to be able to decipher what may have happened at the scene. Samples of porcine long bone, specifically femurs, were burned at a multitude of temperatures ranging from 300–900 degrees Celsius. This involved the use of a muffle furnace for the application of intense heats, burning at 300–900 degrees Celsius, in increments of 150 degrees, and a realistic fire scenario, burning at ~612 degrees Celsius, in which the samples could come into contact with open flames. This scenario was presented as a fire that had broken out in a living room due to careless discarding of a butane lighter. The samples were each analyzed through the use of FTIR, SEM, and XRF to collate data such as mineral content, elemental content, and changes to the bone's structural integrity. The results found the outputs of burning butane to have been absorbed into the boned that were placed in the open fire, elements that were not present in the control samples or in those burned in the muffle furnace. The elements absorbed included Neodymium and Bromine, both outputs of burning butane, but also Cerium, an output of lighter flints. This coupled with the results of the FTIR and SEM in comparison with that of the samples burned in the muffle furnace at 600 degrees Celsius provided a great insight into the changes that can occur to bone depending on temperature, but alight to the ignition source, allowing for the results to potentially be applied to forensic investigations and medicolegal situations.

Reference:

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Forensic; Fire Investigation; Skeletal Remains

F28 Postmortem Thermal Injuries Due to Muzzle Flash Ignition

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Learning Objectives: Attendees will understand the potential for muzzle flash ignition of objects in fire investigations involving gunshots, as demonstrated by a case study of a residential fire ignited by muzzle flash following a suicidal gunshot. This includes recognizing the significance of the Bureau of Alcohol, Tobacco, Firearms and Explosives' (ATF's) experimental findings, which highlight the ability of muzzle flash to ignite natural materials and the importance of considering this factor in forensic and arson investigations.

Impact Statement: This case underscores the importance of considering muzzle flash ignition in fire investigations involving gunshots. The ATF's thorough experimental approach and digital documentation provided crucial evidence, highlighting the potential hazards of muzzle flash and its role in fire ignition.

Abstract Text: Muzzle flash is the flame created subsequent to the discharge of a firearm due to the combustion of gunpowder and remaining unburned ejecta oxidized by ambient air. It is widely known to mark the skin and clothing in close-range firings. Nonetheless, muzzle flash ignition of objects is not a well-documented sequela of gunshots. Herein we report a case of a smoldering residential fire ignited by muzzle flash, following a suicidal single gunshot injury to the head of a 74-year-old male with the aid of a .22 caliber revolver. The local Office of the Chief Medical Examiner performed an autopsy. A single bullet was recovered embedded in the decedent's left temporal skull, and extensive upper body thermal injuries were noted at autopsy. Arson investigation conducted by the local fire department at the scene did not reveal the presence of any accelerants, and there were no apparent signs of a criminal act. Hence, there was no clear indication of the origin of the fire.

The revolver handgun was sent into evidence at the ATF. ATF conducted testing using the handgun at the highly specialized Fire Research Laboratory (FRL) to observe the potential for muzzle flash ignition when a revolver is fired into and near natural and synthetic clothing materials. A series of 31 experiments was performed in which the different materials were positioned in various configurations. The experiments were captured utilizing digital still photography, High-Definition (HD), and high-speed video, which will be presented. In two experiments in which rounds were fired into a tight pattern and natural materials were used as pillowcases, ignition and sustained burning of the pillow were observed. The investigation conducted by the ATF FRL discovered the origin of the fire at the scene and showcased via digital imaging the ability of muzzle flash ignition when a revolver is fired near natural materials.

Handguns; Muzzle Flash; Ignition

F29 Pyrolysis Products Obtained From Household Substrates at Crime Scenes: An Analytical Challenge for Forensic Fire Investigation Experts

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Learning Objectives: The audience will be given a thorough awareness of common household products and their analytical strategies based on samples collected at crime scene. The detailed methodology involving result-oriented outcome will be helpful for a forensic expert in solving crime scene related to such products.

Impact Statement: The study will help in studying the exact chemical profile of pyrolysis products of household objects and improving the analytical strategies and methodologies for better understanding and conclusive outcome of the crime scene. It will also help in making policy regarding crime scene investigation and improving fire debris examination protocols in forensic laboratory.[3]

Abstract Text: This study explores the detection and persistence of ignitable liquid residue traces originating from petroleum products found in household materials. These materials include PET bottles, plastic materials, polyvinyl chloride pipes, win boards, synthetic fabrics, and acetate sheets. The main objective of the study was to investigate thoroughly the chemistry of pyrolysis products that arise during violent fires and how these products interfere with the trace detection of flammable liquid residues at arson crime scenes.[1]

Methodology: The samples of above mentioned products were spiked with 50µL of commercially available ignitable liquid, kerosene, and diesel. The substrates were then set on fire, and once approximately three-fourths of the substrate had burned, the fire was extinguished. The remaining semi-burnt debris was carefully collected in lined airtight metal cans for further analysis using the American Society for Testing and Materials (ASTM) 1618 E-19 method.[2] Solvent extraction (ASTM E1386) and gas chromatography/mass spectrometry was used for the chemical analysis.[3]

Results and Discussion: The results of the analysis revealed the successful detection of ignitable liquid residues in the samples without significant challenges arising from the chemistry of pyrolysis products from the semi-burnt substrates. However, some ignitable liquid-like products were observed in the data, requiring careful handling and adherence to the ASTM 1618 approach to mitigate interferences. It was also noted that a few substrates did not retain ignitable liquid residues, and some residues from the ignitable liquids were not fully identified, suggesting the need for further research in this area.

Conclusion: In summary, this study demonstrates the detection and persistence of ignitable liquid residue traces in household materials, highlighting the challenges and interferences encountered during the trace detection of flammable liquid residues at arson crime scenes. The findings provide valuable insights for forensic investigations and pave the way for future research endeavors. It will also give an insight into improving the analytical strategies and protocols for analysis of fire debris at crime scene to elucidate conclusive insight.

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Fire Debris Analysis; Chromatography; Crime Scene Investigation

F30 The Detection of Accelerants (Petrol and White Spirit) From Cadaver Flies That Fed on Spiked Meat

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WITHDRAWN

F31 The Refinement of a Thermodynamic Model to Explain the Weathering Patterns of Ignitable Liquids on Household Substrates at Elevated Temperatures

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Learning Objectives: After attending this presentation, attendees will understand how evaporative processes (weathering) of ignitable liquids known as weathering change at different temperatures, and how a thermodynamic model can be used to accurately model casework by easily accounting for resistance to mass transfer in different substrates.

Impact Statement: These refinements of the thermodynamic model will allow fire debris analysts to better understand the factors that influence observations in casework, including the temperature of evaporation, the duration of evaporation, and the apparent porosity of the matrix. The model can be used to compute any weathered chromatogram from a chromatogram of a neat liquid sample, which can be very helpful for generating reference chromatograms for comparison to casework samples.

Abstract Text: Analyses employed regular (87 octane) gasoline that was collected and weathered to various extents of weathering ranging from 20-99.5%. Weathered residues were exposed to passive headspace extraction using activated carbon strips (ACS) at 60°C for 18hrs before extraction using pentane and analysis using Gas Chromatography/Mass Spectrometry (GC/MS).

Substrates such as vinyl flooring, drywall paneling, tufted carpet, plastic laminate, and polyurethane (PU) foam were used as substrates for the evaporation studies. These materials are common in residential and commercial properties and frequently appear as fire debris in arson cases. The substrates were spiked with a known quantity of gasoline, which absorbed for a predetermined time of two minutes, and underwent weathering at 210°C for predetermined amounts of time to obtain residues that have been weathered to various extents (e.g., 20-99.5%) before passive headspace extraction. The Total Ion Chromatograms (TICs) underwent retention time alignment before statistical comparison to chromatograms simulated by the refined thermodynamic model. The fitness of the measured chromatograms to the refined thermodynamic model with and without the correction factor was determined using Pearson Product-Moment Correlation (PPMC), Mean Absolute Residuals (MAR), and Root Mean Square Error Predictions (RMSEP).

The original/pure model assumes perfect thermodynamic equilibrium between an ignitable liquid and the headspace and adequately predicts weathered chromatograms with PPMC values on the order of 0.99 between modeled and measured chromatograms. However, in porous substrates, like tufted carpet and PU foam, the weathered chromatograms are significantly less well-predicted because they deviate from ideal behavior. By incorporating one term to account for the resistance to mass transfer in the condensed phase, MARs between modeled and measured chromatograms can be improved from values of ~0.09 for the original model to ~0.01 for the refined model, regardless of the substrate or extent of weathering. By incorporating one term to account for the resistance to mass transfer in the condensed phase, the absolute difference between the predicted and measured extents of weathering shrank from ~15.0% to 1%, regardless of the substrate or extent of weathering.

The resistance-to-mass-transfer term in the refined model provides a physical/chemical foundation for why casework samples may appear to be only slightly weathered, even after prolonged periods at elevated temperatures.

Arson; Gasoline; Fire Debris Analysis

F32 Advancing AI-Enhanced Topic Modeling With BERT in Forensic Text Analysis

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Learning Objectives: This presentation aims to provide attendees with a comprehensive understanding of the application of advanced AI-enhanced topic modeling, specifically Bidirectional Encoder Representations from Transformers (BERT), in forensic science. By showcasing its ability to uncover latent themes and interpret evolving trends, attendees will gain insights into leveraging AI for evidence analysis, fostering interdisciplinary collaborations, and advancing forensic science practices.

Impact Statement: The application of BERT in forensic science offers profound implications for research and practice. By enhancing topic coherence and capturing subtle semantic relationships, BERT enables efficient identification of key forensic topics and emerging research areas. This capability supports evidence-based decision-making, promotes interdisciplinary collaborations, and contributes to advancing forensic science in solving complex cases and ensuring justice.

Abstract Text: The field of forensic science plays a pivotal role in the criminal justice system, requiring a nuanced understanding of its thematic landscape to drive advancements. This study explores the application of advanced topic modeling techniques, specifically BERT and Latent Dirichlet Allocation (LDA), in the analysis of forensic texts.[1-5] Utilizing Artificial Intelligence (AI) and Natural Language Processing (NLP) technologies, our primary objective is to compare these methodologies in uncovering and interpreting latent thematic structures within a corpus of forensic science abstracts.

BERT harnesses state-of-the-art neural network architectures to enhance topic quality by capturing intricate semantic relationships, thereby enabling the discovery of latent themes with heightened precision and interpretability. In contrast, LDA employs a traditional probabilistic approach, which, while established, may struggle with the complexity and specialized nature of forensic texts.

Through extensive evaluations using metrics such as topic coherence and diversity, our findings demonstrate that BERT surpasses LDA in identifying more coherent and diverse topics. Notably, BERT exhibits superior performance in handling the specialized language and nuances prevalent in forensic science literature. This research underscores the potential of embedding-based topic models in enhancing the analytical capabilities crucial for forensic investigations.

The study is structured to first review related works, situating our research within the existing literature, followed by a detailed description of the forensic text dataset employed. We then outline the methodology used to implement and evaluate both BERT and LDA models, presenting a comprehensive analysis of the results. Our findings highlight BERT's ability to effectively detect dynamic shifts in research interests over time, thereby facilitating evidence-based decision-making in forensic science.

In conclusion, our research contributes significantly to advancing forensic science's analytical toolkit through AI-enhanced topic modeling. By leveraging advanced neural network architectures like BERT, our models capture nuanced semantic relationships within forensic texts, paving the way for continual advancements in forensic practice, policy, and education. Future research directions include integrating Large Language Models (LLMs) and lifelong learning techniques to further enhance forensic analysis methods in response to evolving challenges and data dynamics.

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Forensic Science; Topic Modeling; BERT

F33 AI-Enabled Systems and Applications in Forensic Science: A Landscape Analysis and Future Directions

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Learning Objectives: After attending this presentation, attendees will learn about several strategic initiatives by the National Institute of Standards and Technology (NIST) to advance the responsible use of trustworthy AI in forensic science.

Impact Statement: This presentation will impact the forensic science community by providing an overview of a landscape analysis and catalog of current and emerging AI-enabled technologies having potential applications in forensic science. Furthermore, it will present future directions for developing the infrastructure needed to support the responsible adoption of trustworthy AI systems in forensic science.

Abstract Text: AI has become a prominent form of next-generation technologies offering transformative potential across industries and domains, including forensic science. AI-enabled technologies may create opportunities for improved accuracy and reliability of forensic evidence analyses, streamlined operational workflows, and investigative insights across large data systems. Additionally, AI-enabled technologies have the potential to strengthen quality management systems, improve training and education, and advance research and development using its automation and generative capabilities.

Although the emergence of AI-enabled tools for forensic science is nascent relative to other industries, such as health care, customer service, cyber-security, surveillance, finance, and fraud detection, many emerging AI-enabled tools have dual-use capabilities and applications relevant to forensic science use-cases. As such, there has been increasing desire and possibilities for integrating AI-enabled technologies into forensic science. However, use of AI-enabled technologies for high-stakes decisions in sensitive domains, such as criminal justice, requires assurances that they are valid, reliable, and trustworthy. Further, such applications must conform to legal restrictions, privacy requirements, and ethical concerns affecting the design and deployment of AI-systems.

The National Institute of Standards and Technology (NIST) has established partnerships with industry experts and launched a series of strategic initiatives to advance the responsible use of trustworthy AI in forensic science. These initiatives include conducting a landscape analysis and development of a catalogue of current and emerging AI-enabled technologies having potential applications in forensic science; convening AI-technology experts, academia, forensic scientists, attorneys, and others across the criminal justice community to discuss opportunities and challenges to the use of AI in forensic science, including key operational, technical, or legal implications impacting policies, procedure, and practices; and developing a roadmap and the infrastructure needed to support the responsible adoption of trustworthy AI systems in forensic science, including risk management profiles, standards and guidelines, and testing and validation frameworks.

This presentation will provide an overview of the steps NIST has taken to understand the use of AI in forensic science, including an overview of the landscape analysis and catalogue of current and emerging AI-enabled technologies that have potential applications in forensic science and encourage forensic scientists to learn more about how they can take advantage of AI-enabled technologies to improve technical and operational practices.

Artificial Intelligence; NIST; Technology Transition

F34 Artificial Intelligence, Forensic Science, and the Criminal Justice System: New Challenges and Ethical Implications for Innovative Technologies

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Learning Objectives: After attending the presentation, attendees will understand what ethical issues need to be considered and what responsibilities are involved in the use of AI in forensic science and the criminal justice system, including from an anthropocentric perspective.

Impact Statement: This abstract presents a reflection on the ethical, philosophical implications and responsibilities related to the use of AI in the field of forensic science with reference also to the criminal justice system, emphasizing which elements should also be considered for the prevention of possible risks related to the use of innovative technologies.

Abstract Text: What is the possible relationship between AI, the criminal justice system, and forensic science? In the current international panorama, it becomes very complex to provide a definition of AI that can be considered exhaustive, since it is a dynamic concept in continuous transformation, closely related to technological development, which must, however, be harmonized with the protection needs of both the individual and the community, even from an anthropocentric perspective.

AI in general can be defined as a set of systems that exhibit intelligent behavior by analyzing their environment, performing actions with a certain degree of autonomy to achieve specific goals, and it is one of the innovative technologies that is widely used in forensic science.[1] Within the criminal judicial system, on the other hand, AI can be considered as a part of functional technology for the realization of devices which, through the perception of the environment by acquiring data, interpreting structured or unstructured data collected reasoning about the knowledge or subsequent processing of information derived from all acquired data, they are able to identify the best actions to be taken to speed up activities and avoid possible human errors within the jurisdiction, "with the aim of strengthening the certainty of the legal system, through the containment of judicial subjectivism."[2] In developing AI, however, it becomes critically important to examine its ethical and philosophical implications and to develop a set of categorical imperatives, or morale rules, that can be applied universally.

Security, transparency, and ethics are key aspects that must be evaluated by different professionals, who must also operate a control within standardized procedures so that AI is used responsibly, since the use of the AI profoundly impacts privacy, data protection, bias, fairness, and reliability of the system related to it.[3]

Professionals working in various capacities in the field of forensic science must be able to maintain high ethical standards during the use of AI. Ethics, compliance with applicable regulations, collaboration among different professionals, integrity of data of interest, training standardization, human oversight, and the impact on society sustainability constitute essential elements that must be constantly evaluated during the practice of the profession (4) because human critical thinking, the exercise of acquired expertise, can actually be the added value within a highly complex field.[4-5]

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Artificial Intelligence; Ethics; Forensic Science

F35 Lessons Learned From the Development of Sexual Assault Kit Evidence Machine Learning Models

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Learning Objectives: After attending this presentation, attendees will: (1) understand key lessons learned from the development of sexual assault kit evidence machine learning models, and (2) explore future strategies for the application of machine learning models in sexual assault kit evidence selection.

Impact Statement: This presentation will impact the forensic science community and align with the focus of the American Academy of Forensic Science 2025 conference theme by expanding our understanding of machine learning models in evidence selection, specifically from sexual assault kits.

Abstract Text: The findings shared in this presentation resulted from a National Institute of Justice grant (2019-NE-BS-0001) entitled "Assessment of Sexual Assault Kit (SAK) Evidence Selection Leading to the Development of SAK Evidence Machine Learning Model (SAK-ML Model)."[1] The presenters will review the background of utilizing machine learning models for sexual assault kit evidence selection.[1-3] With the increased submission of sexual assault kits to publicly funded crime laboratories, many crime laboratories have moved to a selective swab approach in which swabs are chosen for analysis based on assault history and forensic analysts' experience. The goal of developing a machine learning model to guide swab selection was to automate the process to save time and resources. Yet, several factors negatively impacted the development of a successful machine learning model for implementation in practice. These factors will be explored through findings from three publicly funded crime laboratories and the barriers faced in machine learning model development. The machine learning models developed in this study will be reviewed and the open access links to the models will be shared. This presentation will utilize PowerPoint to facilitate discussion on the promise of machine learning models in sexual assault kit evidence selection and strategies to mitigate barriers.

This presentation will also highlight the importance of collaboration between forensic nurses/examiners who collect sexual assault kit evidence, forensic scientists who select and analyze sexual assault kit evidence, and researchers in developing best practice guidelines and implementing machine learning models.

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Sexual Crimes; Machine Learning; Forensic Analysis

F36 The Application of Artificial Intelligence in Medicolegal Assessments

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Learning Objectives: After attending this presentation, attendees will understand the potential of Artificial Intelligence (AI) in the forensic field.

Impact Statement: This presentation will impact the forensic science community by showing how technology can facilitate the interpretation of complex information, improving the accuracy and efficiency of the assessments.

Abstract Text: AI is a rapidly evolving tool that is revolutionizing multiple fields, including medicine. Using advanced algorithms and machine learning techniques, AI can quickly process large volumes of clinical data, medical histories, and psychometric test results, identifying patterns and correlations that may elude human analysis. In forensic medicine, AI can help draft medicolegal reports. Thanks to its data analysis capabilities, it can interpret complex information more effectively, improving the accuracy and efficiency of the assessments. In assessing damages, AI can more precisely determine the impact of an injury on an individual's life, estimating the future evolution of the damage and care needs, by analyzing socioeconomic, psychological, and medical factors. In forensic psychiatric evaluations, AI can support professionals in the diagnosis and understanding of mental disorders, standardizing the assessments of criminal liability, reducing inter-individual variability, and increasing the reliability of the evaluations. In forensic pathology, AI can quickly and accurately estimate the time of death or hypothesize the weapon used to produce a specific lesion, starting from an image.

We evaluated three different medical-legal cases using ChatGPT and compared its conclusions with those of practitioners. In the first case, AI was asked to evaluate the percentage of permanent biological damage of a man injured in a car crash. In the second case, it was tasked with evaluating the subject's capacity of understanding and will, only on the basis of the mental examination. In the third case, AI was used to estimate the time of death by analyzing the thanatological phenomena. The AI was not trained before.

Case 1: After a car accident, a man suffered head trauma, displaced spinal fracture at D6, and multiple rib fractures resulting in complete paraplegia, neurological bladder, and erectile impotence. AI assessment: 95-98% at first evaluation (Italian's criteria). Upon request for a more precise and cautious estimation, it revised the estimate to 85%. Medicolegal doctor assessment: 80-85%.

Case 2: Cooperative individual during the psychological interview. No ongoing hallucinations but reported past experiences. Poverty of thought content, lack of insight, dysphoric anxiety, fragile personality. Reported sporadic use of cannabinoids. Apathy and abulia. No projects for the future. AI assessment: the person may have a preserved capacity for understanding but a compromised capacity for will, due to his apathy and poor consciousness of the ill's condition and care needs. Forensic psychiatrist evaluation: the person, unfit to plead and incapable of comprehending, is suffering from a severe and persistent psychotic spectrum disorder, currently in remission, which requires constant psychopharmacological treatment.

Case 3: A corpse with a rectal temperature of 35.5°C. Lividity is still developing, blanching after thumb pressure. Temporomandibular joint stiffening, no rigor in other muscles. Normal intraocular tension and no corneal opacity. Ambient temperature of 29°C detected at 6:32 pm on July 25. AI assessment: estimated time of death approximately 3-4 hours before the rectal temperature was taken, therefore around 2:32 m-3:32 pm on July 25. Medicolegal doctor assessment: the time of death can be traced back to 3-5 hours before the time of detection of the cadaveric phenomena.

In the cases presented, the AI's evaluations closely matched those of the medical examiners. AI represents a promising opportunity to improve the effectiveness and fairness of medical-legal assessments, and it's a useful tool in supporting practitioners. However, it is currently unable to replace the doctor's clinical experience and judgment. Its role should be seen as complementary, not as a replacement, to ensure that assessments are complete, accurate, and respect the rights and dignity of the people involved.

Artificial Intelligence; Forensic Psychiatry; Forensic Analysis

F37 The European AI Act: A Risk-Based Approach to Regulating AI

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Learning Objectives: By attending this presentation, participants will gain knowledge of the recent European regulations governing the development and deployment of Artificial Intelligence, justice and forensic fields included. Given the increasingly widespread use of Artificial Intelligence (AI) in the legal field, it is crucial to understand the rules that grant its use only if they comply with given conditions.

Impact Statement: This presentation will impact the forensic science community by making it aware of the main points on which the recent AI Act issued by the European Union to regulate AI is based. An important point is related to risk classification, with applications in the legal field that are ranked as high risk. The presentation will hence focus on those risks and, consequently, on the risk of developing AI systems that might not be compliant with the European Union regulations and might be banned from the European Union.

Abstract Text: AI constitutes a valuable tool also in the forensic field for supporting experts. Similarly to various technologies, the development of AI systems has occurred without any specific regulatory constraint, provided the pursued objectives were legal, and this gap has highlighted the weaknesses of such a self-regulating system without adequate legal limits. The risks that may arise can increase possible violations of fundamental individual rights, so that this technology may be perceived as unreliable in the eyes of the community, consequently instilling fear about its use in certain fields.[1,4].

In order to establish a legal framework that defines the limits of these new technologies, the European Union introduced legislation in 2024 aimed at regulating AI systems, "Regulation (EU) 2024/1689 of the European Parliament and of the Council of 13 June 2024 laying down harmonized rules on artificial intelligence and amending Regulations (EC) No 300/2008, (EU) No 167/2013, (EU) No 168/2013, (EU) 2018/858, (EU) 2018/1139 and (EU) 2019/2144 and Directives 2014/90/EU, (EU) 2016/797 and (EU) 2020/1828 (Artificial Intelligence Act)".[5]

The approach used by the European Union, which mirrors the so-called New Regulatory Approach previously adopted by the European Commission (EC) (product directives that set safety requirements and mandate European Conformity [CE] marking on compliant products), involves identifying different levels of risk for AI applications to ensure that only safe products are placed and used on the market: Technologies that present an unacceptable risk are excluded, without any prohibition on research and innovation activities.

The goal of the European legislator is to ensure the dissemination of AI that conforms to specific predefined standards based on risk levels, which are evaluated in advance by manufacturers/designers (and other similar parties), thereby ensuring their full responsibility; in addition to these parties, further responsible entities, known as deployers, are included. Deployers must ensure the compliance and safety of the AI systems they make available to their users.

The legislative act pays particular attention to high-risk AI systems, which include, "AI systems intended to be used by a judicial authority or on their behalf to assist a judicial authority in researching and interpreting facts and the law and in applying the law to a concrete set of facts, or to be used in a similar way in alternative dispute resolution" (defined in ANNEX III High-risk AI systems referred to in Article 6).

It is therefore necessary to understand the actual impact of this new European regulation on forensic activities: will they need to fully comply with the requirements, potentially impending innovations that could be useful, or will they be granted a greater level of flexibility? Furthermore, it is likely that this regulation will have an impact also on AI systems developed outside the European Union and, in an era of high globalization and digitization, these rules should be carefully analyzed to avoid that useful applications developed outside the European Union are banned inside the European Union.

This presentation aims to explain this new risk-based approach followed by the recent European Union AI Act.

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Artificial Intelligence; European AI Act; AI Risks

F38 Technology: A Tool for Transformation of Data Element Exchange in Specialized Types of Medicolegal Death Investigations

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Learning Objectives: After attending this presentation, attendees will understand the data elements that should be collected by the Medical Examiner's or Coroner's (MEC) office during specialized Medicolegal Death Investigations (MDIs) to include item origination/source during the investigation. The attendees will also recognize the downstream users of all MDI data elements, specifically federal mortality data collection efforts, and tools available to help others understand the impact of the data.

Impact Statement: This presentation will impact the forensic science community by creating greater awareness of data elements to be collected during specialized MDI and where that data originates. This presentation builds on past research and delves into identifying and defining data elements commonly collected by MECs during specialized investigations, where that data originates, and data users. Those in MEC offices will be able to ensure that they are collecting all relevant data points, and data users will be able to recognize if they are getting complete data sets, as well as understanding the primary information source.

Abstract Text: The National Institute of Justice (NIJ), in partnership with its Forensic Technology Center of Excellence (FTCOE) at RTI International and the Center for Disease Control and Prevention (CDC), convened the Medicolegal Death Investigation Data Exchange Working Group (MDI-Data-WG) from 2020-2023. The MDI-Data-WG was formed to advance forensic science and ensure communication between medical examiners, coroners, and other stakeholders. Publicly available reports of MDI-Data-WG efforts were developed relating to data collected and exchanged in MDI, and the initial report focused on core data elements that apply for all investigations. This presentation will focus on more recent guidance for data collection in five types of specialized death investigations (infant, suspected drowning, fall, substance, or firearm), as well as their data exchange workflows.

The MDI-Data-WG was comprised of forensic pathologists, medicolegal death investigators, and other specialized interested parties. The MDI-Data-WG focused on five specialized death investigations and identified the core elements to be collected when a case of that type is suspected to have occurred in a jurisdiction. The WG obtained consensus to define these elements as necessary components of a comprehensive MDI. When nuances of elements were identified, exemplars were provided. Data elements were then integrated into the previously established core data elements list.

To correlate with additional efforts other than map the MDI data flow, the primary high-level source for each data element is identified. Finally, several federal mortality data collection efforts were identified, and each core and specialized data element was mapped to the identified users of that data within those efforts. The identification of data used by a small subset of data collection efforts shows how impactful the use and communication of data collected by an MDI office can be for public health, public safety, and the decedent's family.

Graphical representations were developed to help visually communicate the identified elements. The ongoing standardization of identified data elements can be viewed as necessary for modernized data exchange with public health and public safety entities, including the multitude of forensic practitioners and interested parties that MDI interacts such as researchers, media, organ procurement organizations, and families.[1,2]

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Medicolegal Death Investigation; Data Elements; Coroner and Medical Examiner

F39 The Impact of Automated Asylum Determination Processes on Asylum Applicants Subjected to Acts of Torture

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Learning Objectives: This presentation will consider the impact of automated asylum determination processes including the use of Artificial Intelligence (AI) for those seeking asylum who have been subjected to acts of torture. After attending this presentation, attendees will appreciate that the integration of automated asylum determination processes in a decision-making process that is defined by human vulnerability overlooks the negative consequences such tools may have on not only the asylum applicant's human rights but also the right to a fair and transparent decision-making process.

Impact Statement: This presentation will impact the forensic science community by reinforcing the importance of medical experts within the asylum decision-making process. Medical experts play a key part in assisting asylum decision-makers with determining who qualifies for refugee status, and they should be involved in the design of any forms of technology aimed at automating asylum determination processes. Through greater collaboration amongst the different actors involved in this process, the rights and welfare of those seeking asylum will be safeguarded.

Abstract Text: This presentation seeks to determine whether automated asylum decision-making processes can aid in determining the credibility of asylum applicants who have been subjected to acts of torture and what the risks are of such forms of technology.

A significant proportion of persons seeking asylum have been subjected to acts of torture either in their country of origin or during their onward journey to a host country where they have applied for international protection.[1] Physical traces of torture have been labelled as representing "the last form of truth telling" with asylum applicants required to validate their account of torture through medical evidence, which, at its core, is a "corporal inscription of their persecution."[2] Modern practices of torture, however, often take the form of psychological torture to circumvent the prohibition of torture in international law as such acts leave no overt physical marks.[3,4] According to a systematic review on the prevalence of torture and other war-related traumatic events in forced migrants undertaken in 2016 by Sigvardsdotter and colleagues, at least 27% of refugees and asylum applicants in high-income countries are likely to have experienced torture.[5]

In order to qualify for refugee status, asylum applicants are required to demonstrate that they have "a well-founded fear of being persecuted for reasons of race, religion, nationality, membership of a particular social group or political opinion."[6] For asylum applicants who have been subjected to psychological acts of torture, establishing that their fear is well-founded is a complex hurdle to cross as it requires consideration of subjective fearfulness and the objective validation of that fear.[7] The subjective element imposes an additional burden on those suffering from the psychological effects of torture whether that may be Post-Traumatic Stress Disorder (PTSD), complex PTSD, anxiety, or depression.

Expert systems to automate credibility assessments are being developed using data from previous asylum claims to counter the difficulties faced by state authorities in assessing credibility. Such systems, however, carry an inherent unconscious bias and may impose a larger burden on the asylum applicant to demonstrate that their fear is well founded.[8] These systems risk creating a standardized process that removes the discretionary element of asylum decision-making.[8] As AI systems are designed to replicate human decision-making processes, they are at risk of reproducing prejudiced decisions by perpetuating or amplifying biases.[9] The opacity of such systems further impacts the legality of the decision-making process as human logic remains hidden from the process.[9]

Technology that has the potential to affect the rights and obligations of vulnerable populations must be considered within a human rights framework. Although there is no doubt that AI systems can improve the efficiencies of asylum decision-making, their design and implementation must be carried out with meaningful and human control over such processes. This presentation advocates for medical experts to continue to play an active role in the asylum decision-making process for victims of torture through close collaboration with the other actors involved in determining refugee status. This will help ensure the adoption of transformative forms of technology that promote a fair and just asylum decision-making process that upholds the human rights of asylum applicants.

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Asylum; Artificial Intelligence; Human Rights

F40 Developing Standards and Risk Management Profiles for the Responsible Adoption of Artificial Intelligence in Forensic Science

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WITHDRAWN

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Artificial Intelligence; Standards; Risk Assessment

F41 Improving Polygraph Screening Examinations With Computerized Scoring Algorithms and Minimum Data Standards

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Learning Objectives: Attendees will (1) understand how polygraph test data is evaluated for decisions of truth or deception; (2) better understand the limitations of polygraph related to test formats and issues to be tested; and (3) recognize the value for objectivity in automated computer scoring and test decisions for polygraph.

Impact Statement: Polygraph testing and its reputation is one of the oldest yet most misunderstood of forensic sciences. Far too often polygraph is erroneously described as a "lie detector" with all of the burden that label imposes. Education that illuminates evidence-based practices for polygraph will support a significant increase in appreciation for polygraph as a credibility assessment tool for most investigative purposes. This research provides achievable changes in improving outcomes with polygraph testing.

Abstract Text: In-depth pre-employment screening of police applicants offers significant promise for law enforcement agencies in reducing police misconduct. However, identifying inappropriate past behavior that casts doubt on the applicant's integrity is almost impossible to identify by conventional methods. Falsifying work history, aggressive behavior, sex crimes, and drug use are often completely hidden from public knowledge and discovery. The use of polygraph testing for forensic credibility testing would seem to be ideally suited for identifying negative behaviors. Unfortunately, the National Research Council's 2003 Report rejected the use of polygraph screening exams as being too inaccurate.[1] Thus, law enforcement agencies often limit or avoid the use of polygraph for identifying undesirable conduct by applicants.

Conversely, the American Polygraph Association's (APA) 2012 meta-analytic survey describes research studies supporting mean accuracies in the low 90th percentile for polygraph testing.[2] Reading into the APA's survey, these results were only obtained from single-issue test formats over event-specific issues with enhanced inspection of test data. Inexplicably, the widespread practice for administering a polygraph over non-specific issues for applicants commonly uses multiple-issue test formats, ambiguous relevant test questions, and visual inspection of test data. These practices adversely impact polygraph test accuracy and are incongruent with evidence-based testing practices over specific identified crimes.

This research study utilizes a single-issue test format, the Single-Issue Screening Test (SIST), as an efficient testing method to provide a testing process similar to event-specific crime testing and utilizes computer scoring algorithms for test decisions. The study collected significant numbers of field polygraphs sessions conducted as standardized examinations from a law enforcement agency with a peer-review process for determining truth or deception coupled with an extensive background investigation. These polygraphs sessions were then subjected to automated computer scoring algorithms for concurrence with human evaluators. The computer algorithms utilized have been subjected to two or more validation studies, and the results published in a peer-reviewed journal. This research facilitates an analysis of polygraph data for identifying minimum data quality standards and reduced subjectivity in test decisions.

This research demonstrates increased accuracy for polygraph testing of police applicants that is similar to event-specific crimes. While doubt may linger over polygraph accuracy, Honts, Thurber and Handler 2020 note that arguments against the polygraph test in event-specific crimes are completely lacking in support.[3] However, the Honts meta-analysis notably elaborated on the lack of standards and regulations in the polygraph profession. The criticisms in the Honts study on lack of standards are applicable to currently observed polygraph testing of police applicants. This current study directs the polygraph profession and law enforcement agencies to adopt the same evidence-based practices used in event-specific polygraph testing for credibility assessment testing of police applicants. In so doing, the concerns of the NAS 2003 Report can be rejected, and the desired outcome of reducing police misconduct can be realized.

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Police and Forensic Psychology; Screening Methods; Fitness for Duty Evaluation

F42 The Benefits and the Challenges of an Independent Crime Lab

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Learning Objectives: In 2012, the executive leadership of Washington, DC, established the Department of Forensic Sciences and moved the Crime Scene Sciences Unit from the Metropolitan Police Department to the newly established independent crime laboratory. After attending this presentation, participants will learn how this transition occurred, the advantages of a civilian crime scene unit, and the challenges faced during the transition.

Impact Statement: The presentation will impact the forensic science community by providing a model to separate a crime scene unit from a law enforcement agency and incorporating it into an independent crime laboratory, a step toward mitigating potential bias.

Abstract Text: In 2012, Washington, DC, pioneered a significant shift in forensic practice by establishing the Crime Scene Science Unit (CSSU) under the Department of Forensic Sciences. This move, driven by the mayor's vision, aimed to separate forensic analysis from traditional law enforcement, challenging the United States norm where police officers typically collect forensic evidence.

The CSSU's transfer to the newly established Consolidated Forensic Sciences Laboratory (CFL) marked a new era in forensic effectiveness. The CFL integrated various forensic units, including the CSSU, Forensic Sciences Laboratories, Office of the Chief Medical Examiner, and Public Health Laboratory, into a single technologically advanced facility.

This transition aimed to create an independent crime scene science laboratory, mitigating potential bias concerns. The process involved implementing advanced scientific technologies, recruiting skilled civilian staff, and developing and implementing quality measures. Crime scene scientists underwent training and competency testing on various technologies, including cyanoacrylate fuming chambers, alternative light sources, advanced cameras, and 3D scanning systems.

The newly formed unit was not without challenges. It took years to reach full staffing, secure the appropriate resources, and build a professional relationship between the Metropolitan Police Department and the Department of Forensic Sciences proved complex. The conversion of the crime scene response team from a law enforcement unit to a civilian unit met several personnel challenges.

The CSSU's establishment provides a model for creating an independent crime scene science unit separate from law enforcement agencies. This presentation aims to share insights into the transition process, highlighting the advantages of an independent unit and discussing the challenges faced and how to avoid them.

Despite ongoing challenges, the CSSU continues to uphold its mission with professionalism and efficiency. This paradigm shift represents a significant step toward enhancing the credibility and effectiveness of forensic science in criminal investigations, potentially influencing future forensic practices across the United States.

Crime Laboratory; Crime Scene Investigation; Technology Transition

F43 The District of Columbia (DC) Department of Forensic Sciences: A Fall From Grace and a Path Back to Accreditation

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Learning Objectives: After attending this presentation, attendees will learn how an accredited forensic science laboratory lost its accreditation and the steps taken to become reaccredited. The presentation will highlight the many challenges the laboratory faced in regaining accreditation as well as lessons learned.

Impact Statement: This presentation will impact the forensic science community through presenting a model that can be followed by other laboratories working to regain accreditation and rebuild trust among their stakeholders.

Abstract Text: On April 2, 2021, the DC Department of Forensic Sciences (DFS) received a letter from ANSI National Accreditation Board (ANAB) stating, "ANAB has received credible evidence that the DC Department of Forensic Sciences, Forensic Science Laboratory Division, has deliberately concealed information from the ANAB assessment team, violated accreditation requirements, engaged in misrepresentations of fraudulent behavior, and engaged in conduct that brings ANAB into disrepute." In May 2021, ANAB fully withdrew accreditation and DFS stopped all casework in the Forensic Biology, Forensic Chemistry, Latent Fingerprint, Digital Evidence, and Firearm Examination Units. In December 2023, ANAB performed an assessment of DFS' Quality Assurance System, Forensic Biology Unit, and Forensic Chemistry Unit and found zero non-conformances, fully accrediting these units. During the two-and-a-half years between the loss and regaining of accreditation, DFS underwent drastic management and cultural changes.

The first step to rebuild the agency was a full-scale audit by an external vendor. The use of an external vendor was key to mitigating internal bias as well as build confidence with stakeholders. The audit resulted in a written report detailing both identified non-conformances and recommendations to strengthen the agency. The report was made publicly available, and DFS' stakeholders hired independent consultants to review the report. Although the review of the report by consultants was not driven by DFS, the process began to establish trust in the rebuilding of the agency.

During the first-year post-loss of accreditation, the District administration opted to replace DFS executive leadership and, in consultation with the new leadership, to eliminate the Firearm Examination Unit and completely revamp the Latent Fingerprint Unit. DFS relied on an external expert, an established leader in the latent print field, to adopt new approaches that conformed with best practices. Also, DFS instituted a requirement that all upper-level analysts be certified practitioners.

Furthermore, DFS utilized an external consultant to assist with the rebuilding of the quality and operational systems. The consultant conducted an external audit following International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025 detailing all findings and providing recommendations to address the findings. Subject matter experts worked with the forensic biology and chemistry units to prepare staff for the ANAB assessment.

To further build confidence in the laboratory, DFS provided its stakeholders, upon request, access to operational documents, standard operating procedures, training manuals and records, validation studies, and validation data. As a final effort to rebuild trust, DFS has fully embraced the DC Science Advisory Board by welcoming the members and committing to full transparency.

Accreditation; Quality Management System; Systemic Review

F44 Washington, DC, Department of Forensic Sciences (DFS): A Unique Approach to Developing Impactful, Strategic Testing Plans While Minimizing Cognitive Bias

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Learning Objectives: DFS has created a model to stakeholders' testing requests that helps mitigate contextual bias, streamlines external communication, and ensures appropriate use of laboratory resources. This model can be applied in other forensic laboratory systems. After attending this presentation, the participants will learn the unique approach DFS has taken to receive and process forensic analysis testing requests that protect against contextual bias as well as the perception of it.

Impact Statement: The impact of the presentation on forensic science is to educate laboratory practitioners on the DFS approach that creates a degree of separation between the analysts and the stakeholder while ensuring appropriate use of the laboratory's resources and is a model that can be followed in other jurisdictions.

Abstract Text: The DFS Forensic Science Laboratory (FSL) consists of three technical units, Forensic Biology, Forensic Chemistry, and Latent Fingerprint, as well as one non-technical unit, the Forensic Intelligence Unit (FIU). The FIU is a team of intelligence analysts who work closely with stakeholders to develop strategic forensic testing plans.

DFS receives forensic analysis requests from local law enforcement agencies, the United States Attorney's Office, and the Office of the Attorney General via an online Request for Testing form. The form has been designed to obtain necessary information to build strategic testing plans while limiting the submission of superfluous details that may cause or create the perception of contextual bias.

Once a Request for Testing form is received, an FIU analyst reviews it to ensure the testing request meets the DFS Testing Submission Guidelines. Then, the analyst ensures the evidence being targeted for testing is the best evidence available to answer the questions being posed, limiting testing on items that are unlikely to result in impactful, scientifically sound results. FIU analysts use success rates reported by the technical units to guide this approach. Finally, the analysts review any time constraints the requestor communicates to ensure the laboratory is prioritizing testing appropriately. When a case or testing request is highly complex, the FIU analyst will coordinate a meeting between the requestor and the technical unit manager to ensure the best testing plan is developed.

The value of having the FIU analysts serve as an intermediary between a requestor and the technical unit is three-fold. First, there is a degree of separation between the analyst and the requestor, which helps mitigate contextual bias as well as the perception of contextual bias. Second, there are fewer points of contact for external stakeholders, streamlining communication. Third, having a unit focused on receiving and navigating testing requests frees the analysts to conduct the testing.

In summary, DFS has created a model to stakeholders' testing requests that helps mitigate contextual bias, streamlines external communication, and ensures appropriate use of laboratory resources. This model can be applied in other forensic laboratory systems.

Crime Laboratory; Quality Assurance; Casework

F45 The National Institute of Justice Research and Development Program

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Learning Objectives: Audience members will learn about new research projects and resources being funded by the National Institute of Justice (NIJ). Participants will also learn: (1) how they can get involved with NIJ initiatives and working groups, (2) how to apply to NIJ solicitations, and (3) how NIJ supports forensic science through federal partnerships.

Impact Statement: This presentation will impact the community by disseminating knowledge about federal resources available to researchers and practitioners.

Abstract Text: 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.[1] As the lead federal agency for forensic science research and development, NIJ, through the Office of Investigative and Forensic Sciences (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.[2] This presentation will discuss NIJ program updates, including an overview of our new awards, and communicate how conference participants, including practitioners and academics, can get involved in research initiatives to improve forensic science. The goal of NIJ is to inform the forensic enterprise about research trends, technologies, partnership opportunities, and federal efforts in hopes that the knowledge leads to increased forensic laboratory capabilities and collaboration.

NIJ will discuss significant projects developed by the Forensic Technology Center of Excellence, the National Center on Forensics, and working groups such as the Forensic Laboratory Needs Technology Working Group and Medicolegal Death Investigation Working Group. In addition, NIJ will provide updates about upcoming projects such as the Needs Assessment of Forensic Laboratories, Medical Examiner, and Coroner Office. OIFS will also present on its collaborations with the National Institute of Standards and Technologies, the National Science Foundation, and other components within the Office of Justice Programs.

Finally, this presentation will demonstrate how NIJ is seeking to advance a culture of research to advance forensic science. The mission of 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. NIJ invites feedback from the AAFS community on ways our agency and partners can further strengthen the current and future practice of the forensic sciences.

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Research; Grants; Crime Laboratory

F46 A Photograph of a Partially Skeletonized Finger Confirms Positive Identification: A Case Study

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Learning Objectives: After attending this presentation, attendees will gain practical insights into using digital photographs for fingerprint analysis to positively identify deceased individuals in medicolegal death investigations. Through a detailed case study, attendees will understand how investigators navigated the complexities of advanced decomposition and employed cutting-edge technology to achieve rapid and accurate identification.

Impact Statement: This presentation will impact the forensic science community by illustrating the practical application of recent advancements in fingerprint analysis within medicolegal death investigations. This case study will detail how investigators successfully identified a 40-year-old male, despite advanced decomposition, by utilizing a digital photograph of a partially usable fingerprint.

Abstract Text: Investigators in Tippecanoe County, IN, responded to a call regarding the discovery of a deceased 40-year-old male in his van on a seldom-used service road behind a business. Upon arrival, the investigators observed that the body exhibited advanced decomposition, likely accelerated by rodent and insect activity. The stages of decomposition varied, with the head, face, arms, and hands showing the most advanced stage of skeletonization. This level of decomposition rendered visual identification impossible, necessitating alternative identification methods.

Fingerprint analysis emerged as the most efficient method available, though there were concerns about obtaining a viable print. During the autopsy, one finger was found to have a partially usable print. A digital photograph of this fingerprint, taken with scale, was promptly submitted to the Indiana State Police Laboratory for analysis. Within 24 hours, the results confirmed the decedent's identity.

Advancements in fingerprint analysis over recent years have transformed the identification process. Formerly reliant on physical inked fingerprints, the process now often requires only a digital photograph with scale. This innovation has significantly expedited the positive identification process, reducing the time and resources needed for physical handling and submission. Such developments not only enhance the efficiency and accuracy of medicolegal death investigations but also serve as a compelling example of how modern technology continues to elevate forensic practices.

Death Investigation; Decomposition; Fingerprint Identification

F47 Overlooking the Utility of the Tattoo as an Investigatory Lead: Comparing Tattoo Documentation Practices Among Investigators and Medical Examiners

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Learning Objectives: After attending this presentation, attendees will be informed about how tattoo documentation practices vary significantly between agencies. Attendees will become aware of how uniform and detailed reporting of tattoos can be valuable for providing investigatory leads in cold cases, missing persons cases, and unidentified remains cases.

Impact Statement: This presentation will impact the forensic science community by educating forensic science professionals about the importance of tattoos as traces—when properly identified and documented, tattoos are valuable as investigatory leads in both criminal and non-criminal investigations. This presentation will demonstrate not only how tattoo documentation is inconsistent across agencies and jurisdictions, but also how overlooked and underused the tattoo is as an investigative tool. Attendees will gain a better understanding of the need to create a systematic method for tattoo documentation that is consistent across forensic science and the broader criminal justice system.

Abstract Text: Tattoos have not only served as a subject of interest across cultures and disciplines for centuries, but also serve as a critical component of forensic investigations. By examining the location, dimensions, colors ,and overall imagery of a tattoo, investigators have been able to use tattoos as a method of rapid, tentative identification of human remains.[1] Although tattoos have proven to be useful in a wide variety of cases and contexts, they can be exceptionally useful in cold cases in particular since they allow for the generation of investigatory leads when other traces are unavailable or insufficient for identification. Cold cases, which are defined as cases in which all efforts have been exhausted by investigators thus rendering the case inactive for a period of time, can remain inactive for periods ranging from weeks to decades.[2] These cases have always been a demanding concern to law enforcement and remain so as the number of unsolved cases increases by 6,000 each year.[3] For centuries, it has been a difficult task to find strategies to solve cold cases; however, through the use of academic collaborations with individuals such as criminalists, forensic anthropologists, and forensic psychologists, it is possible to develop new investigatory leads on many cold cases.[4] Through the use of forensic experts, especially those specifically focused on tattoos as traces, police investigators and forensic scientists can work together to identify solutions to cold cases, specifically those pertaining to missing persons and unidentified remains.[4]

To address the issue at hand, this presentation will compare data collected from redacted records provided by the Camden County Prosecutor's Office along with data collected from public databases such as the National Missing and Unidentified Persons System (NamUs) and state police agencies across the United States. The redacted records provided by Camden County Prosecutor's Office such as crime scene reports, medical examiner/autopsy reports, police reports, and photographs were examined to obtain references referring to tattoos. The records were focused on missing persons, unsolved, and cold cases. In addition to demographic information, each case with a reported tattoo was evaluated to assess the type and quality of tattoo documentation along with whether or not there are discrepancies across different reports. Each instance of a reported tattoo was assessed for its potential value as an investigatory lead; this was done in an attempt to provide investigators with information that may shed new light on open cases.

Thus far, there is no mandated method for tattoo documentation for crime scene investigators or medical professionals, which has caused inconsistencies to arise both inter- and intra-agency. Upon analyzing the legal reporting procedures for these agencies, issues with tattoo documentation formalities (e.g., lack of space to document on reports) were revealed, which led to a lack of documentation in general. Additionally, preliminary analysis of the data collected indicated that there are significant reporting discrepancies of tattoos among police reports, medical examiner reports, prosecution files, and photographs. Primary discrepancies included differences in descriptions of tattoos, number of tattoos, and the general reporting of whether or not there is a tattoo present. Additional data (e.g., age, sex, condition of the body, color of tattoos, quality of tattoos, and region of body tattooed) was also analyzed to generate statistics on tattoo data collection procedures. All data collected was thoroughly evaluated to assist in generating a systematic method for tattoo documentation to eliminate discrepancies moving forward with the ultimate goal of recognizing the value of tattoos in providing investigatory leads in criminal and non-criminal cases.

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Tattoo; Cold Case; Generalist

F48 A Study in Distortion on Determining Latent Print Classification

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Learning Objectives: After attending this presentation, attendees will understand the impact that common types of distortion can have on common classification issues by latent print examiners and Automated Fingerprint Identification System (AFIS) operators.

Impact Statement: This presentation will impact the forensic science community by examining the data results of this study, including erroneous classification for AFIS databases and potential false exclusion or inclusion of contributors.

Abstract Text: Fingerprints have features that are quite useful when it comes to identifying humans. The premises of friction ridge skin identification include the proliferation of friction ridge detail and uniqueness of the orientation and spatial relationship of features in the print. Fingerprints can be systematically classified despite their unique finger ridge features because they have similar patterns in their overall ridge flow. Historically, there have been a variety of systems designed to classify fingerprints. This study did not involve these systems, but it is important to note that fingerprints can be generally classified as arches, loops, and whorls. This general classification is commonly used by latent print examiners for AFIS, database search parameters, and comparing prints side-by-side. This level of classification is commonly known as Level 1 Classification and looks at the general friction ridge flow. Level 1 Classification cannot be used to individualize fingerprint patterns, but it can be used to exclude or include prints for further searching.

Generally, the best latent prints are those with minimal distortion and maximized surface detail. Distortion can occur due to a variety of issues such as the elasticity of the friction ridge skin, pressure, surface texture, or movement of the finger or surface. There are several different types of distortion that can occur together, including circular distortion, double tap or overlaid, smeared/smudged, and pressure. Circular distortion occurs when the finger moves in a circular fashion while in contact with a surface. Double tap or overlaid distortion occurs when areas of fingerprint impressions are overlaid with additional impressions of either the same fingerprint or different fingerprints. Smeared/smudged impressions are created when the friction ridge skin moves laterally while the impression is deposited. Oftentimes, this can result in a significant portion of the pattern to either be missing or smudged.

Distortion is an important area of research because latent prints found at crime scenes can often be distorted, which may lead to misclassification errors. Latent print examiners are the professionals who examine and classify latent prints found at crime scenes. The most common method used to examine latent prints is the Analysis, Comparison, Evaluation-Verification (ACE-V) method, which describes the steps for examination in order: analysis, comparison, evaluation, and verification. It is postulated that if a print can be misclassified due to distortion, it can interfere with AFIS database search results and potentially result in suspects being excluded or innocent people included on a suspect list.

In this study, we evaluated whether specific distortion of latent fingerprint impressions result in misclassification of prints by certified and uncertified latent print examiners. Fifty latent fingerprint impressions were created and developed that contain various forms of distortion. Forty-five of the prints were control prints and five were loop classifications. The five test-prints were intentionally distorted using circular, pressure, and double tap or overlaid distortion. This was performed in a very simple and repeatable manner that could be found naturally at a crime scene. This resulted in a different pattern that appeared to be a double loop whorl.

With Institutional Review Board (IRB) approval, an approved survey was distributed to certified and uncertified latent print examiners. The survey asked for consent, non-identifying demographic information, and asked the latent print examiners to classify the 50 fingerprints. Each participant was allowed to fill out the survey once. A total of 102 individuals participated in the study, and the results provided robust data that indicates distortion can have a significant impact on classification determination.

Fingerprint Identification; Fingerprint Distortion; Fingerprint Examination

F49 DNA Identification: Lessons Learned From the Largest Mass Casualty Event in Israel's History

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WITHDRAWN

F50 Seasonal Patterns of Fly-Associated *Ignatzschineria* in Human Decomposition-Impacted Soils

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Learning Objectives: After attending this presentation, attendees will gain an understanding into seasonal abundance patterns of the fly-associated bacterium *Ignatzschineria* in human decomposition and how these seasonal differences might affect developing microbial models for time-since-death estimations.

Impact Statement: This work improves our understanding of decomposition-induced soil microbial community assemblages and their successional patterns by focusing on the seasonal contributions of fly-associated microbes, thereby providing a means to refine the predictive capability of microbial models for estimations of the Postmortem Interval (PMI).

Abstract Text: Human decomposition results in a pulse of decomposition products, producing widespread changes to soil chemistry and microbial community assembly and succession.[1,2] Season influences both decomposition rate and the magnitude of nutrient deposition, which in turn drives a proportional response in soil chemistry and microbial communities.[3] Insects present during decomposition are another source of microbes; of these, the fly-associated *Ignatzschineria* is the most commonly reported in microbiome data and was recently recognized as a potentially substantive contributor to PMI models.[4] The contribution of insect-specific microbes has the potential to be unique compared with soil and host microbiomes and thus affords the opportunity to tie forensic entomology-based data directly to forensic microbiome datasets.

The goal of our study was twofold: (1) to explore the relationship of fly-associated *Ignatzschineria* across seasons where insects were present in high quantity (spring/summer) and low quantity (winter), and (2) to determine if sampling methodology resulted in consistent identification of *Ignatzschineria* in surface swabs of human donors and in soil samples. Two year-long surface human decomposition trials were conducted at the University of Tennessee's Anthropology Research Facility, Knoxville, TN. A total of six (n=3 per season) donated human subjects (hereafter: donors) were simultaneously placed at the facility in direct contact with soil not previously used for decomposition experiments. Sampling was performed at 20 (spring) and 19 (winter) time points over a year and consisted of skin surface swabs and soil samples taken directly underneath donors at two depths: 0-1cm (interface), and 1-16cm (core).

In the spring study, fly eggs were present in nasal and oral cavities within 24 hours. Relative abundances of *Ignatzschineria* reached a maximum of 16.0 ± 4.2 percent relative abundance based on 16s rrna gene amplicon libraries in interface soils (0-1cm) on day 8 (164 Accumulated Degree Days [ADD]), falling to 4.8 ± 5.8 percent by day 16 (346 ADD), and below one percent thereafter. These increases coincided with the movement of decomposition fluid into the soil. In core soils (1-16cm), *Ignatzschineria* only exceeded one percent of sample relative abundance on day 8. During the winter study, minimal insect activity was present, with only a few larvae observed. *Ignatzschineria* was detected at >1% relative abundance on days 55 (495 ADD) and 75 (822 ADD) in interface soils, and in skin swabs at donor placement (day 0, 0 ADD).

This work demonstrates that *Ignatzschineria* abundance dynamics support previous findings that seasonality plays a role in determining microbial successional patterns and offers new insights into sampling methodologies for forensic microbiome modelling. This work also corroborates the viability of *Ignatzschineria* as a modeling organism during early decomposition and highlights the potential benefit of coupling entomology and microbiome datasets to refine time-since-death estimates.

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Decomposition; Soil Microbiome; Forensic Entomology

F51 A Preliminary Study: The Application of 3D Models, Simulations, and Finite Element Analysis (FEA) in Shooting Reconstruction

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Learning Objectives: After attending this presentation, attendees will receive a more developed understanding of how 3D models can be used in firearms analysis for shooting reconstruction. Attendees will also learn about terminal ballistics in materials typically encountered in shooting scenes, simulation software applications in reconstruction, and modeling software applications.

Impact Statement: This presentation will demonstrate the utility of 3D modeling and FEA when not constrained to merely an engineering context, but as applied within the forensic sciences. When FEA is utilized in forensic trajectory analyses, more objective methods will be used to support interpretations, and it will act as a way to visualize evidence as per the needs suggested by the National Institute of Justice (NIJ) Strategic Plan under the Priority I objectives.

Abstract Text: Current research in the firearms examination of shooting reconstruction emphasizes error rates, bullet deflection, and evaluation of current measurement techniques (e.g., probing and the ellipse method).[1,2] Current measurement techniques are directly impacted by the experience and training of the examiner, which can drastically change the outcome of the estimated trajectory. However, while examiner error is a concern, it is not the only issue that requires attention. Currently, a significant knowledge gap exists in the reconstruction of the trajectory of the bullet and the effects of terminal ballistics.

Terminal ballistics is an often-forgotten subcategory of ballistics in terms of research. The interaction between the target and the bullet can provide important information to the examiner. The resulting appearance of the target can direct assessments regarding ricochets, grazing, Gunshot Residue (GSR), or potential deflection upon bullet impact. Ricochet and grazing events typically happen due to scene geography or extremely small impact angles.[3] Understanding when and how these conditions occur may give insight into the reconstruction of the shooting event. The presence of visible GSR may indicate that the shot was fired from a relatively close range. Such conditions will allow for distance determination techniques to be applied.[4,5] There may even be potential damage or some abnormality in the bullet hole that could suggest deflection occurring within the target. Generally, the geometry of the bullet hole can aid in the determination of the caliber of the bullet. However, when less rigid targets (e.g., drywall and glass) are damaged such that important angular information is unobtainable. While providing some information regarding impact angles, examiners will need to perform laboratory testing to develop an understanding of the events. This is neither cost-effective nor time-efficient for an examiner.

Our central hypothesis is that simulated impacts will be sufficiently realistic to provide a direct comparison between the measured angular information and known deformation information. Given improved accuracy in the assessment of the impact angles, improved trajectory reconstructions will be obtained.

FEA is the application of the finite element method and is used in many types of simulations. This method uses mathematical equations to break more complex systems into smaller pieces, called elements. It applies differential equations to each element to solve the problem at hand.[6] FEA can be used for terminal ballistics applications to develop a fundamental understanding of bullet-target interactions, their measurement, and the influence thereof on shooting scene reconstruction. Empirical testing of these interactions was completed using a Ruger SR9 and compared to simulated shots at the same impact angles. Sixty-four test fires on plywood targets at a variety of azimuthal and vertical angles were collected. The same angles were repeated in the simulations. The bullet and target interactions were visualized using ANSYS Workbench, a commonly used FEA software package. The impact angle measurements of the empirical tests and simulations were directly compared.

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Ballistics; 3D Technology; Reconstruction

F52 A Couple of Air Fresheners Ought to Do the Trick! A Case Report on Human Remains Found in an Occupied Residence After Ten Years

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Learning Objectives: The first goal of this presentation is for attendees to understand the forensic process for identifying remains discovered long after death. Attendees will gain insight into the forensic procedures and methodologies used to identify skeletal remains that have been skeletonized and undetected for an extended period. This includes the role of anthropological examination, toxicology, and the use of circumstantial evidence in establishing the identity of the deceased. The second goal of this presentation is for attendees to recognize the implications of long-term undetected deaths on forensic investigations. Attendees will learn about the challenges and implications associated with cases in which a decedent's death remains unnoticed for several years. This includes exploring issues related to welfare checks, family communication, and the impact of such cases on forensic and legal processes.

Impact Statement: The discovery of remains in an occupied residence after over a decade of undetection highlights critical gaps in welfare monitoring and family communication. This case underscores the profound impact of unaddressed isolation and the challenges forensic professionals face in identifying and investigating long-term undetected deaths. It serves as a stark reminder of the need for improved systems to ensure regular welfare checks and effective communication within families to prevent similar situations. The findings also emphasize the importance of interdisciplinary collaboration in forensic investigations, combining anthropological analysis, toxicology, and circumstantial evidence to piece together the truth in complex cases.

Abstract Text: On June 1, 2018, a perplexing case of long-term undetected death emerged when local police were summoned to investigate suspected human skeletal remains discovered in an occupied residence. The investigation revealed that the skeletal remains had been situated in a bed within the residence for over a decade. The scene, a one-story single-family home, was accessed following a welfare check initiated by the homeowner's daughter, who had not been in contact with her father for several months. After failing to reach him by phone or at the door, the daughter's husband observed the remains through a window, prompting a 911 call.

Upon arrival, the police observed that the front door had to be forcibly opened to gain entry. Inside, they found the homeowner alive but unresponsive to inquiries about the remains. Consequently, a search warrant was obtained and executed, leading to the discovery of the skeletonized body in a bedroom. The body was found clothed in a red sweater, t-shirt, blue jeans, and white socks, with no visible signs of trauma or injury.

The examination at the Montgomery County Coroner's Office (MCCO) and subsequent forensic anthropological assessment revealed that the body had been in a state of advanced decomposition and skeletonization. Despite extensive analysis, positive identification was primarily achieved through circumstantial evidence and the matching of prescribed medications found in the residence. The presence of surgical implants in the remains (screws in the right proximal femur and wires in the left patella) supported this identification, despite the absence of known medical history or next of kin.

Attempts to locate the decedent's family proved unsuccessful, as the emergency contact information from the decedent's pre-arranged funeral paperwork either led to deceased contacts or could not be traced. The remains, which exhibited signs of mummification and insect activity, did not show any signs of trauma or foreign bodies upon X-ray examination.

The investigation underscores a rare and unsettling case where an individual's death remained unnoticed for an extended period, highlighting significant gaps in welfare monitoring and family communication. The forensic process included a detailed analysis of the remains and circumstantial evidence, leading to identification despite the absence of direct familial or medical documentation. This case presents a poignant example of the complexities involved in forensic identification and the challenges faced when addressing long-term undetected deaths.

Skeletal Remains; Scene Investigation; Entomology

F53 Cigarette Butt DNA Solves a 30-Year-Old Homicide Cold Case: A Case Study

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Learning Objectives: After attending this presentation, attendees will understand the crucial role of DNA technology in solving cold case homicides. The presentation will also demonstrate how technological advancements over time have significantly enhanced the capabilities of evidence testing, allowing investigators to revisit and solve cases that were once considered unsolvable.

Impact Statement: This presentation will impact the forensic science community by demonstrating how advancements in forensic DNA technology played a vital role in solving a 30-year-old homicide cold case with minimal forensic evidence. This presentation will highlight the transformative power of modern DNA technology in forensic investigations, emphasizing its importance in overcoming challenges associated with old and difficult cases.

Abstract Text: On July 16, 1980, investigators were called to a hotel in Lafayette, IN, for a report of a 49-year-old male who had been robbed and fatally stabbed in his hotel room. A cigarette butt recovered at the scene was collected as evidence at that time and sent to the Indiana State Police Laboratory for saliva testing. However, the technology available in the 1980s could only determine that the saliva on the cigarette belonged to someone with ABO Type A, while the victim was known to have ABO Type O. Unable to conduct further tests at the time, the cigarette butt was returned to the investigating agency and stored as evidence. Investigators were not able to find any leads or make an arrest, and the case went cold for the next 30 years.

That is until 2009, when a Detective Lieutenant with the Tippecanoe County Sheriff's Office found an unmarked box in the evidence room and inside was the decades-old cigarette butt. Knowing that advancements in forensic DNA technology had been made, the detective reopened the case and sent the cigarette butt to the state lab to be retested. This time, a DNA profile was found that was consistent with a potential suspect who was currently incarcerated in Texas. Detectives traveled to Texas to interview the suspect and extradite him back to Indiana for trial. The suspect ultimately pled guilty to voluntary manslaughter charges and was given an 18-year sentence.

Unfortunately, the Detective Lieutenant did not live to see how advancements and utilization of forensic sciences solved this 30-year-old cold case, as he passed away from pancreatic cancer just before the case was adjudicated. However, his legacy was honored by his widow, a fellow police officer and deputy coroner, who was there to escort the murderer out of the courtroom following the conviction.

At this point, his widow believed that her involvement with this case had finally come to an end. However, in June 2024, after serving his time, the murderer was discovered deceased at his residence. In accordance with Indiana law, this case required the involvement of the Coroner. Ironically, the Coroner at that time was none other than the widow of the Detective Lieutenant who had solved the case. This development offered her the unique opportunity to see her husband's case come full circle, right to its conclusion.

This case exemplifies the value of preserving evidence and the significant impact of technological progress in achieving justice, even decades after a crime has been committed. The successful attainment of a conviction in this case would not have been possible without modern advancements in technology over time.

Cold Case; Homicide; Forensic DNA

F54 In the Wrong Place at the Wrong Time

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Learning Objectives: After attending this presentation, attendees will gain knowledge and understanding regarding the difficulties of processing a desolate outdoor crime scene and the investigative complexities of working a missing person(s) case turned into a homicide investigation. In addition, attendees will gain knowledge and understanding of the difficulties of working on a multi-jurisdictional case involving two different police agencies.

Impact Statement: The information will impact the forensic science community by providing an understanding of the complications outdoor crime scenes present and the difficulties of locating potential evidence in this environment. Additionally, the forensic science community will learn about the difficulties of working a scene involving separate law enforcement agencies.

Abstract Text: In early January 2007, a young couple was referred to a mechanic's shop in Oak Cliff, a neighborhood of Dallas, TX, to have their vehicle repaired. They drove a family vehicle to pick up their repaired vehicle, and that was the last time anyone heard from or saw them again. The families reported the young couple missing when they failed to show up for a party later that evening and were unable to reach them. When police arrived at the mechanic's shop, the family vehicle was found parked on the street near the shop with the driver door wide open, cellphones on the front seat, and keys in the ignition. After working on the case and questioning the mechanic, in early February 2007, it was eventually revealed that the young couple had likely been the victims of foul play.

It was discovered that the mechanic bought and sold narcotics to individuals associated with a mid-level drug cartel, and that he and a group of individuals robbed the home of a drug dealer, resulting in a number of guns and large amounts of drugs and money being stolen. The drug dealer stated he would reward anyone who brought him the individuals responsible for this robbery. The mechanic told the drug dealer he knew who was responsible for the robbery as he was repairing their vehicle. As soon as the young couple showed up to pick up their vehicle, they were abducted and taken to a separate location where they were questioned about the robbery of the guns, drugs, and money. When they weren't able to provide answers, their abductors tortured and beat the young couple until they died.

A search team consisting of K-9 handlers from surrounding law enforcement agencies eventually located the deceased couple in a desolate area in an unincorporated section of Dallas County. The female victim, who was pregnant, had been sexually assaulted and beaten. The male victim had been beaten and tortured.

The location where the victims were located was a desolate area under a bridge along Dowdy Ferry Road and the Trinity River. The victims had been thrown over the bridge to the ground below, which consisted of nothing but leaves, branches, and low-lying grass. A search for evidence produced very little, other than the victims themselves. This is an important factor because anything not a part of the natural environment may be considered potential evidence, and thus, a more in-depth search is necessary. The search of an outdoor crime scene in a desolate area requires the use of more methodical techniques (grid search, multiple people, etc.) not to mention the potential for loss, contamination, and damage to potential evidence in a short period of time as a result of exposure, weather, and/or animal activity.

The other issue with this particular case was that it involved two different police agencies. The original abduction and beatings/torture occurred in the City of Dallas, whereas the location of the deceased victims was in unincorporated Dallas County. Both the Dallas Police Department and the Dallas County crime scene sections responded to the location, with Dallas County taking the lead. When it was realized that these were the victims missing from the city of Dallas, the county crime scene unit left the crime scene partially processed, which made completion of the scene all the more difficult.

Ultimately, two of the three individuals were apprehended shortly after the bodies were discovered and convicted of murder. In 2012, the third suspect was apprehended attempting to cross the border and in 2016, a plea deal was reached resulting in a 15-year prison sentence.

Crime Scene Investigation; Violent Deaths; Outdoor Crime Scenes

F55 When All You Have Is a Foot

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Learning Objectives: The goal of this presentation is to discuss an unusual, long-term, unidentified remains case, the work done to identify the individual, and lessons learned once the investigation was complete.

Impact Statement: This presentation will impact the forensic science community by analyzing a single body part case, showing that, while it is preferable to have as many remains as possible, a whole body is not needed to determine identity and confirm the death of an individual. This presentation will discuss the investigation into the death, the identification, and the missing persons investigation, tying everything together.

Abstract Text: On December 16, 2022, after a day of heavy rain and flooding, a couple returned to their home to find a severed right foot on the private bridge to their residence. The recent rainfall had flooded the bridge, but within a few hours, it had dropped, exposing the remains. Normally, the creek is five to six feet deep; however, the rain had caused the creek to rise to approximately nine feet prior to the remains being found. A cursory search was done to locate additional remains, but none were found. Early in the investigation, it was determined that this was not a biological specimen, hospital waste, or severed limb from a traumatic event such as a car crash.

At the time the Office of the Chief Medical Examiner (OCME) was notified, there were no active missing persons in the area and the case was referred to the OCME's Long-Term Unidentified Coordinator (LTUC). The case was entered into the National Missing and Unidentified Persons System (NamUs) and National Crime Information Center (NCIC) with no potential matches. The LTUC submitted several bones to the Virginia Department of Forensic Science for DNA examination and Combined DNA Index System (CODIS) entry. Due to the case backlog at the time, the investigating law enforcement jurisdiction decided to send the remains to a private lab for Short Tandem Repeat (STR) completion as they had a Memorandum of Understanding (MOU) with the lab and the Virginia Department of Forensic Science that STR profile was searched against CODIS prior to entry, and there was a hit from the convicted offender database.

Once the information from CODIS was retrieved, and law enforcement was notified, it was determined that the person whose foot was found had been reported missing less than two miles from where the remains were recovered. This individual had a lengthy criminal record, mental health history, and had a history of substance abuse. The possibility of the individual being alive somewhere was discussed, but due to his history and habits, it was determined that the rest of his remains could still be in the area. Subsequently, the Virginia OCME issued a death certificate for the individual, released the remains to the family, and finalized the case with cause and manner of death being undetermined. The STR profile was uploaded to CODIS and will continue searching in the event additional remains are found at a later date.

Early in the investigation, OCME investigators requested information regarding missing persons in the area and were told there were none. This individual should have been reported to the OCME as a possible match; he went missing approximately a year prior to when the remains were found and was still considered an active missing persons case. Had this information been given to OCME investigators, a direct DNA comparison could have been completed, resulting in the identification of the individual after a few weeks, instead of over a year (487 days) after the remains were found.

Unidentified Persons; Skeletal Remains; DNA

F56 Shots Fired! The Escalating Rise of Violence Against Law Enforcement by Youth Street Gangs After the COVID-19 Pandemic

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Learning Objectives: Attendees of this presentation will be able to learn about new youth street gang activities confronting law enforcement and death investigators. Moreover, attendees will be able to identify 10 distinct manifestations of these shootings against law enforcement and death investigators and 12 solutions to help keep officers and death investigators safe while out on the streets investigating these gang-related shootings.

Impact Statement: The impact that this presentation will have on the forensic science community is that it will demonstrate what an impact that youth gangs still have on the community, as law enforcement, death investors, coroners, and medical examiners continue to risk their lives in investigating the deaths of these youth gangs and how to keep themselves safe while doing so.

Abstract Text: In recent months, after the COVID-19 pandemic, violence toward law enforcement has increased by 90% according to the Department of Justice.[1] In some metropolitan areas of the country, this figure is nearly 125%. One cannot turn on the television or radio without hearing about another officer-involved shooting. In Los Angeles County, CA, alone, the Los Angeles Police Department (LAPD) and the Los Angeles County Sheriff's Department (LASD) have increased helicopter patrols and 911 screenings in the wake of the recent police ambushes by youth gangs, as there were 20 in the past three months.

Youth street gangs throughout the United States still continue to terrorize the neighborhoods that they claim as their own, causing the citizens in these gang-infested neighborhoods to live in constant fear of their lives every single day. However, a new trend out on the streets is making a fake 911 call and then ambushing law enforcement as they respond to these fictitious calls for help. As law enforcement responds to the location of the scene, youth gangs are now using urban-style tactical warfare learned from the military and using that training against law enforcement as they respond to the scene, seriously injuring or killing officers. Whereby in the past, youth gangs would retreat when confronted by law enforcement, now they are advancing toward law enforcement while shooting, using the same tactics as the officers themselves use such as "slicing the pie" or "button hook."

There are other various forms of urban tactical warfare learned in the military and the police academy that the gang members are learning on a daily basis through watching social media and using against the police. Five years ago in Los Angeles County, CA, there were a total of 473 shots fired at police in 2019, and between January 1, 2023, to December 31, 2023, there have been a total of 768 shots fired at police; 92% of those shots fired were "gang related," compared to the year 2019 where only 83% were "gang related." Moreover, the number of shots fired in Los Angeles County almost doubled from 2019 to 2023. This is a serious "officer safety" concern for law enforcement and death investigators who respond to these gang related violence calls on a daily basis.

Why are these gang members shooting at law enforcement? The author interviewed 200 Los Angeles and Philadelphia gang members out on the streets and in the jails in person prior to the COVID-19 pandemic and, more recently, in person between January 1, 2023, to December 31, 2023, as to why they would decide to shoot at law enforcement. This study identified 10 distinct manifestations of these shootings against police and 12 solutions to help keep officers safe while out on the streets investigating these gang-related shootings. A sample of the findings includes: distinct cultural differences between African American, Latino, and Asian American gangs as to why they engage violently with the police; state of mind (motivation) of the various gangs; disrespect felt toward police while being questioned, detained, or arrested; covert and overt racism experienced by the gang members; a "getting even" mentality; being male or female in the gang. All of these findings culminate in the recent influx of violence against law enforcement by gang members. In Los Angeles County alone, there are 1,425 documented gangs with a gang membership of over 750,000. Across the country are seen similar results according to the United States Department of Justice.[2] There are over 28,800 gangs in the United States with a total gang membership of 950,000.[2]

References:

1. US Department of Justice, *VALOR Officer Safety and Wellness Initiative*. Washington D.C., U.S. Department of Justice, April 2024.
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Youth Gangs; Youth Violence; Personnel Safety

F57 A Simple Water Laboratory Experiment to Teach Students Good Laboratory Practices and Quality Assurance

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Learning Objectives: This presentation will inform forensic science educators how a simple weighing water laboratory experiment can be used to teach students about good laboratory practices such as minimizing error and the need for device calibration. After this presentation, attendees will gain an understanding of how students can benefit from an experiment that aims to help students understand the possible sources of error in their laboratory measurements.

Impact Statement: This presentation will provide educators with new information about a simple experiment that can be used to teach students about areas of competency related to laboratory skills such as device calibration, accuracy of measurements, and errors. The experiment provides students the opportunity to apply these quality assurance concepts in the laboratory, which helps them to develop their competencies in these topics.

Abstract Text: In the field of forensic science, the understanding of the concepts associated with good laboratory practices and the documentation of device calibration is critical for quality assurance.[1-3] While it is important for students to be able to understand and identify sources of error and the need to document device calibration and tolerance as part of their experimental procedures, students often have difficulty applying these concepts in a laboratory setting. This project aims to enhance the understanding of measurement errors by prompting students to consider potential errors and their mitigation during a simple water measurement experiment.

As part of the pre-lab and post-lab assignments, students were given the following scenario: A student pipettes 1.00mL of water in a weighing container and determines the mass using an analytical balance. They were then asked to identify sources of error as well as ways to minimize errors related to taking the weight of water. The student responses were analyzed using an inductive approach.[4] Preliminary results of the analysis show that students exhibited a deeper understanding of the errors they must consider when making measurements after the conclusion of the experiment. For example, in the pre-lab assignment, sources of error were described using novice terms such as spillage during the transfer, misuse of pipettes, incorrect tare of the balance, and miscalculations. In the post-lab assignment, students described the ways to minimize errors using more sophisticated terms such as ensuring proper calibration of the balance and pipettes, accounting for the accuracy/tolerance of the balance and pipettes, and conditioning the pipette tip.

This project shows how a straightforward experiment can be used to provide students an opportunity to reflect on how good laboratory practices should be considered when making laboratory measurements. The experiment can be used to improve students' understanding and competencies regarding laboratory measurements, systematic error, and the importance of device calibration.

References:

1. *World Health Organization Handbook: Good Laboratory Practice (GLP): Quality Practices for Regulated Non-clinical Research and Development*. World Health Organization; 2009.
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3. Dux, J. *Handbook of Quality Assurance for the Analytical Chemistry Laboratory*. Springer; 1990.
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Education; Laboratory; Quality Assurance

F58 CSIxR: Creating a Virtual Reality CSI Application

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Learning Objectives: After attending this presentation, attendees will be informed about the creation of a Virtual Reality (VR) application that focuses on the learning and skills assessment of the process, techniques, and tools used by Crime Scene Investigators (CSIs).

Impact Statement: This presentation will impact the forensic science community by familiarizing the audience with how cross-disciplinary collaboration between different colleges at a polytechnic university created a VR app for CSI training along with an instructor's component to assess mastery of concepts from students.

Abstract Text: Recreating a crime scene can be challenging as every detail can be important in understanding what took place. As a result, CSIs take many pictures and make detailed notes. No matter how thorough the recreation is, however, it can never replace the actual experience of being at a real crime scene. It is important to allow students an opportunity for practical training at a crime scene in as realistic an environment as possible. VR crime scenes allow forensic science students, CSI's in training, attorneys, and jurors an opportunity to explore the crime scene without worrying about accidentally altering it. Virtual reality scenes can be used to supplement classroom education in crime scene investigation as well.

Many times, it is difficult to create a mock crime scene due to a lack of space, time, or human resources, and even more if the organization intends to create several scenes. To solve these problems, a multidisciplinary team at New Jersey Institute of Technology developed an extended reality application: ForensicXR. ForensicXR is an educational platform that supports crime scene processing in virtual or augmented reality environments. Both options provide a consistent and repeatable view of a recreated crime scene. The scene is either a 3D-scanned scene or a scene recreated using accurate measurements from a 3D scan. These scenes can either be viewed in VR, which entirely replaces the surroundings with the recreated scene, or in augmented reality, which overlays virtual objects and interactions over the existing surroundings. The application mirrors many of the actions that take place in real investigations: taking pictures of the scene, lifting fingerprints, collecting and labeling evidence, and communicating with other departments and labs via a virtual tablet interface. Each scene contains a briefing and debriefing, which is stored on a database alongside the scene, allowing the application to scale and accommodate a variety of different scenarios. The application also contains an instructor's module to assess progress and mastery of concepts by students.

Crime Scene Investigation; Virtual Reality; Augmented Reality

F59 Evaluating the Scientific Validity of Forensic Methods: Guidelines for Study Design and Analysis

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Learning Objectives: After attending this presentation, attendees will be able to: (1) understand key principles of study design and analysis, which will enhance their understanding of existing studies and which they can apply to future studies across a range of different forensic disciplines; (2) define and apply key terminology related to scientific validity, including accuracy, precision, repeatability, and reproducibility; and (3) apply key concepts from guidance documents (e.g., by the American Statistical Association) on how to conduct studies and how to interpret and report results.

Impact Statement: We aim to inform future studies and to foster more communication between the statistics community and forensic science community.

Abstract Text: Reviews conducted by the National Academy of Sciences and the President's Council of Advisors on Science and Technology concluded that new studies are needed to evaluate the scientific validity of some forensic pattern-matching methods, including, for example, firearms examination and DNA mixtures.[1,2] Designing such studies, called validation studies, is challenging but necessary to establish the scientific foundation of forensic methods. As statisticians with expertise in study design and analysis, we aim to contribute by providing guidance on the design of future validation studies.

The presentation will be given by statisticians but will be relevant to forensic practitioners who rely on scientific studies in their work. After attending this presentation, attendees will understand key principles of study design, which will enhance their understanding of existing studies and which they can apply to future studies across a range of different forensic disciplines.

We start by discussing the goals of validation studies. We then define key terminology such as accuracy, precision, repeatability, and reproducibility. A validation study must follow sound experimental design and statistical analysis principles to provide reliable results. Some standard design principles that we will cover in our presentation include the following: selecting participants, materials, and testing conditions that are representative of the full spectrum of casework; including sufficient numbers of such participants and materials to answer the relevant scientific questions; estimating error rates along with their uncertainty; preventing and addressing missing data due to dropout or incomplete responses; and addressing contextual bias.

We illustrate the principles above in the context of validation studies for firearms examination and latent fingerprint analysis. We will draw on key ideas in guidance documents from the American Statistical Association (ASA), the National Institute of Standards and Technology (NIST), the United States Food and Drug Administration (FDA), and the National Research Council (NRC).

Our overall goal is to inform future studies and to foster more communication between the statistics community and forensic science community.

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Validation; Scientific Evidence; Accuracy

F60 Examining Social Belonging and Connectedness in an Online Forensic Science Course

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Learning Objectives: After this presentation, participates should be able to: (1) evaluate students' sense of belonging and connectedness in an introductory forensic science course; (2) examine learning technology tools that can foster a sense of belonging and connectedness; and (3) incorporate effective practices to foster a sense of belonging and connectedness in an online forensic science course.

Impact Statement: As a forensic science educator, it is important to have an understanding of best practices to improve student success in higher education. Students that have a strong sense of belonging and connectedness to the course, program, and institution have shown themselves to be more successful in their college career. This presentation will introduce effective learning tools that can be used to foster a sense of belonging and connectedness for students, which will give specific forensic science-related examples in inclusive teaching practices, communication, and building interpersonal relationships among students and the course instructor.

Abstract Text: There is a desire to improve science education in the online learning environment in which the focus is on students' connectedness and belonging and, with the use of learning technologies, can enhance the current teaching climate of the large enrollment, online, forensic science course. In a popular introductory forensic science online course, students were given opportunities to engage and interact with learning materials, peers, and the instructor using various learning technologies. Specific pedagogies, such as inclusive teaching methods, communication, and building interpersonal relationships, used throughout the course were evaluated in the success of building an online learning community, focusing on the students' perception on their sense of social connectedness and belonging. Various technology tools were used in the online course to deliver learning materials, engage students in course content, and as a platform for student interaction with peers and the instructor, which were focused on promoting students' sense of social connectedness and belonging.

With the increase in learning technologies used in the online learning environment, discovering the effectiveness of learning tools will help others choose appropriate tools depending on the desired outcome. Students were asked at the start of the course what social belonging and connectedness meant to them and were surveyed to evaluate their current sense of belonging and connectedness at multiple points throughout the course. Specific learning activities were designed to build on students' sense of belonging and connectedness based on student feedback, focusing on creating inclusive teaching practices, communication, and interpersonal relationships. At the end of the learning experience, students were asked again to share their perception of belonging and connectedness with the addition of individual student interviews. The students' perception of social belonging and connectedness increased from the beginning of the course. Specific learning technology tools, such as InScribe, peer-review assignments, and recorded instructor videos, were effective in building community through a sense of social belonging and connectedness to both the others in the course as well as the course content.

Education; Forensic Science; Student Success

F61 Project-Based Learning in Undergraduate Forensic Chemistry: Standards Development and Professional Practice

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Learning Objectives: Attendees will learn an effective means for teaching undergraduate forensic science students about professional practice in the forensic field. The project described can be implemented in any upper-division undergraduate forensic science course to effectively teach future forensic scientists about: (1) effective communication, (2) critical evaluation of new technologies, (3) professional organizations in forensic science, and (4) the process of standards development and implementation.

Impact Statement: By implementing the project described in this presentation, forensic science educators can better prepare future forensic professionals for successful employment. Students who complete this project will enter the workforce with foundational knowledge of professional organizations, standards development and implementation, and critical evaluation of emerging technologies. This will lead to a workforce of forensic scientists who are prepared for the legal and ethical aspects of the profession in addition to the scientific foundations.

Abstract Text: For undergraduate students preparing for a career in forensic science, their understanding of the professional landscape is as important as their understanding of the scientific foundations of the field. Students entering the forensic science workforce should have knowledge of the legal and scientific standards that will apply to their work as well as the professional organizations that develop and maintain these standards. For many students, their knowledge of the profession is largely based on television shows, which do a poor job of representing the limitations of the science and the restrictions on how it can be applied in legal settings, particularly regarding new and emerging techniques.

This presentation will detail how these topics are introduced through a semester project in an undergraduate forensic chemistry course at Metropolitan State University of Denver. The project is introduced at the start of the semester, immediately following a lecture on ethics and professional practice in forensic science. Students are provided with examples of forensic scientists who were found to be incompetent or unethical, highlighting the importance of following ethical and legal scientific standards. Students are then provided with the executive summary of the *Strengthening Forensic Science in the United States: A Path Forward* Report and are introduced to the Organization of Scientific Area Committees (OSAC) and the Forensic Science Standards Board (FSSB).[1] For the project, students are asked to assume the role of an FSSB member to evaluate a new forensic technique in terms of its: (1) cost and ease-of-use, (2) accuracy and reliability, and (3) statistical significance (probative value). The students must search the literature to find an emerging technique that suits their interests along with a current accepted standard for the same type of analysis; they must determine whether the new technique could be a suitable replacement for the accepted standard. They are required to share their findings in both written and oral formats, with a report in the style of a *Journal of Forensic Sciences* article and a presentation to the FSSB (a.k.a. their classmates). This project allows the students to gain knowledge and skills in several key areas including oral and written communication, the professional landscape of forensic science in the United States, the critical evaluation of data quality and value, and the process of standards development.

Through completion of this project, students gain an understanding of the field of forensic science that goes beyond the basic scientific foundations and introduces them to the ethical, legal, and professional aspects of their future careers.

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Education; Standards; Professional Responsibility

F62 Using Virtual Reality in Crime Scene Education, Training, and Proficiency Testing

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Learning Objectives: The goals of this presentation are to: (1) identify the key benefits of using Virtual Reality (VR) simulations in forensic science education, including cost-effectiveness, standardization, and accessibility; (2) explain how VR-based practical sessions provide a safe, controlled environment for students and professionals to practice essential crime scene investigation skills; (3) describe the advantages of VR simulations in recording and analyzing trainees' actions for comprehensive feedback and assessment; (4) recognize the potential of VR technology in enhancing proficiency testing and evaluation in forensic science; (5) evaluate the potential impact of VR technology on improving the skills and readiness of future forensic professionals; and (6) compare the effectiveness of VR-based training to traditional methods in crime scene investigation education.

Impact Statement: Compared to traditional methods, VR-based training offers a more flexible, repeatable, and data-driven approach to crime scene investigation education. While it should not completely replace hands-on experience, VR technology has the potential to significantly enhance the preparedness of forensic professionals for real-world scenarios, ultimately contributing to more effective and accurate crime scene investigations.

Abstract Text: VR technology is revolutionizing crime scene investigation education, training, and proficiency testing, offering innovative applications that enhance learning outcomes, reduce costs, and standardize training experiences. This presentation explores the development and implementation of interactive VR crime scene simulations, which provide students and professionals with a safe, controlled environment to practice essential skills.[1]

Crime scene investigation is a field where hands-on experience is crucial, as most learning occurs through practical application rather than theoretical study. However, active crime scenes are not suitable training grounds due to the potential for critical errors that could impact the justice system. VR-based practical sessions address this challenge by offering a risk-free alternative that closely mimics real-world scenarios.[1-3]

The benefits of VR in forensic science education are numerous. Cost-effectiveness is a significant advantage, as creating physical training crime scenes can be time-consuming, difficult, and inconsistent. VR simulations can be easily reset and reused, allowing for standardized experiences across multiple trainees. Additionally, VR offers enhanced accessibility, enabling remote training and reducing the need for physical facilities.[1,4]

One of the most powerful features of VR crime scene simulations is the ability to record and analyze trainees' actions. This capability allows for comprehensive after-action debriefings, providing valuable feedback and assessment opportunities. The recorded simulations can also be utilized for testing purposes, including proficiency evaluations, offering a more immersive and realistic experience compared to traditional methods that often focus on individual skill sets or require expensive one-time use physical crime scenes.[1,2,4]

The presentation will showcase videos from VR crime scenes, demonstrating the level of detail and interactivity achievable with this technology. It will also discuss real-world applications, such as Australia's implementation of virtual proficiency testing tools like the After the Fact (AtF) crime scene proficiency test developed by the National Institute of Forensic Science.[5]

While the potential of VR in forensic science education is significant, challenges remain in integrating this technology into existing curricula and professional training programs. We will address these obstacles and explore strategies for scalability, ensuring that VR can be effectively implemented across various institutions and agencies.[1-3]

In conclusion, VR presents a transformative opportunity to enhance crime scene investigation education, training, and proficiency testing. By providing immersive, standardized, and cost-effective experiences, VR technology has the potential to significantly improve the skills and readiness of future forensic professionals, ultimately contributing to more effective and accurate crime scene investigations.

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Virtual Reality; Crime Scene Investigation; Proficiency Testing

F63 Grizzlies, Guns, and Glaciers: Interesting National Park Deaths

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Learning Objectives: National parks fall under various jurisdictions, to include federal, local, and reservation. These different landscapes not only include glaciers, large bodies of water, forests, mountains, caves, and deserts, but also encompass all four seasons. Attendees will learn of the complex nuances needed to complete investigations and hear interesting cases that are unique to our national parks.

Impact Statement: Presenting on national park deaths will impact the forensic science community by highlighting not only the intricacies of interjurisdictional collaboration, including local police, tribal police, national park rangers, coroner offices and medical examiners offices, but also the challenges of completing death investigations in the absence of a body. It highlights the differences in resources, training, environment and types of visitors that are unique to each national park.

Abstract Text: National parks, located in the United States, are visited by over 300 million people a year. From 2014-2019, a total of 2,149 deaths were recorded.[1,2] Despite deaths drawing media attention, the parks are relatively safe. Mortality data, collected by the National Park Service, reported a mortality rate of 0.11 death per 100,000 recreational visits, substantially lower that the 715 deaths per 100,000 people of the overall United States population.[2,3]. Death investigations that take place in our national parks cover a multitude of breath-takingly beautiful, yet harsh, terrains. There are even cases where the topography is so harsh, bodies are not recovered and individuals are "presumed" dead. National park investigations are also complex in the sense that they often cross not only multiple states but multiple jurisdictions.

The National Park Service reports their deaths as "unintentional," "intentional," "medical," and "undetermined." The leading "unintentional" deaths were from motor vehicle collisions, drownings, falls, and environmental. By far the leading "intentional" death is suicide. Most medical deaths were related to environmental and physical activities. The most common recreational activities leading to death were driving, hiking, and swimming.[2]

We present multiple cases from our national parks from Denali National Park in Alaska to Gateway Arch National Park in Missouri.[1] Deaths resulted from multiple different causes, including but not limiting to, environmental exposure, accidental firearm discharge, animal attack, airplane crashes, and falls. Cases were shared by coroner, death investigators, and medical examiners. Manner of death was determined by not only autopsy examination, but from thorough scene investigation by either national park rangers or other death investigators, including the police. These cases also highlight the principle of "presumed death" and the challenges of body recovery in the setting of rough terrain.

The collection of mortality data is used to increase park visitor safety and fund programs to include high-visibility traffic safe enforcement campaigns, Preventative Search And Rescue (PSAR) programs, injury prevention campaigns to combat drowning, and education on heat safety and safe distances from wildlife.[2] Reflecting upon these deaths is also imperative to highlight the nuances and complexities of death investigations in our national parks.

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Death Investigation; Environment; Scene Investigation

F64 PHD ETDC—The Forensic Reconstruction Methodology: A Scientific Process to Solve Cases and Avoid Wrongful Convictions

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Learning Objectives: After this presentation, attendees will learn about the forensic reconstruction methodology and the application in cases and the scientific results that changed the path of the investigation, that sometimes are biased by the police or the investigators.

Impact Statement: This presentation will benefit the forensic science community to understand the importance of teaching and applying this scientific methodology to minimize the human error in the evidence interpretation and the conclusions. The conclusions that the forensic scientist can get after the application of this methodology can help to provide the key answer that the investigator needs to lead to the author of the crime or exonerate the suspect.

Abstract Text: Changes in case classification, such as suicide to homicide, or sometimes wrongful convictions happen. According to the statistic of the Innocent Project, between 4-6% of people incarcerated in the United States prisons are actually innocent.[1]

This methodology is an update of the methodology published in 2009 by Ross Gardner and Tom Bevel in their book, *Practical Crime Scene Analysis and Reconstruction*.[2] This methodology uses six steps: Problem, Hypothesis, Data, Expectation, Test, and Conclusion (PHD ETC), but after I applied this forensic reconstruction methodology in several cases and in classroom with my student in mock cases, I felt that this methodology needed an update. Although it is a good method, I knew that they needed a new step, a step where the forensic expert can express and discuss their expert and professional opinion before the conclusions.[2,3] This new methodology includes a new step: Problem, Hypothesis, Data, Expectations, Test, Discussion, and Conclusions (PHD ETDC).

Problem:d The problem is when as an investigator you present a problem or question that needs to be solve in the case.

Hypothesis: The expert presents the possible hypothesis based on the problem.

Data: All the information you have related to the case, such as case file, forensic reports, witnesses statement, victim statement, scene visit, if possible, among other data.

Expectations: What do you expect with all the information that you as an investigator have at this stage.

Test: When you test your hypothesis.

Discussion: The expert opinion relates with the findings you obtained during the past stages and the possible answer to the problem.

Conclusions: Are the conclusions related to your problem?

This new methodology is taught in the Forensic Reconstruction class and Laboratory at Chaminade University of Honolulu and is also already used in cases.

During this presentation, the attendees will learn about one case that was ruled as a suicide, but after I applied this new Forensic Reconstitution methodology, the case changed to homicide.

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PHD ETDC; Forensic Reconstruction; Wrongful Conviction

F65 Video Technology for Training and Testimony Preparation

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Learning Objectives: After attending this presentation, attendees will have a greater understanding of how to use technology to modernize forensic training programs, including testimony preparation.

Impact Statement: This presentation will impact the forensic science community by highlighting ways to include modern technology in training programs that may have been based on more traditional approaches. Examples will include using online platforms such as Microsoft Teams to record interactive lectures for later review and as a way for individuals to practice speaking. By using these platforms, trainees can record themselves expressing concepts that are then available for both the trainee and trainer to review in order to improve the trainee's confidence, subsequent performance, and awareness of how he or she may appear when communicating concepts in settings like testimony.

Abstract Text: Before COVID-19, the Seized Drugs section of the Houston Forensic Science Center (HFSC) utilized a traditional training program that relied heavily on readings, in-person lectures, written examinations, and hands-on practical exercises culminating in a mock trial. The expectation from the general public and jurors for forensic analysts, regardless of years of experience, is to "perform" as expert witnesses, which requires significant preparation. Section management recognized the need for modifications to the training program to include methods for trainees to practice expressing their understanding of concepts orally as a way to better prepare them for confident testimony.

With the appearance of COVID-19 in 2020, education in schools and the workforce changed significantly, which required people to adapt to learning using non-traditional approaches. Remote technology-based systems using computers and applications like Microsoft Teams suddenly became the primary avenue for learning to a generation of students coming out of academic settings in place of traditional, in-person methods of learning. Therefore, HFSC needed to change their training approaches for new hires to become forensic analysts seamlessly and had to adapt to new ways of communicating through technology.

Updates to the training program began by consolidating training materials to an electronic format that were then readily available to trainees and all section staff. The next step included using Microsoft Teams to record interactive lectures that trainees could review later for study and clarification. Oral reviews were implemented for the trainer to gauge the trainee's understanding of material. Videoing these reviews became a way for the trainees to see and hear their responses and work with the trainer to identify areas for improvement. This resulted in trainees becoming more confident in themselves so that they were better prepared for the final mock trial—the ultimate test of the ability to explain forensic practices and concepts clearly and accurately.

Training; Expert Testimony; Education

F66 How Research Improves Public Crime Lab Efficiency and Accuracy: The National Institute of Justice-Sponsored Outcomes

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Learning Objectives: After attending this presentation, attendees will understand the purposes/areas under which research applications can be submitted and examples of each. Attendees will also be aware of metrics associated with successfully applications to research funding for public laboratories.

Impact Statement: This presentation will impact the forensic science community by providing detailed information and graphics that are evidence-based on applying for research funding for public laboratories.

Abstract Text: Starting in 2015, the National Institute of Justice (NIJ) initiated an annual solicitation specifically aimed at providing funding to public forensic laboratories titled *Research and Evaluation for the Testing and Interpretation of Physical Evidence in Publicly Funded Forensic Laboratories*. The goal of this solicitation is to identify and inform the forensic community of best practices through the evaluation of existing and emerging laboratory protocols and/or have a direct and immediate impact on laboratory efficiency and assist in making laboratory policy decisions. Awards under this solicitation direct findings of this research and evaluation toward the identification of the most efficient, accurate, reliable, and cost-effective methods for the identification, analysis, and interpretation of physical evidence for criminal justice purposes and produce deliverables that include best practices as well as protocols that can be adopted by the community. Over the past eight years, this solicitation has provided funding for 38 awards across multiple disciplines, including DNA, impression and pattern evidence, seized drugs, and toxicology. Historic output includes:

- Assess analysts' approaches to decision making for electron impact (EI) MS data comparison.
- Decision-making protocols on which swab and how many swabs should be analyzed in sexual assault kits.
- Results that evaluated modifications to the technical and administrative review processes without effecting the identification of Type 1 and Type 2 errors.
- The validation of a confirmatory body fluid assay using proteomic mass spectrometry.
- The systematic exploration of the feasibility of sharing images across 3D imaging systems and the usefulness of 3D technology to reduce the probability of inconclusive determinations.

Output from these awards will be highlighted as part of the presentation. In addition, NIJ will recommend and provide resources to encourage public laboratories engagement with research and contributions toward building a positive research culture within the forensic sciences. NIJ will explain how these activities can ensure improvements to both scientific integrity and quality.

Research; Crime Laboratory; Technology Transition

F67 Old Legal Frameworks and New Technologies: The Baltimore, Maryland, Experience

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Learning Objectives: Attendees will learn how new technologies are being deployed on the street and that courts struggle with technologies that do not fit neatly into existing jurisprudential doctrines.

Impact Statement: This presentation will bring wider attention and scrutiny to the problems of legal frameworks being outpaced by technological advances and detail how this can be addressed through developing flexible forward-looking policies and laws.

Abstract Text: As technology continues to develop unabated, the legal system struggles to keep up with the implications of these new developments. We become accustomed and dependent on these new technologies and services, and traditional expectations of individual privacy have to be revisited and balanced out against previously unimaginable facets and arrangements of modern life.

Here in Baltimore and Maryland, we are acutely aware of this. In addition to the unsettling knowledge that our primary mode of communication doubles as a tracking device that also contains data about every aspect of our existence, we also live in a city that has become a veritable testing ground for surveillance technologies. While the law has been slow to catch up, these technologies were deployed using case law that were outdated and inapplicable to the current state of the art technology. This talk will explore the use of surveillance technology in Baltimore over the past 15 years with an eye toward how courts, legislators, and other stakeholders addressed the issues and constitutional implications that arose from the use of these technologies.

Handling these technologies on an individual basis does not address the fundamental problems with the overuse of these invasive and often questionable technologies. While a systemic approach that involves the courts and the legislature is needed, it is important to recognize that courts are inherently backward-looking institutions. An institution bound by precedent and tradition is not necessarily well equipped to wrestle with issues related to new technologies. Local boards made up of stakeholders should be created to establish both the constitutional and reliability parameters for the use of surveillance technologies before these technologies are deployed by law enforcement in any widespread manner. While this concept may seem cumbersome and bureaucratic, the alternative of allowing the surreptitious use of questionable technologies is a far worse alternative.

Court; Baltimore; Technology

F68 The Stability and Variability of the Korean Saliva Microbiome Over a Period of Time

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Learning Objectives: This study examined the daily, weekly, and annual stability of the Korean saliva microbiome. Although the saliva microbiome is stable within individuals, it can be affected by certain factors such as oral disease.

Impact Statement: Saliva microbiome analysis can support investigation by providing information such as age, lifestyle, disease, and geographic location of suspects or victims when Short Tandem Repeat (STR) profiling is unobtainable due to trace amount or poor quality of DNA. Therefore, it is essential to confirm the stability of saliva microbiome for its application in forensic investigations.

Abstract Text: Compared to other biological evidence, the microbiome has the advantage of being ubiquitous and easy to collect. In addition, it can support DNA analysis if the DNA is degraded or in low quantity for detection.[1] It can also provide supportive information such as geographic location and ancestry if a suspect or victim cannot be identified.[2] Due to these advantages, microbiome is rapidly emerging as a novel forensic tool for human and body fluid identification.[3] Saliva microbiome is individual-specific and remains stable over time.[4,5] However, it can be influenced by internal and external factors, such as individual's dietary habits and oral diseases.[6,7]

The purpose of this study is to determine the stability and variability of the Korean saliva microbiome over a certain period and which factors influence saliva microbiome composition. Saliva samples were collected from five Korean participants on a daily, weekly, and annual basis. Seventy questionnaires on lifestyle, diet, and disease were administered to collect data on changes in internal and external factors between sampling intervals. DNA from saliva samples was purified using ARA MagNA Tissue DNA Isolation Kit, followed by Next Generation Sequencing (NGS) using the MiSeq platform. Taxonomic classification was performed using SILVA database provided by QIIME2, and the data were analyzed using MicrobiomeAnalyst. As a result, saliva microbiome remained stable for most participants in weekly and daily comparisons. However, in annual comparisons, significant changes were observed in most participants. These findings are assumed to be related to: (1) oral diseases such as stomatitis, cavity, and oral warts; (2) dental treatment; and (3) changes in dietary habits such as frequency of sugar intake. The study suggests that the saliva microbiome is generally stable within individuals but can be altered by certain factors related to the oral health. To improve the data, a comparison of seasonal, monthly periods and additional recruitment is going proceed.

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Oral Microbiota; Microbiology; Saliva

F69 The State of Forensic Science in India: Progress and Pitfalls

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Learning Objectives: This presentation will examine the current state and growing importance of forensic science and the role of forensic science in the Indian criminal justice system. This presentation identifies key challenges and opportunities facing the field of forensic science and the impact of recent legislation on forensic science in India.

Impact Statement: The increasing integration of forensic science in India's criminal justice system, driven by technological advancements, expanded infrastructure, and supportive legislation, has the potential to significantly enhance investigation efficiency, improve conviction rates, and ensure justice for victims. By addressing challenges in funding, infrastructure, and legal frameworks, India can fully harness the power of forensic science to create a safer and more equitable society.

Abstract Text: Forensic science is the application of scientific methods and techniques for the purposes of justice. Due to advances in technology, forensic science in India looks like an up-and-coming field in the science with collaboration of law enforcement. There are 300 universities and colleges in whole India who are pursing a forensic science degree in a separate department as it has tremendous opportunities and to rule out the exact duties and code of conduct of a forensic expert during a crime scene investigation. There are 7 Central Forensic Science Laboratories (CFSLs), 32 State Forensic Science Laboratories/Union Territory Forensic Science Laboratories (SFSLs/UT FSLs), 80(+10) Regional Forensic Science Laboratories (RFSLs), and 529 Mini and Local Forensic Science Laboratories (MFSLs) that has increased the number of forensic professionals, and integrating advanced forensic methods into criminal investigations is expected to significantly impact the effectiveness of the justice system in India. New laws have been introduced in the Indian justice system, which has been thought to put a strong bone to the justice system to reduce the crime rate and to provide equality and justice to the people. First, the Bhartiya Nagrik Suraksha Sanhita (BNSS) act, 2023, has introduced new law that implies that any offence punishable with seven years of imprisonment or more needs a mandatory forensic investigation. Second, the Bhartiya Nyaya Sanhita (BNS) act, 2023, replaces the IPC, which helps to increase the punishment toward heinous crimes. It also targeted the organized crimes. The third newly added law in criminal law is Bhartiya Sakshya Adhiniyam (BSA), 2023, has taken the place of the Indian evidence act. It helps to introduce the digital evidence as documents that can be kept under opinion by an expert in criminal justice system. In spite of a promising future, it still faces several challenges that include lack of funding from the government just like other laboratories and education department are provided, inadequate infrastructure in laboratories and research centers, legal framework, data is complex and bulky and hinders its full potential.[1,2]

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Law Enforcement; Justice; Forensic Investigation

F70 The Will to Conceal the Evidence? A Suspicious Case of a Restraint-Related Death That Occurred in the Hospital

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WITHDRAWN

F71 You Take My Breath Away! An Unusual Case of Uxoricide-Suicide by Double Choking

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Learning Objectives: The goal of this presentation is to report a rare case of combined homicide-suicide by choking.

Impact Statement: This presentation will impact the forensic science community by showing a peculiar case of combined uxoricide-suicide by double choking. To the best of our knowledge, similar cases have rarely been reported in the literature.

Abstract Text: An 82-year-old man, exhibiting signs of confusion, reported to the local police the death of his wife. The man presented two bloody stab wounds on his left hand, that he made himself by striking a mirror, no details were recalled. Police investigation of the men's house uncovered numerous bloodstains and his wife's corpse in the bedroom. A full forensic autopsy was ordered by the public prosecutor: the postmortem examination revealed various injuries on the woman's body, including bruising, cuts and swelling on the face and upper limbs. Notably, significant injuries were identified in the perioral region and oral cavity, such as swelling, bruising, and abrasions. In addition, the fracture of the dental element 4.3 and a blunt force wound of the lingual frenulum and the vestibular mucosa of the lower lip were observed. The inspection of the oropharynx revealed the presence of a foreign bod—a blood-soaked tissue. The autopsy further elucidated that this foreign body was a fabric handkerchief measuring 41x28cm, folded up to occlude the laryngeal aditus completely. Bruising was also noted on the arytenoid and cricoid cartilage. The lungs were expanded and congested, with pleural petechiae. The histological examination confirmed the presence of pulmonary edema and the vitality of the perioral and laryngeal lesions. The cause of death was attributed to choking.

After the autopsy results, the old man was questioned and admitted to killing his wife because he suspected that she wanted to poison him. After being arrested, the old man showed aggressive behavior toward the prison employees and once he attempted suicide by trying to strangle himself with surgical gauze. The prison's health director asked for a psychiatric evaluation, but in the meantime, the man was discovered dead in his cell the same night. During the examination of the jail by the medical examiner for the public prosecutor, it was noted that there were bits of toilet paper on the ground. The examination of the corpse revealed material in the oropharynx similar to that on the floor, obstructing the laryngeal aditus as confirmed by the autopsy. In addition, the autopsy examination showed pleural petechiae, pulmonary edema, and a contusion on the laryngeal mucosa. The reason for the death was determined to be self-inflicted choking.

Choking is a violent mechanical asphyxia due to obstruction of the airways caused by food or objects.[1] In most cases, it is an accidental occurrence associated with alcoholic intoxication, neurological pathologies, childhood, and old age.[2] Suicidal and homicidal choking cases are exceptional, often occurring in mentally ill people.[3] The peculiarity of the present case lies in the fact that homicidal and suicidal choking, both rare occurrences, were observed together in a case of uxoricide-suicide. The medicolegal evidence, in conjunction with the police investigation, was decisive in reconstructing the dynamics of events in this complex case.

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Choking; Murder; Suicide

F72 An Efficacy Catalytic Hydrated Photo-Oxidation Tool in Reducing Biocontaminants During Autopsy Procedures: A Pilot Study

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Learning Objectives: This study evaluates the iSanity's (an Efficacy Catalytic Hydrated Photo-Oxidation tool) effectiveness in reducing biocontaminants during autopsy environments. Participants will learn about tool functionality, methodology, practical implications, and future research directions.

Impact Statement: The study on iSanity demonstrates a significant advancement in forensic science by addressing the challenge of biological contamination from the decedent to the autopsy suite, air, and staff during autopsy. This tool offers a promising solution for reducing airborne microbial contamination, which is crucial for maintaining a sterile environment and ensuring accurate forensic analysis. The findings underscore the importance of ongoing innovation and research in forensic decontamination methods.

Abstract Text: **Background:** This study aimed to evaluate the efficacy of the iSanity, an innovative device, in reducing biological contamination from the decedent to the autopsy suite, air, and staff during autopsy. The need for effective contamination control in autopsy rooms is critical to ensure the safety and accuracy of forensic investigations.

Methodology: Environmental conditions were monitored, and surface and air samples were collected at various stages of the autopsy to assess microbial load. Surface sampling was conducted at four different locations within the autopsy room: floor near the autopsy table (F), the autopsy table near the right arm (S), the observational table (O), and the wall opposite the left arm of the corpse (W). Each swab was performed at four different time points: T0 (before autopsy), T1 (1 hour after the start of the autopsy, instrument off), T2 (2 hours from the start of the autopsy, instrument on), and T3 (corpse released from the autopsy room, instrument on). DNA was obtained and quantified from each swab. Air sampling was conducted using a SAS sampler, with results expressed in Colony-Forming Units per cubic meter (CFU/m³).

Results: Results from surface sampling indicated that while the iSanity tool showed potential in reducing contamination, the differences were not statistically significant. DNA levels on the organ table were highest at T2 and decreased by the end of the autopsy, suggesting some efficacy of this tool. However, on other surfaces like the autopsy wall and floor, contamination levels fluctuated without clear statistical significance. The air sampling results were more conclusive, showing statistically significant improvements in air quality after the activation of the Efficacy Catalytic Hydrated Photo-Oxidation tool. Initial air quality, which started with very low contamination (< 500CFU/m³), deteriorated to intermediate levels (1,000-5,000CFU/m³) during the autopsy but improved significantly to low (< 1,000CFU/m³) and very low (< 500CFU/m³) levels post-activation.

Conclusions: The findings suggest that while this tool is particularly effective in air purification, achieving significant decontamination on surfaces may require a multifaceted approach, combining advanced air purification technologies with robust surface cleaning protocols. Further research with larger sample sizes and additional cleaning protocols is necessary to confirm these findings and optimize decontamination strategies. Overall, this tool shows promise in improving air quality in autopsy rooms, but comprehensive decontamination strategies are essential for effective contamination control.

Autopsy; Forensic Analysis; Forensic Professionals

F73 Child Abuse or an Accident? The Role of the Forensic Expert in Pediatric Emergency Departments: A Case Series

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Learning Objectives: After attending the presentation, attendees will understand the pattern of injuries in accidents and child abuse. Attendees will also learn about common cases brought to pediatric emergency suspected of child abuse.

Impact Statement: This presentation will impact the forensic science community by helping to sort child abuse cases from accidental cases. Also, this will give a better approach to child abuse.

Abstract Text: Child abuse remains a major problem throughout the world. In the United States in 2022, there were 558,889 reported cases of child abuse. Among them, the most common form of maltreatment was neglect, followed by physical and sexual abuse.[1] A total of 162,449 cases of crimes against children were registered during 2022, showing an increase of 8.7% over 2021 (149,404 cases). In percentage terms, major crime headings under "Crime Against Children" during 2021 were Kidnapping and Abduction (45.7%) and Protection of Children from Sexual Offences Act, 2012 (39.7%), including child rape. The crime rate registered per lakh of child population was 36.6 in 2022 in comparison with 33.6 in 2021[2]

Case 1: A seven-month-old male infant was brought to the hospital and admitted with a history of accidental firearm injury by air gun on the abdomen. The pediatrician suspected it was a homicide attempt by someone and hence sent a call to a forensic expert. After evaluating the infant and detailed history from a relative, we confirmed that firearm circular shape entry wound on the left side of abdomen hypochondriac region, measuring diameter of 5mm with a circular-shape abraded collar. Diagnostic laparoscopy was performed to remove the pellet but could not recover the pellet. On further Ultrasound Sonography (USG), it was found that the pellet passed along the intestine. The pellet was recovered in feces, handed over to the concerned investigating officer. The pellet was 0.22 caliber. After evaluating the crime scene photograph and statement of the family member and investigation of police, we concluded it to be an accidental firearm injury.

Case 2: A 4-year-old girl was brought to the hospital with a history of injuries to the genitals due to a fall, but the pediatrician suspected sexual assault. A forensic expert was called to provide an opinion on the case. After detailed examination, we found the examination findings were consistent with the history.

Pediatricians face major problems in emergency to sort out child abuse cases from accidental cases. Multidisciplinary approach should be implemented in a tertiary care hospital to provide treatment and opinions to investigating authorities.

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Child Abuse; Accident; Pediatric Emergency

F74 Sodium Nitrite Poisoning: "Suicide Kits" Sold on the Internet

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Learning Objectives: After attending this presentation, attendees will understand the toxic effects of Sodium Nitrite ($NaNO_2$) and the use of police, hospital, and coroner investigative reports to determine the cause of death. Attendees will also understand the importance of proper reporting and the significance of a thorough scene investigation.

Impact Statement: This presentation will impact the forensic science community by raising awareness of $NaNO_2$ poisoning and its accessibility to persons via the internet.

Abstract Text: $NaNO_2$ is a compound found in food preservation, coloring agents, and automotive maintenance.[1-3] Ingestion of $NaNO_2$ can be fatal as the substance creates an increase of methemoglobin, which interferes with the ability of red blood cells to transport oxygen throughout the body, resulting in systemic hypoxia, metabolic acidosis, and cyanosis.[2,3] Methemoglobin is a form of hemoglobin that cannot carry oxygen. It is naturally occurring and is continually produced in humans; however, an enzyme in the body eventually converts it to hemoglobin. Methemoglobin commonly occupies less than 1% of the total circulating hemoglobin in a healthy adult. Methemoglobin levels can increase due to injury, drugs, chemicals, or foods, and if increased too much, can cause a condition known as methemoglobinemia.[4,5]

$NaNO_2$ is most used for food preservation and is easily accessible for purchase on the internet. For example, a one-pound bag of $NaNO_2$ labeled as curing salt can be purchased via online retailers such as Amazon for less than $10 USD. The only disclaimer provided with the purchase of this product is that it was not evaluated by the Food and Drug Administration (FDA) for dietary supplement purposes. Otherwise, this product is unregulated and can be delivered within 24 hours. There are several online forums and literature such as "SanctionedSuicide." and "Final Exit" that advocate how to utilize the substance to successfully commit suicide. The "SanctionedSuicide." forum has a specific thread labeled "SN Death Protocols," which outlines two different options on how to use $NaNO_2$ and hosts open discussion of research and opinions on the different "recipes."[6] As of February 2024, there are 12 sets of parents across the United States suing Amazon for selling "suicide kits" to their now-deceased children.[7]

The following two case studies provide in-field experience and insight to $NaNO_2$ deaths.

A 23-year-old White anatomical female, with a past medical history of anemia, depression, and restless leg syndrome, was pronounced deceased on September 8, 2022, at 1:25 p.m. The decedent had called the suicide hotline, stating that they were going to kill themselves by ingestion of $NaNO_2$. The suicide hotline called 911 dispatching police and EMS to the scene of an individual found unresponsive in their car. Police found a container of $NaNO_2$ and Tagamet pills in the vehicle.

The autopsy found the upper airway contained foam and the parenchyma was congested, dark purple, and exuding large amounts of blood and frothy fluid. Toxicology presented positive for methemoglobin. The cause of death was certified as Sodium Nitrite Poisoning with the manner of death determined to be suicide.

A 40-year-old White female, with a past medical history of Borderline Personality Disorder, PTSD, Ehler-Danlos Syndrome, and chronic pain, was pronounced deceased on September 4, 2024, at 5:31 p.m. The decedent was found in a hotel room with a written suicide note, three small cups of a clear liquid with a label stating "poison", ondansetron, metoclopramide, Rolaids, and recipes written inside a notebook on how to ingest "SN."

The autopsy findings are still pending; however, toxicology presented positive for methemoglobin. The cause and manner of death for this case are still pending.

The basic and expanded forensic toxicology panel by NMS Labs does not test for methemoglobin. Methemoglobin testing must be specifically requested; therefore, evidence and/or suspicion of $NaNO_2$ ingestion must be known.

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Sodium Nitrite; Suicide; Poisoning

F75 The First Fingerprint Conviction: Argentina's Historic 1892 Fingerprint Case

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Learning Objectives: Following this presentation, attendees will understand the historical significance of Juan Vucetich's fingerprint system, how it improved on the more famous European fingerprint systems, and how it led to the first use of fingerprints in the conviction of a criminal.

Impact Statement: By focusing on a little-known but significant event in the early history of fingerprint identification, this presentation will provide members of the forensic science community with a deeper appreciation for the origins of their field and insight into how early innovations have shaped current practices.

Abstract Text: Although history largely credits Europeans for the invention of fingerprint identification systems (primarily William Herschel, Edward Henry, Francis Galton, and Henry Faulds), a Croatian man who emigrated to Argentina created, in the view of many experts, a far superior fingerprint system, and his system was used to convict a murderer in the first recorded use of fingerprints in a criminal case.[1-6] This conviction occurred in 1892, a full ten years before fingerprints figured in criminal cases in England and Paris.

The Bertillon system of identification was in vogue when Juan Vucetich moved from Croatia to Argentina in 1884 and found work in a rural police department in La Plata. He studied Bertillon's system of anthropomorphic measurements but became fascinated with the developing field of fingerprint classification. He focused on Francis Galton's fingerprint system but eventually refined Galton's "single fingerprint" system to extend to all ten fingers and created a sophisticated and near-foolproof method of categorizing fingerprint patterns. Even the famous Edmond Locard gave Vucetich's system his highest praise.

In 1892, a woman named Francesca Rojas accused her neighbor of murdering her two small children. Edward Alvarez, a police inspector from nearby La Plata, was familiar with Vucetich's fingerprint system. When he learned that the accused man had a credible alibi and that Rojas was in love with a man who had refused to marry her because of her children, he focused his attention on Rojas. Finding a bloody thumbprint on the door of the room where the children had been murdered, he cut the section of the door off and took it to the police station, had Rojas' fingers inked and pressed onto paper, and finally saw that her thumbprint matched the print on the door. She confessed to the crime.

By 1901, Argentina was the first country in the world to base its identification methods entirely on fingerprinting, and Vucetich would see his system established through South America and many other non-European countries.

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Fingerprint Identification; History; Conviction

F76 Combating Sexual- and Gender-Based Violence: A Crucial Imperative in the Great Lakes Region of Africa

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WITHDRAWN

F77 Isotopic Signatures of Fossiliferous Limestones From the Crato Formation, Araripe Basin, Brazil: A Review With Forensic Implications

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WITHDRAWN

F78 An Update on Forensic Science Standards Within the Crime Scene Investigation Disciplines

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Learning Objectives: Understand the Development Processes: Attendees will learn about the methods and protocols used by the Academy Standards Board (ASB) and the Organization of Scientific Area Committees (OSAC) in developing and validating forensic science standards.

Explore New Standards: Attendees will gain detailed insights into the latest standards related to evidence collection, documentation, and analysis, and how these standards improve Crime Scene Investigation (CSI) practices.

Implement Standards Effectively: Attendees will learn strategies for overcoming challenges and effectively implementing new standards within their organizations, ensuring consistency and quality in forensic practices.

Impact Statement: This presentation will significantly impact the forensic science community by providing the latest updates on forensic science standards and demonstrating their practical application through real-world examples. By equipping forensic scientists, crime scene investigators, and law enforcement professionals with the knowledge and tools to implement these standards effectively, the presentation will contribute to enhancing the accuracy, reliability, and integrity of CSIs. This, in turn, will foster greater confidence in forensic practices and support the broader goals of justice and ethical forensic science.

Abstract Text: The forensic science community is continually evolving with the implementation of new standards and practices, particularly within the realm of CSI. This presentation aims to provide a comprehensive update on the latest forensic science standards as developed and endorsed by the ASB Crime Scene Investigation Consensus Body and the OSAC Crime Scene Investigation and Reconstruction Subcommittee. Attendees will gain insights into the standard development processes and the newly established standards related to evidence collection, documentation, and analysis. Detailed descriptions will be provided on how these standards enhance the accuracy, reliability, and efficiency of crime scene investigations.

The forensic science community has recognized the critical need for standardized procedures in crime scene investigation to ensure consistency and reliability in forensic practices. The ASB and OSAC have been at the forefront of these efforts, working collaboratively to develop standards that address various aspects of CSI, from initial response to evidence analysis. This presentation will delve into the meticulous processes employed by these bodies to develop and validate new standards. By grounding these standards in scientific evidence and best practices, the ASB and OSAC aim to elevate the quality of forensic investigations across diverse jurisdictions.

One of the key highlights of this presentation will be the discussion on newly established standards that have been introduced in the past year. These standards cover crucial areas such as evidence collection protocols, documentation procedures, and analytical methods. For instance, new guidelines on the systematic collection and preservation of physical evidence are designed to minimize contamination and ensure the integrity of samples throughout the investigative process. Enhanced documentation standards, including the use of digital tools for crime scene recording, aim to improve the clarity and comprehensiveness of crime scene reports, facilitating better communication among forensic professionals and with the judicial system.

In addition to outlining these new standards, the presentation will address the practical aspects of their implementation. Real-world examples and case studies will illustrate the application of these standards in actual crime scene investigations. These case studies will highlight the tangible benefits of adopting standardized practices, such as increased accuracy in evidence analysis, improved case resolution rates, and strengthened credibility of forensic testimony in court. Attendees will learn about the challenges faced during the implementation process and the strategies employed by various law enforcement agencies to overcome these hurdles. This practical guidance will be invaluable for professionals seeking to integrate these standards into their own practices.

Furthermore, the presentation will explore the broader implications of these standards for the forensic science community. By fostering greater consistency and reliability in crime scene investigations, these standards contribute to the overarching goals of justice and ethical forensic practices. They help to build public trust in forensic science by ensuring that forensic evidence is collected, analyzed, and presented in a manner that upholds the highest standards of scientific rigor and integrity.

In conclusion, this presentation will provide a thorough update on the latest forensic science standards in the field of crime scene investigation. It will equip forensic scientists, crime scene investigators, and law enforcement professionals with the knowledge and tools needed to implement these standards effectively, thereby enhancing the quality and integrity of their forensic practices.

Crime Scene Investigation; Ethics; Standards

F79 Progress on Developing Dogs and Sensors Standards: Sniffing Out Success

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Learning Objectives: After attending this presentation, attendees will recognize and gain insight on the advancements 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 create standards across an overarching range of canine detection disciplines designed for use by organizations, including law enforcement, military, and homeland security.[1]

Impact Statement: In recent years, the development and use of forensic science standards has increased dramatically across many forensic disciplines, including the use of dogs and sensors for forensic investigations and forensic evidence. This presentation will impact the forensic science community by informing attendees about the latest Dogs and Sensors standards as well as upcoming standards, technical reports, and terms and definitions. The presentation will also highlight how the standards are being structured and why standards should be adopted across agencies employing canine detector teams.

Abstract Text: This presentation will provide the latest information on standard development 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 SDO-published standards, OSAC Registry posted standards, proposed standards in comment adjudication phase at the ASB, and forthcoming OSAC proposed standards. To date there are 7 published ASB standards that have simultaneously been placed on the OSAC registry. Additionally, this presentation will highlight standard document content and emphasize the importance of dissemination and subsequent implementation to the relevant canine detection community. This presentation will project the future of the group's standardization efforts and inform attendees 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.

Reference:

1. https://www.aafs.org/academy-standards-board/consensus-bodies.

ASB; Dog; Standard

F80 Standards Development Activities in Medicolegal Death Investigation, Including Disaster Victim Identification

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the process for standards development in medicolegal death investigation. The presentation will also highlight the status of discipline specific standards pertinent to the field of medicolegal death investigation, including disaster victim identification. This presentation will cover documents both at the Organization of Scientific Area Committees (OSAC) and Academy Standards Board (ASB).

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. It will also increase awareness regarding training, tools and resources that support implementation, compliance monitoring, and broader understanding.

Abstract Text: The 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. Standards on the OSAC Registry: ANSI/ASB Standard 125, *Organizational and Foundational Standard for Medicolegal Death Investigation.* 2021. 1st. Ed.; ANSI/ASB Best Practice Recommendation 007, *Postmortem Impression Submission Strategy for Comprehensive Searches of Essential Automated Fingerprint Identification System Databases.* 2018. 1st. Ed; ANSI/ASB Best Practice Recommendation 008, *Mass Fatality Scene Processing: Best Practice Recommendations for the Medicolegal Authority.* 2021. 1st. Ed.; ANSI/ASB Best Practice Recommendation 009, *Best Practice Recommendations for the Examination of Human Remains by Forensic Pathologists in the Disaster Victim Identification Context.* 2019. 1st. Ed.; ANSI/ASB Best Practice Recommendation 010, *Forensic Anthropology in Disaster Victim Identification: Best Practice Recommendations for the Medicolegal Authority.* 2018. 1st. Ed.; ANSI/ASB Best Practice Recommendation 094, *Postmortem Impression Recovery: Guidance and Best Practices for Disaster Victim Identification.* 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.

2. Other OSAC proposed standards: OSAC 2023-N-0022, *Best Practice Recommendation for Communicating with Next of Kin during Medicolegal Death Investigations*; 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*; OSAC 2022-N-0027, *Medicolegal Death Investigation Response to Death Locations and Incident Scenes: Best Practice Recommendations* ; OSAC 2022-N-0026, *Medicolegal Death Investigation: Terms and Definitions;* OSAC 2021-N-0007, *Media Communications Following a Mass Fatality Incident: Best Practice Recommendations for the Medicolegal Authority;* OSAC 2021-N-0008, *Victim Accounting: Best Practice Recommendations for Medicolegal Authorities in Mass Fatality Management*; OSAC 2022-N-0020, *Standard for Mass Fatality Incident Management;* OSAC 2022-N-0021, *Family Engagement Following a Mass Fatality Incident: Victim Information Center Best Practice Recommendations for Medicolegal Authority;* OSAC 2022-S-0022, *Standard for Disaster Victim Identification;* OSAC 2024-N-0008, *Mass Fatality Incident Data Management: Best Practice Recommendations for the Medicolegal Authority.*

3. Published standards from the ASB that have yet to go through the Registry approval process: ANSI/ASB Best Practice Recommendation 006, *Best Practice Recommendations for DNA Analysis for Human Identification in Mass Fatality Incidents.* 2019. 1st. Ed.

4. Documents currently in development at the OSAC or by the ASB: REVISION to ANSI/ASB Best Practice Recommendation 009, *Best Practice Recommendations for the Examination of Human Remains by Forensic Pathologists in the Disaster Victim Identification Context.* 20xx. 2nd. Ed, Best Practice Recommendation for Deaths in Custody; Best Practice Recommendation for Assessment of a Decedent on Scene; Standard for Daily Operation Resource Availability Priorities for new documents or work products: Best Practice Recommendations for Fatality Management During a Pandemic; Daily Operations Identification and Next of Kin notification.

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.

Medical Examiner; Medicolegal Death Investigation; Coroner

F81 Standards Development Activities in Wildlife Forensic Biology

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of wildlife forensic biology.

Impact Statement: The presentation will impact the forensic science community by creating greater awareness of standards development activities in wildlife forensic biology.

Abstract Text: The Organization of Scientific Area Committees (OSAC) for Forensic Science was initially created in 2014 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 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 Wildlife Forensic Biology Subcommittee are passed to the ASB Wildlife Forensics Consensus Body for the standards development process, after which they are considered for inclusion on the OSAC Registry.

During this presentation, updates related to standards development in wildlife forensic biology will be discussed. These include

1. Standards on the OSAC Registry:
ANSI/ASB Standard 019, *Wildlife Forensics General Standards*, First Edition, 2019
ANSI/ASB Standard 028, *Wildlife Forensics Morphology Standards*, First Edition, 2019
ANSI/ASB Standard 029, *Report Writing in Wildlife Forensics: Morphology and Genetics*, First Edition, 2019
ANSI/AASB Standard 047, *Wildlife Forensics Validation Standard-Validating New Primers for Sequencing*, First Edition, 2019
ANSI/ASB Standard 138, *Standard for Collection of Known DNA Samples from Domestic Mammals*, First Edition 2022

2. OSAC proposed standards Wildlife Forensics Methods:
OSAC 2021-S-0006, *Standard for the Use of GenBank for Taxonomic Assignment of Wildlife.* November 2, 2021.
OSAC 2022-S-0011, Standard for Construction of Multilocus Databases. June 4, 2024

3. Published standards from the Academy Standards Board (ASB) pending Registry approval process:
ANSI/ASB Standard 046, *Wildlife Forensics Validation Standards-STR Analysis*
ANSI/ASB Standard 048, *Wildlife Forensic DNA Standard Procedures*
ANSI/ASB Standard 106, *Wildlife Forensics-Protein Serology Method for Taxonomic Identification*
ANSI/ASB Standard 111, *Standard for Training in Mitochondrial DNA (mtDNA) Analysis for Taxonomic Identification*
ANSI/ASB Standard 169, *Standard for Clinical Veterinary Forensic Examination*
ANSI/ASB Standard 170, *Standard for Veterinary Forensic Postmortem Examination*
ANSI/ASB Standard 180, *Standard for the Use of GenBank for Taxonomic Assignment of Wildlife*

4. Documents currently in development at the OSAC or by the ASB:
NWP_013_WL: Standard for Construction of Multilocus Databases

Standards Development; AAFS ASB; OSAC

F82 Washington, DC, Department of Forensic Sciences: Achieving Accreditation Through Collaboration

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Learning Objectives: After attending this presentation, the audience will understand how a struggling crime laboratory can team with an external consultant to develop a quality assurance system and quality culture that are **International Organization for Standardization/**International Electrotechnical Commission (ISO/IEC) 17025 compliant and achieve accreditation.

Impact Statement: The impact of this presentation on forensic science is an overview of a model that can be applied in other jurisdictions to successfully achieve accreditation by collaborating with leaders in the field.

Abstract Text: On April 2, 2021, the American National Standards Institute (ANSI) National Accreditation Board (ANAB) suspended the Washington, DC, Department of Forensic Science's (DFS) Forensic Science Laboratory (FSL) accreditation. ANAB cited findings resulting from an investigation into purported errors made by the DFS Firearms Examination Unit and the DFS Management's alleged lack of disclosure as the cause for the suspension. On May 2, 2021, one month after the initial suspension, the ANAB withdrew the accreditation of all five of the DFS forensic disciplines: Firearms Examination Unit, Forensic Biology Unit, Forensic Chemistry Unit, Latent Fingerprint Unit, and the Digital Evidence Unit. The DFS immediately discontinued forensic casework operations.

The loss of accreditation provided an opportunity for all DFS forensic disciplines, even the previously thriving casework disciplines, to conduct an in-depth technical assessment and develop a strategic plan to achieve re-accreditation. The DFS, through an open, competitive solicitation process, entered into a contractual partnership with the independent, quality forensic consultant firm, FACT: Forensic Analysis. Consultant. and Training. The FACT team was made up of crime laboratory practitioners who all served in leadership positions not only within their laboratory system but also on several professional organizational boards. The FACT subject matter expert team members' working knowledge of government laboratories proved to be a critical resource that aided in the development of a robust strategic plan.

DFS worked in collaboration with FACT to develop a strategic plan that incorporated technical elements such as casework assessments and document reviews, as well as changing the quality culture and rebuilding and restoring trust with DFS customers and stakeholders. FACT conducted an in-depth analysis and assessment of the FSL forensic units. Based on recommendations made by the consultants:

- FSL made significant enhancements to technical procedures, including a comprehensive document review of all FSL standard operating procedures, laboratory operating procedures, and DFS agency-wide procedures. Updates to the procedures were made in accordance with ISO 17025:2017 and AR 3125.

- The overall quality management system of FSL was strengthened to include hiring a Chief Science Officer and a Quality Assurance Manager and assigning dedicated quality assurance specialists to each of the forensic disciplines.

- Mock casework was completed and reviewed to ensure compliance with new procedures.

ANAB conducted an external assessment of the Forensic Biology and Forensic Chemistry Units in December 2022. Zero nonconformities were identified in association with ISO 17025:2017, AR3125, and the FBI's Quality Assurance Standards for Forensic DNA Testing Laboratories (QAS). The DFS Forensic Biology and Chemistry Units achieved ANAB accreditation in December 2023. This presentation will take you through this journey and provide insight into how true collaboration and a shift in organizational mindset led to success.

Accreditation; Crime Laboratory; Quality Assurance

F83 A Facial Perspective Atlas: A Tool to Assist Morphological Facial Comparisons

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Learning Objectives: The attendees will learn about how tools such as databases can aid the analysis of facial features.

Impact Statement: This presentation will encourage the use/creation of databases as tools within forensic science, particularly where facial comparisons are being conducted.

Abstract Text: After attending this presentation, attendees will better understand the importance of tools (such as databases) to support analysis work, and how they can be used to make more informed observations. They will be presented with the methodology pertaining to both the creation of a database and analysis of facial features, alongside some results and conclusions in relation to such.

Imagery examiners will often be asked to document the observed facial features of an unknown individual and compare them to those of the defendant. However, the process of morphological analysis is subjective in nature and, with the evaluations drawn having the potential to be highly influential on the final verdict rendered within the courtroom, it is crucial that examiners are able to provide accurate conclusions. Despite its subjectivity, there is an absence of databases and tools that can be used to support the observations made by examiners, as well as numerous factors which can reduce the accuracy of the classifications and subsequent conclusions drawn. This research considers some of these factors, namely the height, the azimuth, and the distance of a camera from a subject in order to better understand the impact of the said variables.

To address the above, a database was constructed with the aim of creating a tool to aid examiners in making more informed and accurate decisions when analyzing facial features. This database was then used to assess the following research questions:

- At which distance are the greatest number of classifications made?
- At which elevation are the greatest number of classifications made?
- At what subject to camera geometry are the classifications most accurate?

Findings show that the database created can be used as an asset/tool in the analysis of facial features. Camera positioning was found to influence the number of classifications made significantly, with all parameters working in conjunction with each other.

CCTV; Morphological Analysis; Facial Features

F84 Forensic Reconstruction and Forensic Taphonomy: An Experiment for a High-Profile Case Conducted for the First Time in Chile

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Learning Objectives: After this presentation, attendees will learn the results of the forensic taphonomy experiment done for first time in Chile and the forensic reconstruction analysis. These conclusions helped the family of the victim and the state prosecutor to have scientific answers to know what happened to the deceased.

Impact Statement: This presentation will help the forensic science community to understand the importance and the answers that the investigators can get using taphonomy experiments and forensic reconstruction method and how this knowledge and skills can help solve hot or cold cases.

Abstract Text: This forensic taphonomy experiment and forensic reconstruction analysis was done because in a rural area in the south of Chile, a three-year-old boy went out with his great uncle to check the cattle; after 29 minutes the great uncle called to his niece, the mother of the boy, to tell her that the boy was missing.

The volunteers for all the country searched for the missing boy. After 9 days of searching, the boy was found deceased. The medical examiner office analyzed the body, and the forensic laboratories of the two national police agencies processed the scene and the evidence. After a few months and a lack of scientific conclusions, the state prosecutor could not establish what happened and could not conclude the Postmortem Interval (PMI) or cause and manner of death.

The forensic work I did in this case was divided into two parts. The first part was related to the forensic taphonomy experiment. The methods used in this experiment were Keough et al. and Megyesi et al., and the experience and methodology used for the Forensic Taphonomy Laboratory from Forensic Sciences Unit at Chaminade University of Honolulu.[1-3] This experiment was based on placed piglet carcasses, with a similar weight as the victim, in three spots that are important in the case investigation. The first scene was where the deceased was found, the other spot close to the river, and the last one was in a wooden area. These three spots were placed in a two-kilometer radius. The duration of this experiment was nine days, the same number of days that the victim was missing.

The objective of this experiment was to find out if the scene where the deceased was found was the primary or secondary scene.

The conclusions of this taphonomy experiment were that the scene where the victim was found was a secondary scene. The deceased was washed at least one time after his death, the body of the victim was hidden in a wooded area and close to the river, which helped to delay the taphonomy phenomena.

The second part of this analysis was the forensic reconstruction. This work was focused on analyzing the scene, the forensic reports, and evidence collected at the scene. For the process of the analysis of the victim's clothes, I did a detailed analysis of each item. The first stage was a visual observation with direct and oblique light, the second stage was with Alternate Light Source (ALS), and the last stage was based on the utilization of the new product called STK sperm tracker. This last stage was made to confirm whether or not there was a possible sexual attack.

The conclusions of this analysis were: I found new evidence (sperm cells) in the underwear, t-shirt, and hoody of the victim, which confirmed that the victim suffered a sexual attack. After the results of the forensic reconstruction analysis, I was able to provide new scientific hypothesis and conclusions to the family of the victim and the state prosecutor related with the PMI, and possible cause and manner of death of the child.

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Forensic Reconstruction; Forensic Taphonomy; Missing Children

F85 The Sad Story of Two Young Lovers: The Truth Lies in the Details

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Learning Objectives: Attendees will learn the importance of the judicial inspection.

Impact Statement: Attendees will understand the use and misuse of technology. It can solve cases, but it can also backfire.

Abstract Text: Discovering two corpses on crime scene always generates a great deal of interest. In order to ensure that no element is overlooked, a forensic inspection must be conducted flawlessly. The case to be discussed below deals with the discovery of the corpses of a young couple, "Renzo and Lucia" (fictitious names), inside the bathroom of the woman's house. At the time the coroner arrived, the crime scene had been polluted: rooms' doors were no longer in their original position, room temperatures had changed, and there was no certainty that bodies had not been moved.

The judicial inspection revealed a woman about 20 years old inside the bathtub full of stagnant water, supine, naked, with her right leg out of the bathtub, and a foamy fungus as well as a lacerated contusion wound in occipital region. A man about 20 years old was lying supine naked on the floor; his genitals had been covered with a pair of jeans and also with a more modest foamy fungus. In the bathroom, an electric fan to heat the room was on, as were the thermostats fixed to the wall. The bathroom's door was described by the first people who entered the premises as partially closed, and the window was closed. Hanging on the wall and above the bathtub was a small methane water heater, which appeared not to be in operation.

The cause of the deaths was established by an external cadaveric inspection that concluded a low-voltage electrocution for both of the subjects. Party family consultants of both subjects disagreed with the cause of death. There was talk of murder perpetrated by the young Renzo against Lucia and vice versa, so an autopsy of the interred corpses was ordered.

In this circumstance, the corpses were in the initial stage of corification in zinc caskets and not yet fully decomposed, so an examination of the injuries of the two young people was possible. Toxicological tests on blood showed a weak positivity to carbon monoxide. Therefore, it was hypothesized that both Renzo and Lucia died of Carbon Monoxide (CO) intoxication. However, toxicological results were objected to on the grounds of their low scientific sustainability. Thus, two years after the first exhumation, new autopsies were performed. The second exhumation concluded that the cause of death was accidental drowning for both of them; homicide and suicide was excluded. However, even the second exhumation did not convince all parties and judges; hence, a new exhumation of the two corpses was ordered 22 years after death.

On this occasion nothing was left to chance: Lucia's occipital injury was not accompanied by skull fractures and Renzo had no fractured bones. A toxicological analysis to find CO in myoglobin was conducted, using femoral and psoas muscle that were partly mummified, but a different method was adopted: fixation with palladium chloride.[1] This technique allowed us to find a high concentration of CO adhered to myoglobin in Renzo's corpse and significantly less in Lucia's muscle.

This evidence allowed us to conclude that the two lovers died in different ways and specifically that Lucia had died of drowning after fainting from inhaling a small amount of CO. Renzo, on the other hand, lost consciousness trying to rescue Lucia, fell on the ground, and continued to inhale the toxic gas until death.

This case has shown us how small mistakes in the initial handling of a crime scene can be followed by a chain of even more serious mistakes that result in the truth being distanced. This case was solved with the help of technology, it is true, but if the technology is not wisely guided, its use can only backfire.

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Crime Scene Investigation; Cause of Death; Remains

F86 Combating Wrongful Murder Statements With Technology: A Wrongful Conviction Case Study

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Learning Objectives: After attending this presentation, attendees will better appreciate how technology, physical evidence, and crime scene reconstruction techniques are used to confirm or refute false statements of guilt.

Impact Statement: This presentation will impact the forensic science community by sharing the experience gained following a crime scene reconstruction of a horrific murder and a wrongful conviction of two young black males.

Abstract Text: A single shot rings out on the fourth floor of an apartment building. The lifeless body of a Japanese national young male was observed on the landing of the stairwell with a single gunshot wound to the head. The police investigation would take some twists and turns that led to the arrest and conviction of two young Black males. They admitted to committing the murder and you would think the story ends there, but their statements did not match each other nor did the forensic evidence.

The suspects' statements also did not match a police theory at that time. Is it possible that the police elicited false statements? While in jail, one of the defendants would plead to the court that he initially confessed because he was hungry and thirsty, and the police threatened to go after his mother. He also believed that the truth would come out in court, but that was not meant to be. As the years passed, the appeals court would affirm the conviction. One suspect filed unsuccessful motions and petitions, seeking to overturn the conviction in 2010, 2013, and 2014. Eventually it would come to be that a bad police investigation would reveal itself. The District Attorney's Office would recognize that the case was not what it had appeared to be and recommended that it be reopened by the Innocence Project. It is the result of an investigation of the erroneous statements that did not fit the forensic evidence, and the reconstruction of events using current technologies, that would break the truth of the case.

This murder case demonstrates how crime scene reconstruction methods can reveal false confessions, confirm sequence of events, and the location of the true crime scene. It would eventually free not one but two men from the bonds of a false murder conviction. One served more than 8 years in jail and the other more than 27 years. In the end, the truth would be revealed, resulting in their convictions being overturned.

This was accomplished through the collaboration and hard work of many attorneys from the Queens District Attorney's Office, the New Jersey Innocence Project, the Center on Wrongful Convictions at Northwestern-Pritzker Law School, law students, and the New Jersey Institute of Technology (NJIT) forensic science practitioners and students.

Reconstruction; Murder; Wrongful Conviction

F87 Forensic Sciences as an Instrument for Improving the Lives of Populations: A Multidisciplinary Theoretical-Practical Case Study in Brazil

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WITHDRAWN

F88 Electric Vehicles: A Technological Firestorm

Stacey L. Chepren, MS, United States Air Force, Tucson, AZ*

Learning Objectives: Attendees of this presentation will learn basic concepts of lithium-ion battery technology, its rise in popularity for use in passenger vehicles, and the extreme challenges this technology is causing for public safety personnel.

Impact Statement: This presentation will impact the forensic science community by providing information about the dangers to public safety personnel responding to fires involving an electric vehicle with a lithium-ion battery as well as the challenges these scenes pose for medicolegal death investigation.

Abstract Text: Lithium-ion battery technology has experienced a sharp rise in popularity for use in a variety of items, including electronic devices and passenger vehicles. There are over 40 models of Electric Vehicles (EVs) available in the United States. These models include cars, trucks, and sport utility vehicles. With more electric vehicles on the road, a dangerous trend has been noted by agencies that respond to vehicle fires involving EVs.

EV fires burn much hotter and faster than a gasoline-powered vehicle. On average, a gas-powered vehicle fire will reach a temperature of around 1,500 degrees, while an EV fire can reach up to 5,000 degrees. An EV can reach a temperature of 1,200 degrees in one-tenth of a second. EV fires are also extremely hard to extinguish and often experience a phenomenon called "thermal runaway." This phenomenon causes an uncontrollable rise in the temperature to the point where the fire becomes self-sustainable, making the fire extremely hard to extinguish.

Occupants of a vehicle have less time to escape an EV fire due to the immediate intensity of the fire. Their chance of survival is also lowered due to firefighters being unable to easily get these fires under control. EV fires will burn for weeks. Additionally, an EV can be much more challenging for occupants to escape due to the power required to operate the door handles. There are manual releases in most models, however, they are not always easily accessible. Once extinguished, they have been known to re-ignite hours later, causing a significant risk to their storage location. Firefighters have been known to contact the vehicle manufacturer directly while on scene in an effort to help get control of the fire.

Special devices have been invented in an effort to combat the severity of EV fires. These include specialized hose attachments that spray water under the battery and heavy fire blankets that are intended to contain the fire. Some of these items are one-time use and are not always feasible to replace due to budget constraints. These fires also strain the fire response throughout an area due to the excess manpower they require. One EV fire can generate a need for a response of 60 firefighters.

The field of death investigation is impacted by the tactics firefighters must employ by causing significant postmortem artifacts that are not related to an occupant's injuries sustained in the initial crash. These tactics can also cause artifacts that mimic injuries, making the investigation more difficult to discern what was caused by an impact and what was caused during the process of extinguishing the fire.

Medicolegal Death Investigation; Fire; Lithium-Ion

F89 Leveraging Analytical Chemistry and Advanced Flow Visualization for the Evaluation of Canine Training Aid Containment Systems

Shawna F. Gallegos, PhD, Oak Ridge Institute for Science and Education, Aberdeen Proving Ground, MD; Matthew Staymates, MS*, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Objectives: This presentation will explore the different types of containment systems used for storage of canine training aids and will provide attendees a thorough understanding of properties of each system. Utilizing analytical chemistry and flow visualization approaches, the data will elucidate the ability of the system to contain materials as well as the potential for contamination of stored materials from the system itself.

Impact Statement: At the end of this presentation, attendees will have a foundational knowledge of the efficacy of a full range of containment systems that are currently in use by the working dog community. This will provide the working dog community with information that will aid in providing an informed approach when choosing a training aid containment system.

Abstract Text: Detection canines are utilized as sensors and trained to the odor (s) required to perform their respective detection tasks. This training involves the use of canine training aids of various types, including true materials such as explosives, seized narcotics, etc., as well as soaks, dilutions, mimics, and sorption aids. To contain these training aids, there are a multitude of systems that are regularly used by canine handlers and trainers, including aluminized bags, plastic and glass snap-ware, plastic zip top bags, mason jars, canvas bags and the Training Aid Delivery Device (TADD) to name a few. While there is extensive focus on the quality of the various training aid materials, little attention has been given to the quality of the containment system utilized for the storage, transport and active training of the canine. The choice of containment system can have a substantial effect on the quality of the contained material, including the potential for cross-contamination from other materials stored in proximity to the aid as well as potential for contamination from the system itself. These issues can contribute to an inferior training aid, thereby directly impacting the quality of training of the canine. As there is no set standard for the containment of canine training aid materials, there is a critical need for extensive evaluation of the containment systems that are regularly employed. Leveraging analytical chemistry, the efficacy of each containment system can be evaluated to better assess the quality of canine training aids when they are enclosed in a containment system.

This presentation will focus on coupling analytical chemistry measurements of training aid containment systems with qualitative flow visualization of vapor and particle transport in a variety of relevant scenarios. Schlieren imaging enables the visualization of odors and vapors in the air and ultimately demonstrates the ability of a system to fully contain a material. Seeing how vapors and/or particles from training aid materials leak out of various objects helps identify the best approaches for the containment of these materials. This work can equip scientists and working dog operators with a bolstered comprehension of best practice containment of training aid materials.

Canines; Training Aid Containment; Flow Visualization

F90 A Validation of Inkjet Printing Methodology in the Development of Non-Detonable Explosive Training Aids

Shawna F. Gallegos, PhD, Oak Ridge Institute for Science and Education, Aberdeen Proving Ground, MD*

Learning Objectives: This presentation will investigate the development of a non-detonable explosive training aid utilizing ink-jet printing technology by employing an all-encompassing approach to the development of non-detonable training aids wherein the quality of the material is chemically evaluated at each step of production, (i.e., the containment system, the true bulk material, the inkjet-printed non-detonable material, shelf life and service life, and operational canine trials).

Impact Statement: In this presentation, attendees will be provided with a prospective alternative to current and emerging explosive threat materials used in canine training.

Abstract Text: Explosive Detection Canines (EDCs) are trained to bulk quantities of explosive threat materials (i.e., grams, pounds). However, due to the hazardous nature of the explosives, there remains a need for a non-detonable alternative that can be used in place of the bulk quantities while still exhibiting the odorant(s) of the threat. This is especially critical when considering Homemade Explosive (HME) materials that may be too sensitive or hazardous for handling in a training scenario. The implementation of inkjet-printing deposition techniques to apply a small amount of explosives onto inert substrates is a potential route to the development of an effective, non-detonable training aid. With a print solution that is made from actual explosives, the odor profile is thereby comparable to that of the bulk material and due to the small quantity of explosive deposit, the training aid is rendered non-detonable. The inkjet-printed training aid is designed as a single-use aid with a known shelf/service life. The risk of contamination or degradation from repeated use is thereby mitigated, and there is no need for special disposal procedures.

This presentation will focus on the implementation of analytical chemistry techniques as well as operational canine trials for a comprehensive assessment and validation of a non-detonable inkjet-printed training aid. Volatile Organic Compound (VOC) collection coupled with two-dimensional Gas Chromatography-Time Of Flight/Mass Spectrometry (GCxGC-TOF/MS) will allow for an extensive snapshot of the odorants associated with the bulk explosive and related print materials. Additionally, operational canine trials will further validate the amount and quality of the odorants present in both the bulk explosive materials as well as the developed non-detonable aid. This method allows for a reduction in the risk associated with the transport and handling of bulk explosives and attainability of a rapid production approach for additional odorants as threats emerge.

Canines; Non-Detonable Explosive Training Aid; Volatiles

F91 Investigating Nuisance Alerts Given by Canines Arising From Substrates Exposed to Composition C-4

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Learning Objectives: The attendees will learn about the volatiles emitting from C-4 that get retained in the substrates that come in contact with explosives. We will explore substrates such as cotton, sheet metal, cardboard, glass, and wood. This presentation will focus on various extraction methods for analysis of these substrates using Gas Chromatography/Mass Spectrometry (GC/MS). Finally, swabbing will also be explored as a sampling method for detection of Volatile Organic Compounds (VOCs) in substrates of interest.

Impact Statement: The impact of this research will help in a better understanding of nuisance alerts that arise as a result of exposure to explosive mixtures. Comparison of different substrates capability to retain the VOCs emitting from C-4 will help in developing a protocol for analysis of these substrates using GC/MS. The best extraction method will also be chosen on the basis of a comparison study of varied extraction methods.

Abstract Text: Explosive Detecting Canines (EDCs) are routinely used for real-time detection of explosives as they can be relied upon for detection of VOCs associated with explosive mixtures. VOCs such as taggants are added to the explosives at the time of manufacture to aid in their detection and identification. Common taggants associated with explosives include energetic VOCs such as 2,3-Dimethyl-2,3-Dinitrobutane (DMNB), 4-nitrotoluene, and Ethylene Glycol Dinitrate (EGDN). EDCs can be trained to detect target odors and show high sensitivity toward them. Their ability to detect VOCs to parts per trillion level makes EDCs an excellent candidate to use for explosive detection. However, the high sensitivity shown by EDCs can allow them to detect invisible residues of explosives on various substrates leading to the possibility of "nuisance alerts." Moreover, training aids and other points of contact that may be exposed to VOCs emitting from explosives during various stages such as manufacturing, transport, and storage run the risk of becoming cross-contaminated. This cross-contamination of substrates can lead to an increase in the instances of nuisance alerts which can be time- and resource-consuming. Therefore, it is important to understand the extent of contamination that various substrates may exhibit on being exposed to explosives.

In this study, commonly encountered substrates such as cotton, wood, cardboard, sheet metal, and glass were exposed to composition C-4 (C4) and analyzed for the presence of VOCs. Common VOCs associated with C4 include DMNB, 2 ethyl-1- hexanol, and cyclohexanone. Triplicates of these substrates were exposed to 1-gram C4 for one week and analyzed using GC/MS. Different extraction methods such as liquid injection, headspace analysis, and Solid Phase Microextraction (SPME) techniques were evaluated to establish a protocol for substrate analysis. It was observed that porous substrates such as wood and cardboard have a higher retention capacity for volatiles in comparison to non-porous substrates such as sheet metal and glass. SPME was found to be most efficient extraction technique for analysis of these substrates as DMNB was observed on all substrates using this technique. In addition, swabbing was evaluated as a sampling technique for VOCs on substrates exposed to C4 and Total Vaporization-Solid Phase Microextraction (TV-SPME) was used to analyze the swabs. No volatiles associated with C4 were found on the swabs, suggesting that swabbing is not an ideal sampling technique for the analysis of these substrates. To the best of our knowledge, this study is the first of its kind in investigating the possibility of nuisance alerts that may arise from commonly found substrates. Future work can focus on exploring other sampling techniques for analyzing the substrates of interest exposed to C4.

Explosive Detecting Canines; Volatile Organic Compounds; Extraction

F92 Micro-CT Imaging and 3D Laser Scanning as Tools for Evidence Presentation in Court

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Learning Objectives: This presentation will cover some of the casework undertaken as part of the partnership between the Warwick Manufacturing Group, the University of Warwick, United Kingdom, and West Midlands Police, through which the advantages and application of micro-Computed Tomography (micro-CT) and surface laser scanning will be highlighted in criminal investigations. The use of high-quality 3D and 2D imaging to accentuate minute details from forensic evidence in court and for a better presentation of evidence to the judge and jury, acquired through the high-resolution micro-CT instrumentation available and surface laser scanning, will be discussed as technological advancements used within the court system.

Impact Statement: The partnership between the forensics team at the Warwick Manufacturing Group, Warwick University, and West Midlands Police has aided over 350 forensic investigations since 2014 and over 30 police forces across the United Kingdom and internationally by providing micro-CT imaging and laser scanning to support police casework. Through the research partnership with the police, barriers to accessing specialist equipment have been overcome, thus bridging the gap between academia and police casework.

Abstract Text: The Forensic Centre for Digital Scanning and 3D printing at the Warwick Manufacturing Group, Warwick University, benefits from five different X-ray micro-CT instruments, each having different specifications, which allow for a wide variety of sample types and sizes to be scanned and analyzed. Through the forensics work performed at the Warwick Manufacturing Group, micro-CT is used as a complementary technique to aid Home Office Pathologists or other experts (e.g., histopathologists) and the police in their (medicolegal) investigations.[1,2] Some of the investigations that the center was involved in included analysis of trauma from strangulation or blunt/sharp force injuries, domestic abuse, arson, and 3D-printed weapons.

Micro-CT as a non-destructive imaging technique is especially useful prior to any further destructive analysis techniques (e.g., histopathology of bones), as a permanent record of the exhibits can be stored. The high-resolution 2D and 3D images acquired can be used for a better presentation of forensic evidence in court, particularly in difficult cases with multiple samples (i.e., dismemberments) or cases of continuous abuse where multiple injuries of varying ages have been identified,[1,3]. Within the forensics center, the most common type of cases submitted for micro-CT scanning are strangulations (~44 %), followed by child abuse cases (~20 %). The latter usually requires a large amount of resources as multiple experts must be involved in an investigation, and there is generally a high number of samples with multiple injuries of varying ages being submitted for scanning.

Histopathology of bones is the gold standard in fracture identification and ageing, though it is time-consuming, particularly when larger samples are involved, such as the rib cage, which is also the most common exhibit submitted for micro-CT scanning in pediatric cases. Therefore, by complementing the histopathology examination with micro-CT scanning, the target sectioning of a sample is made easier by highlighting areas of interest from the micro-CT data. Moreover, by combining the micro-CT scan and the aging of fractures from histopathology, high-quality videos containing 3D views and 2D images can be used to present the evidence in court, particularly when fracture aging is a very important aspect in an investigation where there is evidence of multiple separate events of abuse that occurred prior to death.[3]

With the use of 3D printing technology and the capabilities available within the department, 3D printing of exhibits can additionally be done from the micro-CT data, which has proved imperative in emphasizing the location and severity of trauma and in "sanitizing" the evidence for the jury.[4] The center also benefits from a laser scanner, which can be used, for example, in 3D crime scene scanning and reconstructions, scanning of tools recovered from a scene, etc. Scanning of a crime scene has seen valuable impact in blood pattern analysis, gunshot trajectories, and disproving/approving eyewitness testimony.[5] The 3D scans of a crime scene can also serve as a digital record of the scene, and interactive videos of the scene can be shown in court when evidence is presented, hence ensuring a better understanding and the engagement of the jury. Thus, through this presentation, an overview will be given of the different investigations in which micro-CT and laser scanning were employed, and how the evidence was used and presented in court, as a demonstration of technology as a tool for transformation.

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Micro-CT Imaging; Crime Scene Scanning; Police Casework

F93 The Effects of a Bullet on the Motion and Distribution of Unburnt Gunshot Residue Particles

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Learning Objectives: This presentation will describe the use of high-speed imaging to document the interaction of fired bullets, Gunshot Residue (GSR) particles, and fabric targets during the external (in-flight) and terminal (target impact) ballistic stages. Following this presentation, attendees will have a better understanding of GSR deposition mechanics as well as the sources of variation observed between fired shots for the purpose of close-range shooting-incident reconstruction.

Impact Statement: This presentation will impact the forensic science community by improving the understanding of external- and terminal-ballistic interactions of fired bullets, GSRs, and fabrics. This will allow insight into ballistic behavior and sources of shot-to-shot variation with respect to close-range shooting reconstruction.

Abstract Text: The transfer of GSRs is most-often depicted as a singular event occurring as the bullet exits the barrel (transitional ballistics), with little description of what happens next. It is well understood that variables such as the ammunition, caliber, and firearm barrel-length significantly affect GSR patterns. However, little information exists regarding the external and terminal ballistics of GSRs. This study focuses on two aspects of GSR-pattern formation: (1) the interactions between bullet and propellant particles in-flight (external ballistics); and (2) the interactions between propellant particles and fabric upon impact (terminal ballistics). It is believed that these interactions affect the distribution and retention of GSRs more significantly than is currently recognized.

Targets will be comprised of three fabric materials: cotton, faux leather, or fleece. These will be fired upon at 6-inch (15cm) increments from 6 to 36 inches (15–91cm) using three semiautomatic pistols: .40 S&W caliber Glock model 22; 9mm caliber Glock model 45, and a .22 LR caliber Smith & Wesson model 622 held in a Ransom International Master Series pistol rest. All shooting trials will be recorded on a Phantom TMX 7510 High-Speed Camera with Zeiss 100mm Macro lens. The propellant-particle, vaporous lead, and soot patterns will be subsequently documented using an Image Access WideTek 12 scanner in Blue-Green Fluorescence (BGF) and Infrared (IR) modes. This device allows quantitative analysis of GSR patterns that are not feasible with chemographic methods such as the modified Griess test and sodium rhodizonate test. This combination allows qualitative behaviors observed using high-speed imaging to be compared to quantitative results observed in the corresponding targets. By removing the variation in target processing associated with the chemographic methods, the shot-to-shot variability in GSR patterns can be better controlled and understood. This will allow more accurate measurement of GSR pattern characteristics corresponding to specific ammunition, fabrics, and ballistic behaviors.

This study will demonstrate the degree to which interactions between bullets, propellant particles, and fabrics lead to significant changes in GSR patterns. These results will benefit the forensic science community by improving upon existing models of GSR deposition. Understanding the factors that lead to variation in GSR patterns will allow firearm examiners to better control variables during shooting reconstructions and more accurately estimate muzzle-to-target distance.

Ballistics; Reconstruction; High-Speed Imaging

F94 Process Maps: The Beginning of a Technological Revolution for Fire Investigations

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Learning Objectives: After attending this presentation, attendees will appreciate the reasons for the Organization of Scientific Area Committees' (OSAC's) initiative to develop interactive process maps to facilitate the evidence examination process across forensic disciplines, using fire investigations as an example.

Impact Statement: This presentation will impact the forensic science community by explaining the concept of process maps and examining their advantages and disadvantages for expert witnesses and attorneys.

Abstract Text: The impetus for this presentation came from the work of the OSAC Fire & Explosion Investigation Subcommittee (F&EI Sub.). In March 2023, the F&EI Sub. made public its "Fire Investigation Process Map (Current Practice)."[1] At first glance, it appears daunting, with a complex arrangement of various shapes containing instructions and questions, with arrows and other shapes connecting related sections of this 17-page map.

Before addressing this particular process map, the author begins by describing process mapping, considering its benefits when contrasted with checklists and flowcharts. Next, we explain the growing complexity of the two standards governing fire investigations National Fire Protection Association (NFPA) 921 *Guide to Fire and Explosion Investigations* and NFPA 1033 *Standard for Professional Qualifications of Fire Investigator*.[2,3] The evolution of these standards has given rise to a need for using process maps to effectively manage and document data collection and analysis in the process of fire origin and cause determination.

Every coin has two sides. The same can be said of process maps. We will highlight some of its pros and cons. For example, on the downside, the OSAC process map in its current PDF format may require an investment of considerable time to thoroughly digest it or to apply in a particular fire incident, at least until an investigator becomes familiar with it.

However, even as it now stands, the map is valuable. It incorporates methodologies from NFPA 921, providing a tool to put its recommendations into practice. As such, the map can improve the data collection and analytical processes involved, while facilitating documentation of the considerable information contemplated by both NFPA 921 and NFPA 1033. Further, it represents the combined efforts of several fire investigation experts over a period of years who have broken down the process of fire origin and cause investigations into a logical sequence of steps. Thus, it has the potential to improve the quality of investigations, particularly for those who are new to investigations or not otherwise detail oriented.

The OSAC process map is also a great resource for peer-reviewing investigations. Similarly, in litigation, it will prove useful for attorneys who are evaluating a fire investigation expert's work. It will help to identify gaps in the investigation, including data that was available but not recorded, or hypotheses that should have been developed based on the data available. Investigators who use the process map can minimize such gaps. Courts may eventually look to process mapping as an indicator of reliability.

Most of the steps (actions, decision points) in the map can be traced back to NFPA 921, NFPA 1033, or other authoritative publications. This means that even if investigators do not use the map or acknowledge it as authoritative, they can still be challenged using the underlying principles from industry standards or other reference sources.

The process map represents a major landmark in further improving the fire investigation discipline, but it is cumbersome. Either the government (i.e., National Institute of Standards and Technology [NIST]) or some other organization will transition it into an interactive web-based app or import it into widely available process mapping software. This technology will make it user-friendly and have the potential to further revolutionize fire investigations and how fire or arson cases are litigated.

References:

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Process Maps; Fire Investigations; NFPA 921

F95 Forensic Genetic Genealogy: An Emerging Tool for Identification in Transnational Missing Persons Cases

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Learning Objectives: Attendees of this presentation will gain a broad understanding of Forensic Genetic Genealogy (FGG) as an advanced tool for identifying missing persons in transnational cases. The presentation aims to elucidate the distinctions between FGG and traditional forensic genetics methods in identifying human remains and establishing familial connections. However, the applicability of FGG as an investigative tool hinges on meticulous analysis of technical, operational, legal, and ethical considerations in human identification, particularly in forensic applications.

Impact Statement: Forensic sciences play a pivotal role in the investigation, localization, and identification of missing persons. Among various identification methods, DNA stands out for its ability to establish genetic links between missing individuals and their relatives. A cutting-edge technology in this field is FGG, which offers a more comprehensive approach to kinship analysis.[1,2]

Abstract Text: The quest for innovative methods to identify missing persons has intensified in recent decades. Transnational disappearances present compounded challenges due to factors such as large-scale migrations, human trafficking, and fragmented identification systems. Investigating these cases is crucial not only for fostering social cohesion but also for maintaining peace and ensuring human security.[3]

The principles and methodologies behind FGG, including its integration of molecular marker testing, genetic databases, and genealogical research, will be explained. FGG aims to provide resources for identifying distant family connections, with its effectiveness needing evaluation across both native and immigrant populations.[4,5] It is crucial to rigorously test FGG in diverse demographic contexts and consider its potential limitations.

This presentation will provoke discussion on the impact of FGG within the forensic science community. Attendees will be encouraged to contemplate the practical applications of FGG in identifying missing migrants, thereby enhancing their ability to identify missing persons, especially in migration cases. Integrating FGG into practice may enhance success rates in resolving cases, thereby improving stakeholder outcomes such as providing closure to families and delivering justice to victims of transnational disappearances.

Future prospects and challenges in the field of FGG should be discussed within academic and professional circles. The application of FGG is expected to contribute to the development of comprehensive strategies for identifying missing persons and provide a hopeful outlook for addressing complex issues associated with transnational disappearances.

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Human Identification; Forensic Genealogy; Transnational Disappearance

F96 Watkins Who?: The Use of Forensic Genealogy in a Medical Examiner's Cold Case

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Learning Objectives: After attending this presentation, attendees will better understand the available methods used to identify unknown individuals and the mounting benefits of using forensic genetic genealogy in a modern medical examiner's office.

Impact Statement: This presentation will impact the forensic community by demonstrating the complexity of modern unidentified person cases and the growing scientific technology of DNA and genetic genealogy that allows us to further give a voice to those who have been silenced in death.

Abstract Text: The Hennepin County Medical Examiner (HCME) serves a population of approximately 1.9 million, or roughly one-third of the state of Minnesota. Since its inception in 1963, the HCME has been involved in the investigation and storage of roughly 235 unidentified individuals. In 2021, the HCME opened a state-of-the-art facility with space and technology to specifically address the importance of decedent identification. Concurrent with the design of the new facility and Minnesota State Statute 390.25, the HCME initiated a multiyear project focused directly on unidentified persons. With the advancement of DNA and the rapidly growing field of forensic genetic genealogy, medical examiner's offices can still provide answers to those that have long given up hope.

In this case study, an individual checked into a local Minneapolis hotel in 1996 with no expectation of checking out. The decedent was found in the hotel room several days after her arrival to the hotel and was taken to the HCME for further examination. Through the investigation it was found that the decedent had given a false name and address. Manner of death was ruled suicide, and this decedent has stayed at the HCME throughout the decades.

The various technologies and investigative methods employed from the time of her death in 1996 to the moment of her identification in 2023 will be presented. Through the years, the complexity of unidentified cold cases has increased for various reasons (e.g., degradation of the evidence, loss of the documentation or remains, overall growing caseloads), which will be discussed. This presentation will also address the challenges, such as funding or staffing, that can arise during an investigation of this manner in a medical examiner's office. However, the focus of this case study is that it highlights the value of forensic genealogy to identify those who remain unnamed when all other methods and available technology failed to provide closure.

Medical Examiner; Genealogy; Unidentified Persons

F97 The Development and Validation of the EX38 Kit: A Comprehensive Forensic Tool for Enhanced Genetic Identification

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WITHDRAWN

F98 Wildlife Forensic Proteomics: Approaches and Applications

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Learning Objectives: Attendees will learn the following objectives: (1) be able to generally define "wildlife forensics" and "forensic proteomics;" (2) identify three methods used for taxonomic identifications; (3) describe the importance of interdisciplinary collaborations when implementing novel technology to casework; (4) identify the key features of scientific best practices and standards in the forensic context; (5) understand the limitations of forensic proteomic testing techniques; and (6) describe how forensic proteomics is being applied to wildlife forensic casework.

Impact Statement: The field of forensic proteomics is a nascent field that has the potential to solve previously unsolvable cases. Without sharing the relatively obscure work being conducted in the field of wildlife forensics, the human-based forensic community may be unaware of the possibilities to solve cases that require innovative methods in order to answer challenging forensic questions. Presenting directly to the broader forensic science community allows for awareness and recognition of the power of proteomic testing capabilities and increases the possibility that these techniques will be incorporated into other forensic laboratories. Interdisciplinary communication is critical to ensure that crimes on both humans and wildlife are combatted with the latest available technology. Additionally, open communication ensures that the limitations of forensic proteomics are disclosed and discussed, and that implementation of this novel technology is based on forensic best practices and standards.

Abstract Text: Wildlife forensics is a broad discipline requiring interdisciplinary techniques and expertise to effectively address the massive scale of the illegal wildlife trade—the fourth-largest transcontinental crime with an estimated black market value of $20 billion per year.[1] Traditional DNA or morphology-based methods of identification may fail when the biological source material is degraded, altered in appearance, fragmented, or has been subjected to extreme chemical or heat processes. The implementation of novel forensic proteomic workflows enables taxonomic resolution of samples encountered in wildlife trafficking investigations ranging from leathers, furs, skins, feathers, ivory, shells, and claws to cosmetics, foods, pills, powders, or pastes originating from a plant or animal.

Wildlife Forensic Specialists at the California Department of Fish and Wildlife (CDFW), along with interdisciplinary collaborators, are working to implement proteomic techniques for wildlife forensic casework purposes, including identification of mixtures of distinct species and identification of illegal species contributors within a comingled sample. Current research and casework applications of wildlife forensic proteomics include the identification of ivory, shark, pangolin, fur-bearing, aquatic, avian, and plant species that are protected by state, federal, and international laws and regulations.

Implementation of wildlife forensic proteomic tools requires: (1) identification of species and sample types relevant to law enforcement and conservation, (2) development and validation of methodology for casework, (3) adherence to scientific best practices and standards, and (4) interdisciplinary collaborations to harness the full potential of forensic proteomics. These aspects must evolve apace, as the opportunities for increasing data yield offered by optimized instrumentation and analytical algorithms must be stringently evaluated within a framework of inquiry that maximizes probative value while prioritizing ethical-legal imperatives. Ultimately, the direct application of proteomic tools to the field of wildlife forensics expands casework capabilities to solve previously unsolvable cases, thus supporting the enforcement and prosecution of wildlife crimes.

Reference:

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Wildlife Forensics; Proteomics; Casework

F99 You Can Get DNA Conclusions From That?: Case Studies Involving Animal DNA

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Learning Objectives: After attending this presentation, attendees will understand and be able to recognize opportunities to utilize animal DNA in the cases that come across their desk. They will gain the knowledge needed to pursue this type of testing.

Impact Statement: This presentation will impact the forensic science community by increasing awareness of the applications of domestic animal forensic DNA analysis in a variety of cases, including where the crime is between humans (for example, homicide or sexual assault).

Abstract Text: As forensic scientists, it is our job to speak for the evidence, to articulate what it is telling us to the jury. As technology progresses there becomes more and more information that we can learn from evidence using new and different methods. As forensic scientists, we need to be knowledgeable about what is available outside of our area of expertise in order to give investigators the chance to know the most about their evidence.

Consider these scenarios:

1. After analyzing a submitted tape lift for trace evidence, you conclude that the only thing present is animal hairs.
1. As you start a footwear comparison, you notice what looks like feces in the tread of the shoe.
3. After a positive presumptive test for blood from a visible stain on a blade, there is no quantifiable DNA.

In each of these scenarios, there are DNA conclusions that can be drawn—just not human DNA conclusions. The animals we live with and those in our neighborhoods leave DNA as well. These DNA conclusions can be incredibly probative depending on the nature of the case and with comparably high likelihood ratios as are expected from human DNA analysis. The field of forensic animal DNA analysis is now over 20 years old with more and more laboratories becoming accredited every year worldwide.

The UC Davis Veterinary Genetics Laboratory (UC Davis VGL) was one of the first laboratories to be accredited to do forensic DNA analysis on animal evidence in 2010 and to this day is the only laboratory in the United States to do so primarily for domesticated animals. While there are clearly applications of analyzing animal DNA in illegal wildlife trade or animal cruelty cases, because domesticated animals are those we welcome into our families and coexist with, the VGL often sees cases that are part of violent human-on-human investigations such as homicide or sexual assault.

This report will present multiple and varied case studies of domestic animal DNA being applied in different case situations. All of these cases came from investigators and forensic scientists who, when faced with "just animal hairs" or "feces on a shoe," had the forethought to keep pushing, finding the cutting edge of what is possible and pursuing it.

Casework; Cold Case; Genetics

F100 Safeguarding the Community: Rewriting the Narrative on the Modern Coroner in Today's Society

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Learning Objectives: With this presentation, the learner will understand the critical role of the medicolegal coroner in America today. Attendees will come to understand the necessity of the modern coroner's partnership with the forensic pathologist and their significant role in safeguarding the community. This presentation will highlight the valuable investigative efforts coroners use in determining cause and manner of death, and also how coroners aid other community stakeholders such as law enforcement, protective services, and medical care providers as well as trauma services in protecting the community in which they serve.

Impact Statement: The impact of this presentation will be significant. Recognition of the modern medicolegal coroner as a partner of the forensic pathologist and as an integral component of the medicolegal investigation process allows for better protection of the communities in which they serve. Dispelling the narrative of the coroner as being a glorified transport service will allow for better training, funding, and acceptance. It will also provide for improvements in overall community safety, law enforcement, and medical treatment.

This presentation will discuss the shifting narrative of the coroner's office in America. It begins with a brief review of the historical role of the coroner to the nationally certified professional coroner being instituted today. This presentation will highlight the necessity of the modern coroner and its significant role in safeguarding the community.

Currently, the medicolegal death investigation system in the United States varies between and within states, employing one or a combination of systems, including the coroner, medical examiner, justice of the peace, or mixed systems.

The National Association of Medical Examiners, a proponent of the medical examiner model, has long called for the abolishment of the coroner system, despite the continued and critical shortage of forensic pathologists. Moreover, with a lack of these essential physicians to support such a transition, the feasibility of such a widespread change is most impractical.

The American Board of Medicolegal Death Investigators (ABMDI) was established, in no small part, as an effort to fill the medicolegal knowledge gap at death scenes. In addition, they instituted forensic standards as set forth in the National Institutes of Justice, for all professional medicolegal death investigators to practice. The ABMDI was recognized and accredited as a forensic specialty by the Forensic Specialties Accreditation Board in 2005.

With the advent of the ABMDI and the application of their standards, the modern medicolegal coroner has demonstrative value to the death investigation process. These investigative professionals are not only essential to the forensic pathologist but may also aid law enforcement in directing their own investigative process.

While the narrative of the coroner as glorified body transport or "body snatchers" persists, the recognition of the modern coroner as a partner of the forensic pathologists and a valued component to a complete multidisciplinary medicolegal investigation is now accepted as a national forensic standard.

In many states, coroners remain elected officials with little that prequalifies them for office. However, modern forensic requirements are often being required. Many states now mandate that an elected coroner obtain professional certification after taking office. This significant requirement is not just a bureaucratization of the coroner's role but is of tremendous benefit to the community. By utilizing current medicolegal practices, it allows for informed decision making when it comes to the expenditure of coroner resources, the utilization of the forensic autopsy, and the safeguarding of the community.

The coroner's role has developed beyond its humble roots into a key component of a multidisciplinary medicolegal investigation. Coroners not only obtain valuable information to be used in determining cause and manner of death, but they also aid other community stakeholders such as law enforcement, children and adult protective services, and trauma services.

Cases selected for review will demonstrate how the modern coroner is no longer the "body snatcher" of old but an essential protector of the community. Presented cases will include investigations of communicable disease, mechanical hazards, and criminal investigations. Informational contributions to state vital statistics child fatality review committees, drug interdiction, and trauma services will also be discussed.

Multidisciplinary; Coroner; Medicolegal Death Investigation

F101 Technological Advances to Medical Examiner and Coroner Case Management Systems

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WITHDRAWN

F102 Technology Use Among the Nation's Medical Examiner and Coroner Offices: A Snapshot

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Learning Objectives: After attending this presentation, attendees will have a better understanding of how data from the 2018 Census of Medical Examiner and Coroners show a difference in resources available to a Medical Examiner or Coroner (MEC) office as related to technology. They will also gain insights into the factors influencing MECs' access to, and use of, technologies.

Impact Statement: The presentation will impact the forensic science community by creating greater awareness of the variety of technological resources within or available to MEC offices. The forensic science community will be able to make decisions to improve technology use within MEC offices based on factors that influence its use.

Abstract Text: In the United States, MECs fill critical public health and public safety system 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 roles essential to job-performing duties, technology used among MEC offices is disparate and not well characterized. Resources, operational infrastructure, and MECs' policies and procedures affect technology use and should be better understood to promote technology implementation that is appropriate, ethical, and applied justly in providing a complete death investigation.

The 2018 CMEC was conducted by RTI International on behalf of BJS (2017-MU-CX-K052) from June 2019 through March 2020. A total of 1,648 MECs responded for an overall completion rate of 80.9%. We conducted an analysis of the publicly available 2018 CMEC dataset to evaluate access and use of technologies, like internet and computerized Case Management Systems (CMS), databases, and advanced imaging (e.g., computed tomography, magnetic resonance imaging).[1] This presentation will highlight how key metrics of agency type, population, budget, and geographic location are related to access and use of technologies. Participation in national data collections and databases, like the Combined Offender DNA Index System (CODIS) and National Missing and Unidentified Persons System (NamUS) will also be discussed.

Exemplary key findings of the 2018 CMEC will be discussed. The United States Census-defined New England division (Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont) reported higher levels of access to the internet and CMSs. While 1 in 4 MEC offices are unable to access the internet through work, details about location of internet access, extent of agency websites, in-office computer use for non-internet tasks, and the process of collecting information for MEC offices remain unknown for the 75% of MEC offices that can access the internet through work. Offices serving populations >250,000 have more access to the internet, CMSs, databases, and advanced imaging. Many offices reported limited access to, and low participation in, databases for assessing and sharing case data. Generally, offices with larger budgets reported access to more resources, technology, and infrastructure. Although MEC office technology use has improved over time, it is still disparate.

Without access to fundamental technologies such as computers, CMSs, or the internet, a death investigation can be less comprehensive, and the information provided to public health, public safety, and a decedent's friends and family may be incomplete or even inaccurate. Hence, a transformative state of technology use that is responsible, ethical, and just for every death investigation means the nation's MEC offices must have access to and use of fundamental technologies to perform their duties.

Reference:

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Medicolegal Death Investigation; Technology Use; Medical Examiner and Coroner

F103 How Local Coroner/Medical Examiners Can Contribute to Advancing Forensic Science

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Learning Objectives: This presentation will aim to empower local jurisdictions and academic institutions to partner on projects to advance forensic science. Specifically, this will use the example of Park County, Colorado, Coroners Office partnering with Colorado Mesa University to develop the High-Altitude Human Decomposition Research Facility.

Impact Statement: The impact of this presentation will be to educate those leaders of local coroner/medical examiner offices as well as academic institutions to partner to advance research.

Abstract Text: The presentation will begin by outlining how research is often conducted in a vacuum without real-world data. The need for more collaboration partners in research will be the center focus using the above stated example. Local jurisdictions may have resources that academic institutions do not and vice versa.

In the case of Park County Coroner Office and Colorado Mesa University, both saw a need and worked together to fill it. In 2017, a researcher and Doctorate student at Colorado Mesa University and the Park County Coroner (myself) began discussing how we could work together to bring Human Decomposition research to a high-altitude environment. This discussion followed years of field work that showed high-altitude mountain environments affected forensic cases in ways that current research did not reflect.

Micro climates in high-altitude mountain environments differ greatly in relatively small areas, and there are many other unique factors. One example was where a coyote carried a single arm 1.2 miles in one carry and left it. Research in taphonomy indicated that wildlife scattering should be within 300 yards. With this information and more, we began to look at other cases that did not seem to fit current research. It quickly became obvious that more research was needed in this unique environment of the Rocky Mountains.

I began by meeting with the county commissioners and explaining the situation. To me, it seemed obvious that if we were seeing the need, then the county should be part of the solution, not just sit idly by. The researcher was enthusiastic that maybe we could develop a unique relationship between a county and academic institution to work on this project. After several meetings, the county commissioners began to see this as well. The commissioners asked what they could do and we decided that possibly providing the land for the research would be a great start. I began to research what county-owned properties may be available for this type of research. The researcher and I toured different county-owned options and found a suitable site. At this point, the director of the forensics program, the researcher, and myself presented the idea in more detail to the county commissioners. This was successful, and the county donated 39.5 acres to the university to get things started. Today, the county and university work together to operate the facility and are actively working to bring more academic institutions into further collaboration.

Forensic Science; Decomposition; Research

F104 Cell Phones—Can They Replace Advanced Digital Cameras in Medicolegal Scene Photography Investigations?

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Learning Objectives: This presentation aims to explore the strengths and weaknesses of both cell phone cameras and advanced digital cameras in the context of medicolegal death scene photography. The two types will be compared based on their convenience, camera control, image quality, cost, integrity, and security.

Impact Statement: This presentation will impact the forensic community by introducing knowledge about the differences between cell phone cameras and advanced digital cameras and whether cell phone cameras can effectively replace traditional advanced digital cameras in medicolegal death scene investigation photography.

Abstract Text: Forensic photography plays a vital role in documenting medicolegal death investigations. The photographs offer a permanent visual record of the scene that can be used to recall pertinent information on scene and assist investigators and forensic pathologists with background information, reports, and cause of death. High quality and clarity are of crucial importance when capturing these photographs. Traditionally, advanced cameras like Digital Single-Lens Reflexes (DSLRs) have been the preferred choice. However, the rise of high-quality cell phone cameras has raised questions about their suitability for this purpose. By comparing their performance across various factors such as convenience, camera control, image quality, cost, integrity, and security, it can be determined which type of camera is more effective when documenting medicolegal death scene investigations.

Cell phones, while convenient, cost effective, and easier to handle, offer limiting manual settings and adjustment control and lack the resolution and clarity needed for capturing detailed forensic evidence. Advanced cameras like DSLRs, mirrorless, and compact cameras lack some convenience, have a higher cost and learning curve, provide more control over the image capture process, and offer higher resolution images capturing greater detail and clarity. Furthermore, they often have built-in security features, such as password protection and data encryption, ensuring image integrity and security.

The project will involve using a work-issued 12MP cell phone and a 24MP mirrorless camera with a lens. Photos taken with the cell phone will be saved in its internal memory, while those captured with the mirrorless camera will be stored on an external memory card. Both devices will be used to photograph various settings, including indoor, outdoor, and nighttime death scenes, with identical shots taken by each. The resulting images will be evaluated and compared based on factors such as convenience, camera control, image quality, cost, integrity, and security to assess the performance of each camera. Preliminary findings indicate that although cell phones are convenient and budget-friendly for quick captures, advanced digital cameras provide better image quality, control, and security for death scene photography, ensuring the integrity of the images. The collected images, advanced methodology, and findings will be presented.

Photography; Medicolegal Death Investigation; Camera

F105 Navigating the Challenges: The Struggle of Medicolegal Death Investigations in Sparsely Populated Regions

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Learning Objectives: After attending this presentation, attendees will: (1) Understand the primary challenges faced by medicolegal death investigation offices in sparsely populated areas; (2) assess how inadequate resources affect the accuracy of death determinations in sparsely populated and rural areas; examine the existing methodologies and protocols used by medicolegal death investigation offices and identify areas of improvement; and (4) recognize the broader implications of accurate and reliable death rulings on public health, justice, and the integrity of vital statistics, with the importance of addressing the identified challenges to achieve these outcomes.

Impact Statement: This study examines the profound challenges faced by medicolegal death investigation offices in sparsely populated areas of the United States, highlighting the critical need for systemic reforms. Addressing the multifaceted issues is imperative for improving the accuracy and reliability of death investigations, thereby enhancing public health surveillance, supporting justice, and maintaining the integrity of vital statistics. The research contributes to a broader discussion on public health and forensic science, advocating for a strategic realignment of resources and policies to support medicolegal infrastructure in rural and sparsely populated areas of America.

Abstract Text: Medicolegal death investigation offices in sparsely populated areas across the United States encounter significant challenges in accurately determining the cause and manner of death. These challenges are compounded by limited resources, including financial constraints and inadequate access to advanced forensic technology. This mixed-methods study aims to explore the multifaceted struggles experienced by medicolegal death investigation offices in sparsely populated regions and assess their impact on death investigations.

This study reveals several critical obstacles through a comprehensive review of existing literature, surveys, and interviews with coroners from sparsely populated and underfunded regions. Key among these obstacles is the insufficient availability of training for medicolegal professionals. Many coroners in rural areas rely on outdated methodologies due to the lack of access to continuous professional development and modern forensic tools. This reliance on antiquated techniques significantly undermines the accuracy of death determinations. The lack of standardized protocols and advanced report management systems can contribute to inconsistencies in death investigations. Some rural and sparsely populated offices lack uniform guidelines for death investigations, leading to variability in investigation quality. The absence of modern data management systems hinders efficient case handling and complicates inter-agency collaboration, which is essential for comprehensive death investigations.

The research emphasizes the urgent need for policy interventions to address these multifaceted issues. Recommendations include increasing funding to enable the procurement of advanced forensic technology and the development of standardized training and certification programs tailored to the needs of rural coroners. Additionally, the establishment of robust inter-agency communication frameworks and standardized protocols is essential for enhancing the accuracy and reliability of death investigations.

Addressing these challenges is essential to ensure accurate and reliable death determinations, which are fundamental to public health, justice, and the integrity of vital statistics. Accurate death investigations not only support public health initiatives by providing reliable mortality data but also uphold the principles of justice by ensuring that deaths are properly classified and investigated. This research contributes to the broader discourse on public health and forensic science, advocating for strategic realignments of resources and policies to support medicolegal infrastructure in rural and sparsely populated areas of America.

Medicolegal Death Investigation; Coroner; Medical Examiner

F106 Mass Fatality on a Budget

Robert L. Hunkeler III, MFS, Montgomery County Coroner's Office, Dayton OH*

Learning Objectives: Identify and implement cost-saving strategies for mass fatality equipment: attendees will learn how to utilize unconventional purchasing methods, such as acquiring used or repairable equipment through online resellers and negotiating directly with manufacturers, to significantly reduce costs while maintaining operational effectiveness. This objective will focus on practical steps to maximize budget efficiency and achieve substantial savings in preparing for mass fatality incidents.

Leverage grants and direct manufacturer relationships for equipment procurement: attendees will understand how to effectively use state and federal grants to fund equipment purchases and secure better pricing by establishing direct contact with manufacturers. This objective aims to provide strategies for optimizing financial resources and enhancing preparedness capabilities through grant utilization and strategic vendor negotiations.

Impact Statement: The innovative cost-saving strategies implemented by the Montgomery County Coroner's Office (MCCO) have not only achieved significant financial savings but also strengthened emergency response capabilities. By effectively utilizing online resellers, negotiating directly with manufacturers, and leveraging state and federal grants, MCCO has saved over $100,000 while ensuring robust preparedness for mass fatality incidents. This approach demonstrates that thoughtful resource management and creative procurement methods can substantially enhance response readiness and operational efficiency. The success of these strategies serves as a model for other organizations, highlighting the potential for substantial cost savings and improved emergency preparedness through innovative financial and logistical practices.

Abstract Text: This presentation will provide a comprehensive overview of innovative strategies for achieving significant cost savings while preparing for mass fatality incidents. The MCCO has adopted several unconventional yet highly effective approaches to maximize response capabilities with minimal financial outlay. Key to this approach is the acquisition of essential, high-cost equipment—such as mass fatality shelters, HVAC systems, generators, flooring, and trailers—through non-traditional means.

MCCO has effectively utilized online resellers to procure used or repairable equipment at a fraction of the original cost. For example, mass fatality shelters, typically priced at $30,000 new, have been acquired for as little as $2,000. By mastering the repair of these shelters and other equipment, MCCO has been able to maintain full operational functionality while significantly reducing costs. Staff training in the repair of tent structures and other equipment further enhances cost efficiency, ensuring that response capabilities remain intact despite the lower expenditure.

In addition to leveraging online resellers, MCCO has negotiated directly with manufacturers to bypass the substantial mark-ups imposed by vendors. This direct engagement has enabled MCCO to secure better pricing on critical equipment, leading to savings of over $100,000. The session will also explore how MCCO's strategic use of state and federal grants has complemented these efforts, allowing for the acquisition of necessary equipment without additional financial strain on taxpayers.

Attendees will gain valuable insights into the practical application of these cost-saving techniques, including how to identify and procure used or repairable equipment, negotiate directly with manufacturers, and effectively utilize grants. This presentation aims to equip emergency management professionals with the knowledge to enhance their own mass fatality preparedness efforts, optimize their budgets, and ensure robust response capabilities. By sharing MCCO's successful strategies, the presentation will demonstrate how similar methods can be applied to achieve significant financial and operational benefits in emergency preparedness.

Mass Fatality; Mass Disaster; Search and Recovery

F107 "We Specialize in the Heartbreakers": Practitioner Perspectives on Investigating Long-Term Unidentified Persons Cases Throughout the United States

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Learning Objectives: After attending this presentation, attendees will better understand practitioner perspectives on managing long-term unidentified persons cases in medicolegal agencies around the country.

Impact Statement: This presentation impacts the forensic science community by increasing awareness of practitioner and investigator decision making for identifying the long-term deceased. Understanding what medicolegal professionals find to be the biggest challenges and assets for resolving unidentified persons cases will help improve decedent identification policy and practice across jurisdictions.

Abstract Text: The chronic mass disaster of long-term unidentified persons in medical examiners' and coroners' offices around the country is well known in the medicolegal community, yet little work has been done to examine the root causes of this crisis. Despite ever-advancing forensic technology, approximately 15,000 decedents listed in the National Missing and Unidentified Persons System (NamUs) remain in limbo as the long-term unidentified with growing agency backlogs of unsolved cases and families of the missing waiting for answers. With no national system for death investigation, jurisdictional fragmentation leads to differential caseloads, protocols, and access to tools and resources for identification. While the condition of remains and preservation of evidence may hinder postmortem analyses, decedent demographics and structural vulnerabilities in life may also limit the availability of missing persons reports and antemortem data to reconcile cases. Due to the multitude of factors affecting decedent identification, the present study asks: What do medicolegal professionals believe most impacts the solvability of long-term unidentified persons cases? By gaining insight into practitioner and investigator perspectives on the identification process, this study seeks to target areas of improvement to ultimately increase identifications and case closures nationwide.

Methods: To illuminate professionals' first-hand experiences with long-term cases, 11 semi-structured interviews were conducted virtually and in person with medicolegal death investigators and forensic anthropologists responsible for managing long-term unidentified persons cases around the country. Participants were affiliated with eight different agencies across six states, including both medical examiner and coroners' offices. Questions focused on protocols, procedures, and decision-making processes for decedent identification, including difficulties faced and best practices for long-term case investigations. Interviews also asked about agency resources, use of forensic services, inter-agency collaboration, and approaches to prioritizing open cases. Interviews were coded and analyzed using thematic analysis to inductively discern overarching patterns in practitioners' responses.

Results and Discussion: Several commonalities pervade participant responses, including similar definitions of what constitutes a "cold" or long-term case and poor preservation of remains and "Undetermined" manner of death being reported for the oldest or most challenging cases. However, there was also much variation in terms of demographic trends of the unidentified populations at each agency, as well as in access to forensic technologies, particularly DNA services. Investigative genetic genealogy was a major topic discussed, being desired or used for identification in varying degrees. All participants expressed the need for additional personnel and resources devoted exclusively to long-term case resolution and the importance of thorough and streamlined antemortem data. These results will be further discussed within the broader scope of addressing structural inequities in medicolegal death investigation and the feasibility of mitigating case backlogs at the local, state, and national level.

The objective of this presentation is to spread awareness of investigator and practitioner perspectives on what most impacts decedent identification around the country. It is the overall goal of this research to promote changes to policy and practice to increase identification rates for long-term decedents across jurisdictions.

Identification; Death Investigation; Cold Case

F108 Abuse or Self-Neglect? A Medicolegal Case Study Exploring Multi-Agency Failure to Report a Vulnerable Adult

Ella B. Butler, MS, Charleston County Coroner's Office, North Charleston, SC*

Learning Objectives: After attending this presentation, attendees will grasp key concepts to identify vulnerable adults in death investigations, understand key protective services in place to protect at-risk individuals from harm, and recognize limits within these systems.[1] This case example will expose faults within medical systems that perpetuated neglect and recommend medicolegal death investigators to offer preventative solutions for their local agencies.

Impact Statement: This presentation will impact the forensic science community by featuring an unfortunate example of systemic failure to report suspected abuse, as the case underscores the consequences of subpar interagency communication. The intended outcome for this presentation is to advocate for vulnerable adults and encourage investigators to collaborate with local protective services to promote immediate action from mandated reporters when they suspect elder abuse and neglect.

Abstract Text: Vulnerable adult risk assessments require scrutiny equivalent to child-death investigations. Principally, a thorough death investigation is mandatory to identify the competency of the decedent. They necessitate a thorough review of medical history, detailed and documented interviews, and an understanding of the legal and ethical culpability of individuals responsible for determining the decedent's capacity for decision making. Although nuanced, the distinction between abuse and self-neglect must be clear and precise to impact the manner of death classification. This case study details the complexity of this challenge.

Interagency communication between rehabilitation facilities and hospitals is key to ensure prior incidents of suspicion are reported in tandem with patient medical history. As demonstrated in this case study, the decedent experienced several inpatient and emergency visits through a medical university. Although the decedent was deemed unable to make her own care decisions by an Ethics Committee, proper protective services were not alerted, and she was discharged into a rehabilitation facility. The Ethics Committee did not share their assessment with the facility, and the decedent was ultimately discharged back into an unsafe living environment. Upon investigation, the facility had explicit concerns for her safety and discharge plan; however, they too failed to report their concerns to appropriate protective services.

This case study will present attendees with an example of a thorough death investigation of a vulnerable adult. This example will expose faults within medical systems that perpetuated neglect and recommend medicolegal death investigators to offer preventative solutions for their local agencies.

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Elder Abuse; Death Investigation; Manner of Death

F109 Cases of Child Abuse and Maltreatment: A Retrospective Observational Study From Meyer Children's Hospital IRCCS of Florence, Italy, Between 2010 and 2022

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Learning Objectives: After attending this presentation, participants will understand the key points of abuse and maltreatment in children and adolescents through a retrospective study from an Italian database of a pediatric center of excellence.

Impact Statement: The aim of this presentation is to analyze the incidence of abuse and maltreatment in children and adolescents hospitalized at the IRCCS Meyer Children's Hospital in Florence, Italy, to identify risk factors and to suggest interventions to support affected children and health care professionals, thereby creating effective prevention strategies.

Abstract Text: Health care workers have a legal and moral obligation to report child abuse. All suspected cases must be reported to law enforcement.[1] However, child abuse is often underrecognized and underreported by health care providers. Therefore, it is essential to train health care workers to recognize and manage cases of abuse and neglect.[2] The incidence of child maltreatment and abuse remains high, necessitating improved prevention systems to protect children. This presentation highlights the importance of a multidisciplinary approach for children suspected of being victims of abuse and maltreatment.

According to the literature, the multidisciplinary team that should be enrolled and deployed in cases of child abuses must include pediatricians, radiologists, gynecologists, urologists, social workers, nurses, psychologists, and forensic pathologists.[2-5] According to the World Health Organization (WHO) and recent literature, approximately 1 in 4 children will experience abuse or neglect in their lifetime.[6] The mortality rate for child maltreatment is 2.2 per 1,000 children annually, making it the second-leading cause of death in children under one year of age.[6]

Diagnosing child abuse and maltreatment is challenging because it must be distinguished from unintentional injuries, self-inflicted injuries, or conditions that mimic maltreatment but have different etiologies. For medical providers, including clinical and forensic pathologists, recognizing cases of child abuse/maltreatment is essential because childhood exposure to violence can have lifelong consequences, including poor physical, emotional, and mental health. In this retrospective observational study, cases of child maltreatment involving children aged 0-18 years who attended the Group Unit for Child and Adolescent Maltreatment ("Gruppo abusi infanzia e adolescenza" [GAIA]) of the Meyer Pediatric Hospital from 2010 to 2022 were reviewed. A total of 812 subjects were identified, including 370 males and 442 females. Cases were categorized according to age, gender, nationality, perpetrator characteristics, family environment, and type of maltreatment (physical, psychological, sexual, and neglect). Specifically, 267 cases of physical abuse, 233 of sexual abuse, 129 of neglect, 30 of psychological abuse, and 9 of Munchausen syndrome by proxy were identified. In addition, 144 cases involved two or more of these conditions at the same time.

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Child Abuse; Neglect; Maltreatment

F110 Bridging the Gap: Collaborative Programs and Initiatives That Are Transforming the Interface Between Forensic Science and the Legal System

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Learning Objectives: Upon completion of this presentation, participants will be able to identify how forensic science agencies and academic institutions can use programs and initiatives to support legal professionals, law students, and other individuals in the legal system in their understanding and application of forensic science concepts.

Impact Statement: This presentation will strengthen the interaction between the forensic science and legal communities by:

- Breaking Down Silos: This presentation aims to bridge the gap between science and law by promoting clearer communication and enhancing the understanding of legal matters for forensic scientists and of the capabilities and limitations of forensic science for legal professionals.
- Empowering Justice: This presentation will highlight innovative programs at the University of Central Oklahoma (UCO) that create two-way learning experiences for forensic scientists and legal professionals. By fostering this collaboration, these programs empower both legal professionals and forensic experts to make more informed decisions, ultimately strengthening the justice system.
- Building a Stronger System: By sharing program blueprints, the hope is to inspire similar initiatives at universities and agencies nationwide fostering a more just and accurate legal system.

Abstract Text: The UCO's Forensic Science Institute (FSI) fosters collaboration between forensic science and the law through innovative programs designed to strengthen the justice system and empower professionals and students in both fields.

Collaborative Course on Expert Witness Testimony: A unique initiative brings together UCO forensic science graduate students and Oklahoma City University (OCU) law students in a combined course focused on expert testimony. Law students gain a deeper understanding of the science, limitations and legal presentation of forensic evidence. They explore the ethical responsibilities of lawyers and expert witnesses while developing effective strategies for preparing and presenting expert witnesses during trials, culminating in a mock trial experience. Forensic science students gain the skills and knowledge necessary to effectively communicate scientific findings in a legal setting. They participate in a mock trial for practical experience in expert testimony and develop a strong understanding of how federal rules of evidence impact the admissibility of forensic evidence. Additionally, they sharpen their communication skills by instructing law students in various forensic science disciplines. This collaborative course fosters a unique learning environment where both law and forensic science students gain first-hand experience with the intersection of their respective fields. By working together, students develop a deeper understanding of the challenges and opportunities presented at this critical juncture between science and law.

Continuing Education for Legal Professionals: Recognizing the growing importance of forensic science in criminal cases, the FSI has initiated a Continuing Education program for its community partners, including law enforcement, lawyers, and members of the judiciary. The program leverages the FSI faculty's extensive experience in forensic science and law enforcement to offer valuable training opportunities. This includes online training modules on the latest investigative techniques, developing forensic science workshops, and providing opportunities for case analysis reviews for the legal community.

The Innocence Project: The Innocence Project is a capstone experience dedicated to identifying and remedying wrongful convictions in Oklahoma. This unique program brings together UCO forensic science graduate and undergraduate students, OCU law students, attorneys, FSI and OCU faculty, and the Oklahoma State Bureau of Investigation (OSBI) to review and investigate claims of actual innocence. This program offers an invaluable opportunity for students to bridge the gap between theory and real-world application. Law students and forensic science students are paired on cases, fostering collaboration and a deeper understanding of each other's disciplines. Forensic science students delve into evidence and crime scene documentation, analyzing the forensic aspects of the cases. Law students, meanwhile, focus on uncovering potential legal issues that could have contributed to wrongful convictions. The OSBI conducts court-approved forensic testing deemed necessary and FSI and OCU faculty offer expert insights and ensure students approach the case with professionalism and ethical rigor.

The Innocence Project educates students on social justice, legal complexities, and ethical considerations in law and forensics. It equips them to professionally and ethically evaluate complex cases, conduct focused research, clearly communicate findings, and develop effective investigation strategies. By bringing together diverse expertise and fostering collaboration, the program offers a unique opportunity to apply content knowledge to authentic contexts and to potentially exonerate the wrongfully convicted.

This presentation demonstrates the impact of UCO's innovative programs in bridging the gap between science and law and enhancing forensic science analysis and legal decision-making. By sharing program blueprints, the hope is to inspire similar initiatives at universities and agencies nationwide, fostering a more just and accurate legal system.

Forensic Science; Legal System; Programs and Training

F111 Forensic Intelligence: A Contemporary Application or How to Approach a Paradigm Shift

Joe C. Trevino III, MS, New York Police Department, Queens, NY*

Learning Objectives: Attendees will learn how contemporary applications of forensic intelligence refocus the role of Forensic Science Service Providers (FSSPs) in the investigative process as active and proactive participants. Attendees will learn about creative but tailored intelligence beyond classical case management statistics or justice-oriented strategies. Attendees will learn about the challenges associated with pursuing newer forms of forensic intelligence in a typical crime laboratory infrastructure.

Impact Statement: This presentation introduces novel and useful ways to present or communicate intelligence data that classical statistics or metrics don't address. We create more efficient and effective solutions for modern policing by making better use of our data. This in turn keeps FSSPs flexible and innovative for future problems and needs.

Abstract Text: The National Institute of Justice (NIJ) defines forensic intelligence as, "using forensic data early in an investigation, when that information can accelerate the process of solving the case. It also means using data across cases to understand crime trends and identify links between cases, such as serial crimes. The forensic results might not be confirmed and ready for court yet, but they are a powerful tool for making connections between evidence, suspects, cases, and sites of crime. That information can guide law enforcement away from dead ends and toward meaningful leads."[1] This is quite a modern interpretation of how forensic intelligence can be used, and it signals either a shifting paradigm or a natural evolution in the role of forensic science within policing.

The idea of using forensic analysis to identify patterns or associations has been with us since at least the 1980s, where a shoeprint and an instrument (toolmarks) index were used to identify burglary patterns across police district boundaries. Contemporary forensic science has evolved from shoeprint and instrument databases into a multi-discipline arena with capabilities far beyond what we would experience in the 1980s.[2,3] One of the most recognizable advances in forensic intelligence today is the Crime Gun Intelligence Center (CGIC), a concept that turns shooting offenses and National Integrated Ballistic Information Network (NIBIN) data into useable information for law enforcement investigators.

Associating gun violence cases is especially helpful in the criminal justice system, as stacking firearms cases might impact a case at sentencing or it can mean more or more serious charges. This justice-centered approach is where we start evaluating the effectiveness of forensic intelligence or where we start evaluating its worth.[4] Common and historic metrics like case clearance rates, successful prosecution rates, etc. are an incomplete measure of effectiveness because they do not address the very important role law enforcement and forensics play in an overall process.

Early and modern ideas of forensic intelligence include a laboratory role and a law enforcement role.[5] The true strength of a forensic intelligence program is that both entities work together to enhance investigations, increase investigator effectiveness, and identify case patterns or associations together using their available resources. This is much easier to do when the laboratory and law enforcement parties are under the same organization, but it is by no means the only way to do things.

Sound forensic intelligence program policy starts with understanding local factors like the goals of a law enforcement agency's investigative units. Every city might experience gun violence or gang activity, but not necessarily in the same way. What information do investigators need to do their work? What part of that involves a crime laboratory? How can the crime laboratory meet those needs and how can they give investigators the tools to find associations or links? What does the law enforcement agency need to communicate to the crime laboratory? Who can they call to explain or contextualize the data they are receiving?

Key to implementing a forensic intelligence framework or policy lies with understanding your data, the local factors that shape investigative needs, and how data is communicated properly to the stakeholders and end users who make investigation and policy decisions. This presentation will demonstrate how local factors are incorporated into an interactive data dashboard (Microsoft Power BI) of firearms evidence data for investigative and demonstrative purposes and will include avenues and ideas for including data from other forensic science disciplines.

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Forensic Intelligence; Crime Gun Intelligence; Criminalistics

F112 The Unexpected Role of Pacemaker Data in Solving a Homicide: A Case Study

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Learning Objectives: After attending this presentation, attendees will gain a comprehensive understanding of how pacemaker data can serve as a reliable forensic tool, aiding in establishing precise timelines crucial for investigative accuracy and legal proceedings.

Impact Statement: This presentation will impact the forensic science community by highlighting the use of pacemaker data in determining the precise time of death in medicolegal death investigations. Pacemaker data can provide exact timestamps of cardiac cessation, offering a more reliable and objective measure of the time of death than traditional external observations. This approach is particularly vital in cases where an exact timeline is essential for judicial accuracy, especially when there are discrepancies between investigator observations and witness testimonies.

Abstract Text: This presentation discusses the case study of a 29-year-old man found deceased in his residence with multiple stab wounds to his chest and abdomen. The decedent was discovered by his female roommate, who urgently called 911, stating that he was still talking and breathing. However, upon arrival, first responders determined that the man had been deceased for several hours. During the autopsy, it was discovered that the decedent had an implanted pacemaker. Recognizing the potential value of the device's data in establishing an accurate timeline of events, the pacemaker was collected as evidence and stored at the coroner's office. A subpoena was subsequently issued to the manufacturer to extract and analyze the device's data logs.

The data retrieved from the pacemaker provided precise timestamps of cardiac events, showing the exact moment the decedent's heart likely stopped beating approximately four hours before the 911 call was placed by the roommate. This information not only clarified the timeline but also highlighted significant inconsistencies in the roommate's account of events. Ultimately, this evidence contributed to the successful conviction of the roommate in connection with the death.

This case study illustrates the invaluable contribution of advanced medical technologies in forensic investigations, demonstrating their capacity to provide irrefutable evidence and ensure justice in complex medicolegal scenarios.

Death Investigation; Time of Death; Stabbing

F113 "Follow the Bloodstains and Find the Truth"—The Importance of Site Inspection

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Learning Objectives: Cases of falling from a height are a well-known issue in the forensic field. The following presentation illustrates the case of a fatal fall from a height under circumstances that warranted further investigation, particularly with regard to whether the fall may have been a homicide or a suicide. After reviewing this presentation, attendees will receive additional evidence of the significance of exhaustive forensic investigation and comprehensive site inspection.

Impact Statement: This presentation illustrates the imperative of integrating multidisciplinary approaches in forensic science to accurately reconstruct events and understand the underlying causes of such fatalities. It also emphasizes the need for awareness and intervention strategies for individuals exhibiting acute psychological distress to prevent similar tragedies in the future.

Abstract Text: A 38-year-old man fell from a fourth-floor window of a building where he was staying with his girlfriend and two friends to celebrate New Year's Eve. The initial inspection of the scene showed bloodstains of various sizes on the steps and handrail, increasing in number near the last two staircases leading to the apartment. Inside the house, in the bedroom, we found a couple of slightly bloodstained duvets, a pair of slippers, and a bloodstained garment abandoned on the floor. The living area had numerous bloodstains on the floor. A glass door was broken, with traces of blood. The bloodstains continued onto the living room balcony, with splashes and drops of blood on the door and wall behind it; the balcony floor had copious bloodstains and footprints leading toward the edge and, from this one, we could see blood traces extending to the eaves. The body was found lying supine on the asphalt directly beneath the apartment balcony.

Prior to the autopsy, a Computed Tomography (CT) scan of the body was performed, revealing multiple fractures of the sternum, ribs, vertebrae, sacrum, ilium, ischium, femur, and fibula. External examination documented extensive bruising and lacerations. The autopsy revealed a modest subarachnoid hemorrhage in the left temporal region and cerebellar tentorium. About 160cc of blood was found in the left pleural cavity with some lacerations of the ipsilateral lower lung lobe. Liver and spleen lacerations were also noted, along with a small amount of blood in the abdominal cavity. A subcutaneous hematoma was documented in the left dorsal region. These injuries likely resulted from a fall from a great height (at least 12m) onto an asphalt surface.[1,2] A very deep laceration was also found on the upper right arm; it caused the lacerations of vascular structures, including arterial ones. As a result of the severing of major arterial vascular structures prior to the impact, the subject suffered a significant loss of blood in a relatively short period of time. The acute hemorrhage may well have led to a state of hypovolemic shock which, together with the severe traumatic injuries caused by the impact on the ground, contributed to the death of the subject, which probably occurred within a few minutes.[3]

The analysis of circumstantial data allowed us to perform a thorough reconstruction of the events that led to the man's death. His girlfriend and friends reported that the man woke up agitated, saying strange things like, "Satan called me" and "God doesn't want me to sleep." They stated he attacked his partner and punched a glass door, injuring his right arm, which led to significant blood loss. The friends and girlfriend then fled the apartment, and they were chased down the landing and stairs. He then returned to the apartment and threw himself off the balcony.

Eventually, the convergence of circumstantial data, autopsy results, and findings gathered during the site inspection helped clarify the suicidal nature of the man's fall and the reconstruction of the last moments of his life.

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Hemorrhagic Shock; Fall From Height; Site Inspection

F114 Is Forensic Chemistry Different?

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Learning Objectives: Attendees will learn how changes in the German legal system and chemical industry in the 19th century changed the status of experts in court and how that shaped forensic chemistry.

Impact Statement: Learning about forensic science history informs us of its present status and how it developed as a discipline. Going forward, understanding our history is critical to our future.

Abstract Text: Is forensic chemistry a distinct discipline or is it just a toolkit of methods applied to a different set of samples? The latter definition would make forensic chemistry "merely" a kind of analytical chemistry. The former definition, however, requires differences that carve out a distinct knowledge domain, separate from other kinds of chemistry. This presentation argues that forensic chemistry is a separate discipline, differentiated by having its values and methods altered through being a captive profession.[1] A captive profession is subordinate to and whose work is mediated by a more prestigious or powerful profession, for example, sonographers or clinical scientists, whose work, used by medical doctors for diagnosis and treatment, cannot stand on its own. Forensic chemistry, and all of forensic science, is captive to policing and the criminal justice system. Without those professions, no forensic efforts would exist; forensic results are mediated through those more powerful professions. What makes forensic chemistry different, then, is its relationship to and through a criminal justice system that has altered chemistry's values, methods, and epistemology.

The development of German industry and its legal system in the 19th century changed the status of experts in court.[2] The methods chosen by industry were based on efficiency, not accuracy; "good enough" was just that, given potential profits. Industrial chemists had to meet the needs and values of two systems: science and the economy. This changed values and methods with a trade-off between accuracy and efficiency. After 1848, all German states adopted a reformed legal code based on the French model, including testimony in a jury trial and formal rules of evidence, allowing for free consideration of evidence by the judge and the jury.[3] Testimony meant experts could elaborate on their written reports but had to explain their complicated methods to laypersons in a legal setting. Physical evidence, instead of only witness testimony, could be considered.

This mediation of academic chemistry through a court's system altered its values and methods, much in the way industry had. Although selectivity, sensitivity, and simplicity remained in the wheelhouse of forensic chemists, additional values had to be created to meet the investigative and legal demands of the forensic domain.[4] The first was redundancy, the need to use orthogonal techniques to validate findings and provide additional proof to attorneys and jurors that the results were trustworthy. The second was conservatism, using the smallest sample size possible. Most chemical methods then were destructive and forensic samples are typically limited. The last was comprehensibility, the need to be able to explain what the method did, what the results were, and what significance it had to the issues before the court. Academic chemists talk peer-to-peer, while forensic chemists are required to talk to laypersons, including police, attorneys, judges, and jurors. Academic chemistry's values and methods were altered to meet the needs of police and courts, creating forensic chemistry as a captive profession but with a distinct knowledge domain.

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Forensic Analysis; Forensic Professionals; Expert Testimony

F115 A Comparison of Particulate Collection Methods Using Fluorescent Polyethylene Beads and an Introduction to the Use of a Pulse Lavage for Particulate Evidence Collection

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Learning Objectives: The audience will learn about how fluorescent particulates were used to emulate trace evidence for an assessment of four collection techniques. In addition to learning about how a pulse lavage can be used for the collection of particulate evidence, they will understand the comparative efficacies of swabs, standard and water-soluble tapes, a dry vacuum, and a pulse lavage regarding trace evidence collection. Finally, they will learn about the adjacent considerations of ease of use, cost per sample, and recoverability of the evidence from each collection technique for downstream analyses. This is beneficial for lab, field, and administrative considerations.

Impact Statement: This presentation will introduce the pulse lavage as a feasible alternative to an M-VAC due to its lower cost per sample, better transportability, and the ability to collect DNA and particulate evidence simultaneously from the same sample. This will also benefit those who collect trace evidence in the field or lab by providing insight into the efficacy and cost when considering their collection resources. In total, they will understand the benefits or drawbacks of each method in terms of collection, cost, and downstream recovery for analysis to facilitate the most beneficial collection.

Abstract Text: Using fluorescent polyethylene beads as an analog for trace particulates, four evidence collection methods were compared to determine their efficacy as determined by the percentages removed and recovered from the initial deposition. The efficacy of each method based on the particle's size was also assessed. Finally, each method underwent a cost benefit analysis to take into consideration the financial and resourceful toll that each collection would take on the performing laboratory or crime scene professional.

Trace particulate evidence such as glass fragments, paint chips, fibers, soil, or pollen can establish contextual links between scenes, victims, and suspects.[1] The small size of this evidence can cause them to be unknowingly left behind by suspects, but also be unintentionally overlooked and inefficiently collected by investigating scientists. To improve the collection of this important evidence, four methods were assessed regarding their efficacy of recovering particulates from a fabric substrate: swabbing, tape lifts, dry vacuuming, and a pulse lavage (wet vacuuming).

Three fluorescent bead sizes were used to emulate trace evidence, each with a corresponding color: green, violet, and blue for 10-20µm, 53-63µm, and 125-150µm diameters, respectively. After depositing the beads on a piece of black cotton twill fabric using Teflon and a standard weight, NIS Elements was used to generate photomosaic images under fluorescent microscopy. This photomosaic was used to count the initial and post-collection numbers of beads. The percentage of collected beads from the original quantity was used to assess the removal efficacy of the collection method. This particle-counting method was also used to quantify the particles recovered from the collection method for downstream analyses.

Swabbing for trace evidence was performed by rolling an ethanol-moistened polyurethane swab over the fabric sample. Its flexibility and hydrophobic nature benefitted the collection of hydrophobic particulates in a porous substrate, although swabs are typically used for particle collection from non-porous substrates.[2] Tape lift collections involved applying a standard weight to either a standard tape or water-soluble tape that was applied to the sample and repeating this method for a total of four lifts as the tape systematically changed position on the fabric. The vacuum method involved using a filter cassette attached to a tube on a house vacuum and running it over the sample[3].

The pulse lavage, a medical device used to irrigate and debride wounds or clean tissue during surgery, has been shown to be an effective method of collecting trace DNA, particularly from porous material.[4,5] The pulse lavage introduced pressurized saline to the sample through one nozzle and suctioned the saline and particulates through an adjacent nozzle into a sterile cannister.[4] The particles from the accrued liquid were collected on a membrane filter under vacuum filtration. This study demonstrated that the pulse lavage can efficiently collect both trace DNA and trace particulates and could, by extension, collect both trace DNA and particulate evidence simultaneously from the same sample. Additionally, similar to its medicinal uses, it could provide a means of collecting trace evidence from wounds.

The pulse lavage proved to not only be a viable means of collecting particulate evidence, but it had the greatest removal percentage at an average of approximately 98% across bead sizes. This study proposes the use of a pulse lavage as an effective method for removing particulate evidence from a fabric substrate while also providing a multi-variable comparison to and between the other methods.

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Trace Analysis; Fluorescence; Microscopy

G1 Facial Recognition Technologies in Police and Security Activities: Legal Aspects in the Era of the European AI Act

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WITHDRAWN

G2 The Italian National Cybersecurity Agency (ACN) and Criminal Justice: An Integrated Cooperation Model for Cyber Resilience, Crime Prevention, and Security

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Learning Objectives: Participants will acquire knowledge of the national cybersecurity system and the functions of the main reference bodies: the National Cybersecurity Agency, the Intelligence Services, and, to some extent, the Judiciary. Participants will be able to critically evaluate the various challenges connected to managing cybersecurity scenarios and refine their analytical and judgment skills in relation to public control of technology and the distribution of different powers within the Italian constitutional system.

Impact Statement: The presentation will help the scientific community to: (1) identify the competencies of the various authorities and how they have been distributed in line with the constitutional structure of the separation of powers; (2) understand what national security means in the system of the Italian Republic and how it is differently protected by cybersecurity and judiciary bodies; and (3) understand the relationships between the executive power and the judiciary, and, in particular, the challenges related to the circulation of information between security and judicial authorities.

Abstract Text: At the culmination of a process that began in 2014 to strengthen the state apparatus dedicated to protecting security and national interests in the cyber domain, between 2019 and 2021, the Italian Republic undertook a monumental reorganization of competencies and functions in cybersecurity through the implementation of a comprehensive system called the "National Cybersecurity Architecture."[1] Central to this system is the highly specialized governmental agency, the National Cybersecurity Agency (ACN), which falls under the executive branch, specifically under the responsibilities of the Prime Minister in matters of national security.[2,3]

Before this reform, cybersecurity-related activities were dispersed among multiple state authorities. These authorities, in addition to their regular duties, also carried out functions related to cybersecurity. For example, national intelligence agencies, which traditionally focus on information security, were also tasked with various other functions, such as technology security control and certification, as well as the response, analysis, monitoring, and prevention of cyber threats.[4]

Today, the national architecture is based on four major pillars of diversified yet interconnected competencies: cybersecurity and resilience in cyberspace, entrusted to the National Cybersecurity Agency; intelligence collection and analysis, entrusted to the Security Information System of the Republic; defense and military security of the state, entrusted to the Ministry of Defense; and crime prevention and enforcement, under the jurisdiction of law enforcement and judicial authorities.[5]

The driving force of the new system is the ACN, which has centralized the main competencies in cybersecurity management. For instance, the ACN drafts the national cybersecurity strategy, handles regulatory and administrative matters, develops response and resilience capabilities to threats, and monitors technology operators, cyber incidents, and the security of technologies and systems.[5] These responsibilities come with a set of regulatory, certification, investigative, and sanctioning powers necessary to pursue its institutional mission.[6]

Because the system is structured around diversified yet interconnected competencies, several issues of significant scientific and regulatory interest arise: the role of the National Cybersecurity Agency in relation to national security and public order in cyberspace and, consequently, its coordination with the activities carried out by both intelligence and law enforcement agencies in the comprehensive management of the cyber domain; the position of private entities and the coordination between their economic and technological initiatives and their subjection to government authority oversight; and finally, the relationships between the ACN and the judiciary, particularly with public prosecutors and criminal investigations, under the dual perspective of potential secrecy needs related to national security and the admissibility in criminal proceedings of information collected by the National Cybersecurity Agency during its inspection and monitoring activities.

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Cybersecurity; Cyber Treats; National Security

G3 The SARS-CoV-2 Vaccination Mandate in Light of Italian Constitutional Court Judgments No. 14 and No. 15 of 2023

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Learning Objectives: After attending this presentation, attendees will deepen their understanding of the impact that anti-COVID-19 measures had on the Italian legislative system regarding vaccine regulations.

Impact Statement: This presentation will impact the forensic scientific community by offering a strategic perspective on the relationship between scientific needs and regulatory obligations.

Abstract Text: The Coronavirus pandemic has inevitably impacted legal systems worldwide through measures aimed at curbing the spread of the virus, thus generating heated debates, particularly concerning mandatory vaccination. In this study, we analyzed the measures introduced in each country during the COVID-19 pandemic to contain the spread of the virus. Inevitably, the implemented provisions involved restrictions on personal freedoms; the most divisive decision regarded the COVID-19 vaccination, which became compulsory, especially for some categories of citizens/workers.

The Constitutional Court was called upon to rule on the government's measures to curb the spread of the virus, stating on multiple occasions that, for public health reasons, the law can limit citizens' freedom of movement, including isolating individuals with contagious diseases. Therefore, the restrictions imposed on the population (mandatory quarantine or lockdown) do not fall among measures limiting personal freedom but among those limiting movement for public health reasons, hence being constitutionally legitimate. Specifically, judgments No. 14, 15, and 16 of 2023 concern appeals filed by ordinary and administrative courts related to measures imposing mandatory vaccination on some groups of workers. The Court analyzed the appeals, ruling on the constitutional legitimacy of the provisions.

In judgment No. 14, the Constitutional Court ruled on the constitutional legitimacy issue regarding the vaccination mandate for health care workers, highlighting that the principle of solidarity was the basis of the mandate; without this legal premise, any restriction imposed on the unvaccinated turns into discrimination, as it is not based on the rationale of the mandate. Ruling on this matter, the Constitutional Court always emphasized the temporal nature of the legislator's decision ("at the time of the mandate's introduction," "in light of the knowledge available at that historical moment," "regarding the medical-scientific knowledge of the time"), referring to the "evolutionary dynamics inherent to medical-scientific knowledge that must support legislative choices in the healthcare field." The Constitutional Court thus deemed the legislator's actions in 2021 constitutionally legitimate, based on the principle that the law pursued the aim of preventing the spread of the virus, which, at that specific historical moment, represented the supreme interest of the community.

In judgment 15, the Court ruled on the reasonableness of the vaccination mandate for operators of residential, socio-assistance, and socio-health structures in place of diagnostic tests. For the judges, the vaccination mandate for health care professionals serves a dual purpose: protecting patients (vulnerable members of the population) and avoiding the interruption of essential services; therefore, the suspension of unvaccinated workers is legitimate, as the right to work does not have to constitute a risk for public health and has to maintain safe conditions in health care.

In conclusion, the Italian Constitutional Court upheld the constitutional legitimacy of the measures under appeal introduced by the legislator. The temporary restrictions on personal freedom appear justified by the need to protect the right to health, a fundamental primary good, in the absence of which other legal goods would cease to exist and for which temporary compressions and limitations of other constitutionally guaranteed rights are legitimate.

COVID-19; Vaccine; Court

G4 Transnational Investigations on Encrypted Platforms: The Italian Experience

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Learning Objectives: Participants will acquire knowledge for the correct management of investigative activities aimed at the acquisition of large-scale data, both from a technical-operational and legal point of view. They will know the reference rules, jurisprudential practices, the guidelines of the European and International Courts, and the rules for proceeding with the information-investigative exchange. Participants will be able to critically evaluate the various problems connected to the management of complex scenarios and refine their analytical and judgment skills in relation to the application of the correct intervention models in the technological field.

Impact Statement: The presentation will help the scientific community to: (1) identify the best data acquisition and storage techniques to demonstrate their authenticity and genuineness, respecting the chain of custody; (2) understand the scope of the technical-scientific activities carried out on encrypted platforms and their evidentiary weight in the criminal justice sector; and (3) improve investigative skills in terms of efficiency and effectiveness.

Abstract Text: The activities on encrypted platforms, recently used by criminal organizations to conduct their trafficking, are the last frontier of investigations. Due to the impenetrability of devices that use encrypted communication systems, investigators "penetrate" the server to find relevant information.[1] From the study conducted in recent years by researchers from the University of Foggia and the Italian National Police, it has emerged that these investigations are complex, both technically and legally.

As regards the technical aspects, it is about investigating platforms equipped with important degrees of encryption with servers often located in different parts of the world, exploiting the potential offered by the so-called big data.[2,3] The police need the close collaboration of the investigative bodies of other states than the one in which the investigative need originated, thereby exacerbating the already known dysfunctions of transnational cooperation.[4] As regards the legal aspects, critical issues arise in relation to: (1) the impact on fundamental rights (right to defense); (2) the proper legal framing of activities; (3) the diagnosis of procedural usability of data obtained as a result of decryption of data stored on these platforms; and (4) the transmission of the amount of information to other law enforcement authorities.[5-7]

Moreover, these are critical issues found in all European countries involved in the investigative operations under review. It is no coincidence that, in addition to the multiple pronouncements of the higher Courts of France, Germany, the Netherlands, and Norway, the Court of Justice of European Union (ECLI:EU:C:2024:372) has spoken out. While acknowledging the high rate of invasiveness of investigations on encrypted platforms, it has declared the legitimacy of large-scale communications acquisitions, classifying the activity as "interception of telecommunications."

In Italy, the United Sections of the Court of Cassation addressed the issue in two "twin" rulings (23756/2024; 23755/2024). The Court declared the legitimacy of the operations conducted and, therefore, the usability of the data collected through the European Investigation Order (EIO), but did not clarify the legal nature of the investigations on the platforms, missing the opportunity to outline the category in which to attribute the activities carried out on encrypted systems.[9]

The aim of this research work is to seek a necessary and reasoned balance between investigative needs and rights fundamentals, in compliance with the principle of proportionality.[10,11] In particular, it is necessary to: (1) verify the compatibility of investigations into crypto platforms with fundamental rights in order to prevent large-scale technological operations from turning into mass surveillance; (2) verify the compatibility of the investigations carried out on encrypted platforms with the already-existing evidentiary categories in order to ascertain the existence of suitable regulatory coverage to guarantee the legal integrity of the evidence thus collected; and (3) know the best techniques for acquiring and storing digital data to avoid abuse.

Looking to the future, it is considered essential to introduce a discipline capable of regulating the new forms of investigation with high technological potential, taking into account the balance between the various interests that may come into conflict. Precisely, since it is not conceivable to leave the choice of indiscriminate recourse to new investigation techniques to the availability of the investigators and not even to legitimize their use in jurisprudential terms through extensive interpretations in a matter governed by a rigid principle of mandatory nature, the need is felt for a intervention of the legislator, called to typify the complex of activities that can be carried out through new digital investigative techniques, in such a way as to make the limitations on individual prerogatives "tolerable" in a democratic society.[12,13]

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Digital Evidence; Cooperation; Human Rights

G5 Strategic Opportunities to Advance Forensic Science in the United States: A Path Forward Through Research and Standards

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Learning Objectives: After attending this presentation, attendees will learn about several select activities and reports currently being developed or recently published by the National Institute of Standards and Technology (NIST) Forensic Science Program, including scope, status, and outcomes. This presentation is one of eight that will be presented by NIST individuals.

Impact Statement: This presentation will provide the forensic science community an overview of key findings, implications, and recommendations affecting future directions and laboratory policies and procedures.

Abstract Text: Forensic science efforts at the NIST have been ongoing for the past century since Wilmer Souder's pioneering efforts in handwriting, typewriting, and firearm analysis from the 1920s to the 1950s.[1] NIST has increased its involvement in the field since a 2009 National Academy of Sciences study identified areas for improvement in forensic science.[2] Today, NIST conducts collaborative research, partners with the community to strengthen policies and practices, convenes meetings to examine issues, and explores scientific foundations of forensic methods and practices.[3] The ultimate aim of the NIST Forensic Science Program is to strengthen the scientific basis of forensic disciplines so that evidence may be appropriately collected and accurately analyzed, and results effectively communicated. Through a series of short presentations, this session will provide an overview of select activities and recent NIST reports, including major findings, key takeaways, and recommendations for the forensic science community. These activities range from assessing broad strategic challenges facing the entire forensic science community to discipline-specific recommendations affecting laboratory policies and procedures as part of human factors and scientific foundation reviews.

In September 2024, NIST published a report entitled *Strategic Opportunities to Advance Forensic Science in the United States: A Path Forward Through Research and Standards*, which identifies four "grand challenges" facing the forensic science community in the United States and provides a strategic roadmap for addressing these challenges through scientific research and standards.[4] To complete this report, NIST researchers conducted an extensive literature review and collected input from academic and government researchers, forensic science practitioners, legal experts, statistics experts and feedback from NIST subject matter experts to assess the forensic science environment and discuss the long-term vision and strategic priorities for forensic science in the United States. This presentation will provide a summary of the report, detailing an overview of the findings from those efforts and outline a strategy for the forensic science community to align over the next decade.

Presentations will also cover the use of documentary standards in legal proceedings and some new resources for officers of the court along with recent NIST efforts to assist validation studies.[5]

References:

1. https://www.nist.gov/feature-stories/who-was-detective-x; copies of Souder's notebooks are available in the NIST digital archives: https://nistdigitalarchives.contentdm.oclc.org/digital/collection/p16009coll67/search.
2. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Sci. Int. Genet.* 18: 4-20. [open access].
3. https://www.nist.gov/spo/forensic-science-program.
4. https://www.nist.gov/news-events/news/2024/09/nist-report-outlines-strategic-opportunities-advance-forensic-science-us
5. https://www.nist.gov/programs-projects/nist-forensic-science-training-program.

NIST; Forensic Science; Validation

G6 Communicating Forensic Findings

Sandra Koch, PhD, National Institute of Standards and Technology, Gaithersburg, MD; John Paul Jones II, MBA*, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Objectives: After attending this presentation, attendees will learn about several select activities and reports currently being developed or recently published by the National Institute of Standards and Technology (NIST) Forensic Science Program, including scope, status, and outcomes. This presentation is one of eight that will be presented by NIST individuals

Impact Statement: This presentation will provide the forensic science community an overview of key findings, implications, and recommendations affecting future directions and laboratory policies and procedures.

Abstract Text: Forensic science efforts at the NIST have been ongoing for the past century since Wilmer Souder's pioneering efforts in handwriting, typewriting, and firearm analysis from the 1920s to the 1950s.[1] NIST has increased its involvement in the field since a 2009 National Academy of Sciences study identified areas for improvement in forensic science.[2] Today, NIST conducts collaborative research, partners with the community to strengthen policies and practices, convenes meetings to examine issues, and explores scientific foundations of forensic methods and practices.[3] The ultimate aim of the NIST Forensic Science Program is to strengthen the scientific basis of forensic disciplines so that evidence may be appropriately collected and accurately analyzed, and results effectively communicated. Through a series of short presentations, this session will provide an overview of select activities and recent NIST reports, including major findings, key takeaways, and recommendations for the forensic science community. These activities range from assessing broad strategic challenges facing the entire forensic science community to discipline-specific recommendations affecting laboratory policies and procedures as part of human factors and scientific foundation reviews.

In June 2024, NIST hosted a 2-day workshop on Communicating Forensic Findings: Current Practices and Future Directions.[4] The presentations and discussions focused on: (1) how forensic findings are currently expressed in reports and testimony, (2) the use of likelihood ratios and verbal scales to convey a range of interpretation results, (3) influences on recipient understanding of the weight of different forensic evidence, (4) potential gaps in research or base knowledge that may limit an end user's ability to use or understand the findings, and (5) what the forensic community can do to improve communication and recipient understanding of forensic findings. NIST plans to use the information and insights gained from the June workshop and outreach at forensic meetings to inform a Scientific Foundation Review on this topic. In addition, under NIST's OSAC Program, the Forensic Science Standards Board has been reviewing opinion/interpretation scale standards produced by multiple subcommittees for listing on the OSAC Registry which prompted an effort to unify what these scales should include. Several of these principles will be discussed.

Presentations will also cover the use of documentary standards in legal proceedings and some new resources for officers of the court along with recent NIST efforts to assist validation studies.[5]

References:

1. https://www.nist.gov/feature-stories/who-was-detective-x; copies of Souder's notebooks are available in the NIST digital archives: https://nistdigitalarchives.contentdm.oclc.org/digital/collection/p16009coll67/search.
2. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Sci. Int. Genet.* 18: 4-20. [open access].
3. https://www.nist.gov/spo/forensic-science-program.
4. https://www.nist.gov/news-events/events/2024/06/communicating-forensic-findings-current-practices-and-future-directions.
5. https://www.nist.gov/programs-projects/nist-forensic-science-training-program.

NIST; Forensic Science; Validation

G7 Black-Box and Other Forensic Decision Analysis Studies: Understanding Limitations and Criticisms

R. Austin Hicklin, PhD, Noblis, Reston, VA; Nicole M. Richetelli, PhD*, Noblis, Reston, VA; JoAnn Buscaglia, PhD*, FBI Laboratory, Quantico, VA*

Learning Objectives: The goal of this presentation is to help attendees understand the issues involved in designing and conducting black-box and white-box evaluations as well as the limitations and criticisms of these types of studies.

Impact Statement: This presentation will impact the forensic science community by describing approaches to assess potential areas of strength and weakness in multiple forensic science disciplines, which will foster greater understanding of the use of these studies for evaluating the accuracy and reliability of forensic science examiner decisions.

Abstract Text: In recent years, there has been a great deal of interest in characterizing the accuracy and reliability of forensic comparison decisions using black-box studies, as well as doing detailed evaluations of how decisions are made using white-box studies. 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 guidance on the criteria PCAST considered necessary for such testing to be considered rigorous and suitable for court admissibility.[1]

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. This presentation will discuss the issues involved and lessons learned from designing and conducting 13 decision analysis studies in eight forensic disciplines, including two black-box studies and three white-box studies of latent print examination; two black-box studies of footwear examination; black-box studies of handwriting examination, bullet examination, bloodstain pattern analysis, and tire examination; and an interlaboratory study of DNA mixtures.[2-25]

Each of these studies involves navigating a number of issues and complexities, requiring tradeoffs between the ideal and the practical. This presentation will focus on: discipline-wide issues in the forensic disciplines themselves that limit decision analysis studies; constraints limiting how studies may be conducted; issues and complexities in study design, data analysis, and reporting; and recent criticisms of black-box studies.

These decision analysis studies have been conducted to provide information for laboratory managers, forensic practitioners, policy makers, and the legal community regarding the accuracy, reproducibility, and repeatability of decisions. This presentation is intended to provide stakeholders with an understanding of the complexities and limitations of such studies.

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1. 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). https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/PCAST/pcast_forensic_science_report_final.pdf (accessed March 22, 2023).
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4. R.A. Hicklin, N. Richetelli, A. Taylor, J. Buscaglia, *Accuracy and reproducibility of latent print examiner decisions on comparisons resulting from searches of an automated identification system*, (Manuscript in Preparation) (2024).
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Evaluation; Error Rate; Examiner Accuracy

G8 Standards Use in Legal Proceedings and Primers for Officers of the Court

John Paul Jones II, MBA, National Institute of Standards and Technology, Gaithersburg, MD; Dana Delger, JD*, Casper, WY*

Learning Objectives: After attending this presentation, attendees will learn about several select activities and reports currently being developed or recently published by the National Institute of Standards and Technology (NIST) Forensic Science Program, including scope, status, and outcomes. This presentation is one of eight that will be presented by NIST individuals.

Impact Statement: This presentation will provide the forensic science community an overview of key findings, implications, and recommendations affecting future directions and laboratory policies and procedures.

Abstract Text: Forensic science efforts at the NIST have been ongoing for the past century since Wilmer Souder's pioneering efforts in handwriting, typewriting, and firearm analysis from the 1920s to the 1950s.[1] NIST has increased its involvement in the field since a 2009 National Academy of Sciences study identified areas for improvement in forensic science.[2] Today, NIST conducts collaborative research, partners with the community to strengthen policies and practices, convenes meetings to examine issues, and explores scientific foundations of forensic methods and practices.[3] The ultimate aim of the NIST Forensic Science Program is to strengthen the scientific basis of forensic disciplines so that evidence may be appropriately collected and accurately analyzed and results effectively communicated. Through a series of short presentations, this session will provide an overview of select activities and recent NIST reports, including major findings, key takeaways, and recommendations for the forensic science community. These activities range from assessing broad strategic challenges facing the entire forensic science community to discipline-specific recommendations affecting laboratory policies and procedures as part of human factors and scientific foundation reviews.

The Organization of Scientific Area Committees (OSAC) for Forensic Science, administered by the NIST, supports the development and promotion of sound forensic science standards. This presentation will examine how these forensic standards and others have been used and understood by the legal system in court cases over the last year. It will also introduce new informational scientific primers for officers of the court produced by the NIST Forensic Science Program, designed to help the legal community better understand various scientific principles and become more informed consumers of forensic science information.

Presentations will also cover the use of documentary standards in legal proceedings and some new resources for officers of the court along with recent NIST efforts to assist validation studies.[4]

References:

1. https://www.nist.gov/feature-stories/who-was-detective-x; copies of Souder's notebooks are available in the NIST digital archives: https://nistdigitalarchives.contentdm.oclc.org/digital/collection/p16009coll67/search.
2. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Sci. Int. Genet.* 18: 4-20. [open access].
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4. https://www.nist.gov/programs-projects/nist-forensic-science-training-program.

NIST; Forensic Science; Validation

G9 Direct-to-Stakeholders: One State's Centralized Effort to Improve Crime Laboratory Record Discovery and Increase Transparency in Forensic Science

Leigh M. Tomlin, JD, Texas Forensic Science Commission, Austin, TX; Kevin Callahan, MS, Texas Department of Public Safety, Garland, TX*

Learning Objectives: After attending this presentation, attendees will learn about Texas' effort to improve transparency in forensic science through two major technology initiatives: the establishment of a six-pronged database created by the Texas Forensic Science Commission to provide access to various aspects of its work and, separately, a legislatively mandated statewide crime laboratory records portal in development under the direction of the Texas Department of Public Safety (DPS) to improve long-standing crime laboratory record discovery challenges.

Impact Statement: After attending this presentation, attendees will learn about Texas' effort to improve transparency in forensic science through two major technology initiatives: the establishment of a six-pronged database created by the Texas Forensic Science Commission (TFSC) to provide access to various aspects of its work and, separately, a legislatively mandated statewide crime laboratory records portal in development under the direction of the DPS to improve long-standing crime laboratory record discovery challenges.

Abstract Text: Historically, our criminal justice system has always placed the burden of disclosure on the prosecution. In 1963, the Supreme Court in *Brady v. Maryland* emphasized the government's obligation to disclose exculpatory or impeaching information irrespective of the prosecution's good faith or bad faith.[1] In Texas, article 39.14 of the Texas Code of Criminal Procedure governs discovery rights for defendants in criminal cases, with one particular subsection (commonly referred to as the "Michael Morton Act") focusing on the State's obligation to produce exculpatory, impeachment, or mitigating information. The prosecution must produce evidence if it is material to the case generally, in the State's possession, custody, or control, or under contract with the State.[2] This includes evidence in possession of either the State *or any of its agencies* (including the crime laboratory and any associated law enforcement agency).[3] *Brady* (Michael Morton Act) material must be affirmatively produced, without a request from the defense.[4] The Texas Court of Criminal Appeals recently underscored in *State of Texas v. Heath* that "the State" means State of Texas prosecutors, law enforcement entities, and crime laboratories, and affirmed that a trial court has the discretion to exclude evidence from being admitted based solely on the State's failure to disclose the information in a timely manner, even if the prosecutor was unaware the evidence existed because it was in the possession of law enforcement.[5]

The evolution that accompanied the Michael Morton Act and related case law places a significant strain on agencies at a practical level because information held by law enforcement agencies and crime laboratories is imputed to prosecutors. Crime laboratories working for or under contract with the State must comply with discovery and disclosure obligations, including responding to a significant increase in discovery requests and associated costs of producing voluminous case-related data and other laboratory quality system-related information.

Technology solutions are one pathway to reducing this burden, including the statewide electronic discovery portal described in Senate Bill 991 passed during the 88th Texas Legislative Session, requiring the Texas DPS to create and maintain a centralized, digital crime laboratory records portal for all laboratories (public or private) that perform forensic analysis for Texas criminal cases. The mandate requires DPS to establish by rule a process (and procure a technology solution) for requesting crime laboratory records and for transferring those records among crime laboratories, attorneys, and other parties authorized to access the records as part of discovery. Once complete, the system will give parties to a particular criminal case unprecedented direct and timely access to crime laboratory records. The program is dubbed "Texas Crime Lab Records Connect" or "Texas CLR Connect." Texas CLR Connect will not only bridge the gap between lawyers and crime laboratory records, but also aims to minimize the burden of production on both the prosecution and crime laboratories.

In a parallel effort, the State's oversight body for forensic science, TXFSC, has recently developed a web-based, public database of all public information held by the Commission to increase transparency of its operations, including searchable current and historical records on licensees, crime laboratories, self-disclosures, complaints, Organization of Scientific Area Committees (OSAC) Registry Standard implementation, and crime laboratory quality incident records. The database can be accessed at www.fsc.texas.gov and expects to be fully populated by June 2025. The database has changed the way the public accesses information either received or produced by the Commission, significantly bridging the gap between criminal justice partners and access to the work of the TXFSC.

These efforts are a solid step in increasing the basis for transparency in the criminal justice system and improving the way stakeholders access crime laboratory information.

References:

1. *Brady v. Maryland*, 373 U.S. 83 (1963).
2. Tex. Code. Crim. Proc. art. 39.14.
3. Id.
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Crime Laboratory Records; Discovery; State Oversight

G10 When Are Opinions "Based on My Training and Experience" (BOMTAE) Reliable—And How Can You Show It?

Colin J. Bellair, JD, Travis County District Attorney's Office, Austin, TX; Pate Skene, JD, PhD*, University of Colorado Boulder, Boulder, CO*

Learning Objectives: Recent amendments to Federal Rule of Evidence 702 illustrate ongoing concerns with the way lawyers and courts currently decide whether an expert's testimony in a particular case is based on a reliable application of the expert's training and experience. In this presentation, attendees will learn how to go beyond general assertions that an expert's testimony is "based on my training and experience" (BOMTAE). Attendees will learn: (1) specific criteria that can distinguish whether an opinion or other testimony is or is not based on a reliable application of the expert's training and experience; (2) more effective ways of explaining the basis for expert testimony; and (3) how to structure cross-examination in a way that more effectively tests for limitations or potential errors in the expert's analysis.

Impact Statement: This presentation will: (1) help lawyers, judges, and forensic science understand how and when training and experience provides a reliable basis for expert judgment; (2) describe specific questions that lawyers can use to demonstrate both the reliability and limitations of an expert's training and experience as a basis for a particular opinion or statement; and (3) provide judges and jurors a better framework for evaluating testimony by forensic science experts.

Abstract Text: Training and experience are two of the foundations for establishing a forensic scientist's qualifications as an expert witness in court and the relevance and reliability of their testimony in a particular case.[1]

A common approach for establishing qualifications and (supposed) reliability is for the proponent of the testimony to ask the witness to summarize their training and experience in general terms (e.g, field of training, degrees, years of experience) and then to establish the reliability of their opinions in a specific case by testifying that each opinion is BOMTAE.

However, this approach waters down what is intended to be a substantive probe of relevance and reliability into a rote formula that could be invoked to cover both reliable and unreliable applications of expert training and experience to the facts of a specific case. Research on human expert learning and machine learning shows that this common approach does not provide an adequate basis for a court or trier of fact to evaluate the reliability of expert testimony or for effective cross-examination to probe for legitimate scientific objections or limitations of the testimony.

Human studies show that the amount of training and experience doesn't correlate with performance (proficiency, competence).[2] Decades of research in safety-critical fields like commercial aviation and medicine have found consistently that professional certifications and years of experience correlate with an expert's personal confidence in their skill, but that accuracy and consistency of performance depend on the type of feedback present during training and experience.[3,4] Basic tenets of machine learning show that acquiring expertise in any analytical or cognitive task depends fundamentally on: (1) the scope, variation, and difficulty of cases represented in the training and experience; (2) effective feedback on accuracy and reliability; and (3) the reward structure for accuracy and errors throughout the training and experience.[4,5] These principles are illustrated, for example, by the types and frequency of errors noted for facial recognition algorithms originally trained on images from a sample set with limited racial and geographic variation, and more recently in the widely noted examples of errors and biases in results produced by commercial AI programs built on unsupervised searches by Large Language Models (LLMs).

On top of this, the new revisions to FRE 702 call on lawyers, experts, and gatekeepers to reconsider how they undertake their roles in evaluating this evidence. The inquiry into training and experience needs to move away from a generic approach to a more granular one.[6,7]

When offering or cross examining expert evidence, lawyers need to ask questions such as:

- Does the expert's training and experience cover the specific question/fact in issue in this case?
- Can the expert explain their process in a way that provides a rational basis for another person (the court, trier of fact) to make their own determination of the reliability of the testimony and what weight to give the conclusions?
- Is BOTMAE misleading the trier of fact when it takes advantage of lay expectations that forensic methods or professional training and experience in general provide rigorous feedback AND that the expert's specific training and experience encompasses the type of samples, facts, or data at issue in this case?
- How would you know if you are wrong?

For forensic experts, lawyers, and gatekeepers, this framework provides an opportunity to probe effectively for limitations and errors in the expert's analysis and for meeting the burden of demonstrating that an opinion is based on reliable application of the expert's training and experience to the facts in a particular case.

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Expert Testimony; Human Factors; Machine Learning

G11 DNA Human Factors Study

Melissa Taylor, BA, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Objectives: After attending this presentation, attendees will learn about several select activities and reports currently being developed or recently published by the National Institute of Standards and Technology (NIST) Forensic Science Program, including scope, status, and outcomes. This presentation is one of eight that will be presented by NIST individuals.

Impact Statement: This presentation will provide the forensic science community an overview of key findings, implications, and recommendations affecting future directions and laboratory policies and procedures.

Abstract Text: Forensic science efforts at the NIST have been ongoing for the past century since Wilmer Souder's pioneering efforts in handwriting, typewriting, and firearm analysis from the 1920s to the 1950s.[1] NIST has increased its involvement in the field since a 2009 National Academy of Sciences study identified areas for improvement in forensic science.[2] Today, NIST conducts collaborative research, partners with the community to strengthen policies and practices, convenes meetings to examine issues, and explores scientific foundations of forensic methods and practices.[3] The ultimate aim of the NIST Forensic Science Program is to strengthen the scientific basis of forensic disciplines so that evidence may be appropriately collected and accurately analyzed, and results effectively communicated. Through a series of short presentations, this session will provide an overview of select activities and recent NIST reports, including major findings, key takeaways, and recommendations for the forensic science community. These activities range from assessing broad strategic challenges facing the entire forensic science community to discipline-specific recommendations affecting laboratory policies and procedures as part of human factors and scientific foundation reviews.

The Expert Working Group on Human Factors in Forensic DNA Interpretation has conducted a scientific assessment of the effects of human factors in forensic DNA analysis. The Working Group evaluated relevant bodies of scientific literature and technical knowledge to develop recommendations to improve practice and reduce the likelihood of errors. This report, which was released in May 2024, provides a comprehensive discussion of human factors as they relate to all aspects of DNA analysis, from interpreting electropherograms to reporting results and testifying in court.[4] This expert working group is supported by the National Institute of Justice (NIJ) Office of Investigative and Forensic Sciences (OIFS) and the NIST Special Programs Office.

Presentations will also cover the use of documentary standards in legal proceedings and some new resources for officers of the court along with recent NIST efforts to assist validation studies.[5]

References:

1. https://www.nist.gov/feature-stories/who-was-detective-x; copies of Souder's notebooks are available in the NIST digital archives: https://nistdigitalarchives.contentdm.oclc.org/digital/collection/p16009coll67/search.
2. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Sci. Int. Genet.* 18: 4-20. [open access].
3. https://www.nist.gov/spo/forensic-science-program.
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5. https://www.nist.gov/programs-projects/nist-forensic-science-training-program.

NIST; Forensic Science; Validation

G12 DNA Mixture Interpretation Foundation Study

John M. Butler, PhD, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Objectives: After attending this presentation, attendees will learn about several select activities and reports currently being developed or recently published by the National Institute of Standards and Technology (NIST) Forensic Science Program, including scope, status, and outcomes. This presentation is one of eight that will be presented by NIST individuals.

Impact Statement: This presentation will provide the forensic science community an overview of key findings, implications, and recommendations affecting future directions and laboratory policies and procedures.

Abstract Text: Forensic science efforts at the National Institute of Standards and Technology (NIST) have been ongoing for the past century since Wilmer Souder's pioneering efforts in handwriting, typewriting, and firearm analysis from the 1920s to the 1950s.[1] NIST has increased its involvement in the field since a 2009 National Academy of Sciences study identified areas for improvement in forensic science.[2] Today, NIST conducts collaborative research, partners with the community to strengthen policies and practices, convenes meetings to examine issues, and explores scientific foundations of forensic methods and practices.[3] The ultimate aim of the NIST Forensic Science Program is to strengthen the scientific basis of forensic disciplines so that evidence may be appropriately collected and accurately analyzed, and results effectively communicated. Through a series of short presentations, this session will provide an overview of select activities and recent NIST reports, including major findings, key takeaways, and recommendations for the forensic science community. These activities range from assessing broad strategic challenges facing the entire forensic science community to discipline-specific recommendations affecting laboratory policies and procedures as part of human factors and scientific foundation reviews.

NIST is conducting a scientific foundation review that explores the capabilities and limitations of DNA mixture interpretation methods, including probabilistic genotyping software systems.[4] We have identified scientific principles, reviewed the scientific literature, gathered other empirical evidence from publicly available sources, and collected input from a group of forensic DNA practitioners and researchers. The final version of our report was also informed by public comments received in response to an initial draft of this report released in June 2021. The contents of this report, which is arranged into six chapters and two supplemental documents, will be discussed along with its key takeaways.

Presentations will also cover the use of documentary standards in legal proceedings and some new resources for officers of the court along with recent NIST efforts to assist validation studies.[5]

References:

1. https://www.nist.gov/feature-stories/who-was-detective-x; copies of Souder's notebooks are available in the NIST digital archives: https://nistdigitalarchives.contentdm.oclc.org/digital/collection/p16009coll67/search.
2. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Sci. Int. Genet.* 18: 4-20. [open access].
3. https://www.nist.gov/spo/forensic-science-program.
4. https://www.nist.gov/spo/forensic-science-program/dna-mixture-interpretation-nist-scientific-foundation-review.
5. https://www.nist.gov/programs-projects/nist-forensic-science-training-program.

NIST; Forensic Science; Validation

G13 How Should DNA Analysts Answer the "How" Questions? Pitfalls and Recommendations for Evaluations of DNA Results Given Activity-Level Propositions

Lynn Garcia, JD, Texas Forensic Science Commission, Austin, TX*

Learning Objectives: After attending this presentation, attendees will learn about risks, pitfalls, and a potential path forward for evaluations of DNA results given activity level propositions in the United States.

Impact Statement: This is one of the most critical and urgently needed areas of research in forensic DNA analysis at the current time. The presentation will assist lawyers and scientists alike in understanding the risks and a potential path forward for answering "how" and "when" questions regarding forensic DNA analysis.

Abstract Text: In a recent Texas murder trial styled *State of Texas v. Kaitlin Armstrong*, the State presented results from DNA testing of swabs taken from the victim's bicycle near the crime scene. To raise doubt about the probative value of this evidence given the case facts, the defense called an expert to opine that it was possible the DNA was transferred to the bicycle even though the defendant never touched it. In rebuttal, the State called an expert who performed an evaluation of the DNA results given activity level propositions in advance of trial. The rebuttal expert opined the evidence was "much more likely" if the defendant picked up and removed the victim's bicycle from the house than if the DNA had been deposited through a series of indirect activities.

The Texas Forensic Science Commission (TFSC) investigated a complaint regarding the rebuttal testimony alleging the expert departed from "established best practices" in evaluative reporting given activity level propositions. The TFSC published a report that made observations regarding the integrity and reliability of the activity level analysis and set forth recommendations regarding the challenging questions DNA analysts face every day in court related to determining "how" or "when" DNA may or may not have been deposited on items of evidence.

Because there is very little published guidance on the question in the United States, the report relies heavily on international guidance and best practices documents. The report discusses several areas where the criminal courts in the United States differ from international criminal courts, creating challenges for the application of published international guidance. One such difference concerns the calling of a rebuttal witness to discuss activity level propositions with little discovery or prior notice. While this practice is allowable under applicable legal rules, it thwarts the transparent approach advocated by international guidance. The Commission strongly recommends the preparation (and discovery) of a written report for activity level propositions regardless of whether the expert is associated with an accredited laboratory.

The report also discusses areas that should be addressed by the expert when formulating the mutually exclusive activity level propositions. The expert should use well-formulated propositions, assess the suitability of research to the proposition in question, and provide details regarding the connection between the data in referenced studies and the opinion offered (traceability). There are several unanswered issues and questions that should be addressed if the United States decides to move forward with formal activity level evaluations, including determining whether a numerical likelihood ratio should be required instead of a "notional" qualitative expression, what training and experience an analyst would need to correctly perform such evaluations, and what quality assurance and accreditation measures need to be in place.

The report details numerous recommendations at both the state and national level. The recommendations warn that additional research guidance is needed, and method development (and validation) are critical. While this work is underway, experts should not give opinions on what is the most likely way of transfer. Proposed language is provided to assist practitioners in fielding "how" and "when" questions will be discussed.

Forensic DNA; Activity Level; Expert Testimony

G14 Bridging the Knowledge Gap on DNA-TPPR in the American Court System

John D. Schmid, JD, Sixth District Public Defender's Office, Duluth, MN; Clinton Hughes, JD*, Brooklyn Defender Services, Brooklyn, NY*

Learning Objectives: Attendees will learn about the current knowledge gap among many criminal justice stakeholders concerning research on Transfer, Persistence, Prevalence and Recovery (TPPR) of DNA and the urgent need to bridge this gap. We will introduce attendees to an online, self-administering quiz designed to educate stakeholders about foundational research concerning TPPR and encourage honest self-assessments in a non-punitive and non-adversarial atmosphere. This online quiz will reinforce for attendees the more positive aspect of the annual conference's theme this year because it is a tool of transformation rather than tyranny, and it represents the responsible, ethical, and just use of technology.

Impact Statement: Achieving just outcomes requires attorneys and other stakeholders to responsibly engage in assessing the relevance of DNA collected during criminal investigations. The gaming process will encourage stakeholders to read and absorb the underlying studies and incorporate research findings into assessments of common fact patterns in criminal cases (e.g., executions of search warrants in family homes or cars, complex mixtures found on crime victims' clothing, etc.). Our online, self-administered quiz represents a sensible use of technology to empower all stakeholders to responsibly consider the relevance of DNA evidence.

Abstract Text: The 2021 DNA Mixture Interpretation: A NIST Scientific Foundation Review (Draft Report) highlighted the biological reality that "DNA can be transferred from one surface or person to another, and this can potentially happen multiple times. Therefore, the DNA present on an evidence item may be unrelated (irrelevant) to the crime being investigated."[1] Moreover, the increased sensitivity of DNA testing methods has led to an increase in the risk of prosecutions and wrongful convictions based on the detection of irrelevant DNA during a criminal investigation, as noted in Peter Gill's *How Misuse of DNA Evidence has Led to Miscarriages of Justice*.[2] Preventing these miscarriages of justice is a challenging but essential task of legal practitioners and the forensic DNA community.

The current practice in most American courts and forensic laboratories must evolve. The National Institute of Standards and Technology (NIST) Draft Report recognizes that statistics assigning weight to source and subsource level associations "do not provide information about how or when DNA was transferred, or whether it is relevant to a case. Therefore, using the likelihood ratio as a standalone number without context can be misleading."[3] The Draft Report also laments that "significant knowledge gaps remain" in the "body of DNA knowledge on DNA transfer.[4]

As the 2024 Report of the Expert Working Group on Human Factors in Forensic DNA Interpretation makes clear, the factors related to assessing "how" or "when" DNA may have been transferred are highly variable and distinct from the factors related to source or subsource level assessments.[5] A significant and ever-growing body of TPPR research has identified many different variables that may or may not be knowable in a criminal investigation. Yet, stakeholders in the legal system frequently lack the foundational knowledge necessary to responsibly assess the relevance of DNA recovered during a criminal investigation.[6] As legal practitioners in the criminal justice system, we have observed how this significant knowledge gap has resulted in: (1) misstated or overstated testimony about direct versus indirect transfer, (2) misunderstandings about the strength or weaknesses of a case during plea negotiations, and (3) misunderstandings about the strength of DNA evidence by judicial factfinders. As part of our presentation, we will discuss our experience with problematic testimony and the inability of some stakeholders to gauge the relevance of DNA in particular cases.

We are working to bridge the knowledge gap described above by developing an open access, online exam where anyone interested in DNA transfer can test themselves on knowledge of the basic literature. Stakeholders who participate in the exam will be exposed to findings from seminal TPPR studies. The exam will also allow participants to assess gaps in their own knowledge of TPPR research in a non-confrontational and non-adversarial setting. Considering the recent success of online quizzing in the news industry, we foresee that exposure to TPPR research in an environment allowing for private self-assessments will improve the ability of stakeholders from the legal and forensic DNA communities to responsibly assess the relevance of DNA recovered during a criminal investigation.[7]

References:

1. John Butler et al. (2021) DNA Mixture Interpretation: A NIST Scientific Foundation Review, Gaithersburg, Maryland, *National Institute of Standards and Technology*, at 98 https://doi.org/10.6028/NIST.IR.8351-draft ("Draft Report").
2. See generally Gill P. How Misuse of DNA Evidence Has Led to Miscarriages of Justice. *The Justice Gap*. 2017.
3. Draft Report at 8, 133.
4. Id. at 140.
5. Melissa Taylor et al. (2024) Forensic DNA Interpretation and Human Factors: Improving the Practice Through a Systems Approach, NIST Interagency/Internal Report (NISTIR), *National Institute of Standards and Technology*, Gaithersburg, MD, [online], at 153 https://doi.org/10.6028/NIST.IR.8503 ("Human Factors Report").
6. Id. at 173.
7. See, e.g *Axios*, Games are helping the New York Times thrive amid media chaos, available at https://www.axios.com/2024/01/29/wordle-nyt-games-news-media-layoffs; Daybreak, Hanover-based NewsGames finds a growing market, available at https://bitter-meteoroid-729.notion.site/Hanover-based-NewsGames-finds-a-growing-market-483503aa32074928aa0a9a3a4d4b9439; Niemenlab, The New York Times hits 10 million subscribers by using non-news products as an on-ramp, available at https://www.niemanlab.org/2023/11/the-new-york-times-hits-10-million-subscribers-by-using-non-news-products-as-an-on-ramp/.

Activity Level; DNA; Education

G15 Communicating Complex Topics in Forensic DNA Through Data and Process Visualization

Nathaniel Adams, BS, Forensic Bioinformatic Services, Fairborn, OH; Dan Krane, PhD, Wright State University, Dayton, OH*

Learning Objectives: After attending this presentation, attendees will understand costs and benefits of summary visual representation of data or processes when communicating concepts in forensic DNA analysis.

Impact Statement: This presentation will impact the forensic science community by highlighting opportunities for improving efficiency and efficacy of communication of forensic DNA topics that are challenging to discuss in litigation or trial.

Abstract Text: Informed forensic DNA conversations require that parties understand foundational scientific concepts, guidance and standards, laboratory protocols and validation efforts, and complex data sets. Criticisms of forensic DNA evidence often involve conveying deviations from empirical study, standard practice, protocol, or theoretical expectations. Visual representations can communicate these departures efficiently and impactfully. Various methods of representing data can simultaneously communicate issues and their significance in a case, often with only one or two charts on a single slide or printed page.

This presentation will provide examples of charts included as exhibits or in briefings and expert reports. The following issues will be explored for the "traditional" human review element of forensic DNA profiling: (1) (non-)reproducibility of low-level profiles through duplicate amplifications; (2) discordance between evidentiary and suspect profiles; and (3) human factor analysis of laboratory protocol process flowcharts.

Conversations about strengths and weakness of Probabilistic Genotyping Software (PGS) regularly involve software-assisted complex data analysis or explicitly address the role of software engineering norms and practices in the modern forensic DNA field. Examples from this arena will explore: (1) data visualization used in publications and internal validation summary documents used to support casework use of PGS; (2) underexplored ranges of reliability for mixture complexity "factors" affecting probabilistic genotyping system performance; and (3) illustrations of software development process concerns, including for validation processes.

Each example will be presented within its case context, including a brief background on the identified issue and the competing perspectives held by opposing parties. Good and poor examples will be shown, highlighting the strengths and weaknesses of each, including a breakdown of relevant elements as they would be explained in accompanying text or oral testimony. Finally, a description of the type and amount of work necessary to construct similar charts will be provided so attendees can consider using these tools in future cases.

DNA; Visualization; Court

G16 DNA's Value as Evidence for the Accused: Increasing the Awareness of Potentially Favorable Evidence in DNA Casework

Joseph Cavise, JD, Law Office of the Cook County Public Defender, Chicago, IL; Jessica Goldthwaite, JD, The Legal Aid Society, Mobile, NY; Allison Lewis, JD, The Legal Aid Society, New York, NY; Nicolas Hughes, JD*, Nicolas Hughes Law Firm, Houston, TX*

Learning Objectives: The attendees may be aware that the duty to disclose favorable evidence extends to the crime laboratory, but laboratory personnel receive little guidance on how to identify which information is potentially exculpatory, mitigating, or may have impeachment value. This presentation will seek to bridge the gap between the needs of the defense community and the laboratory by highlighting specific circumstances and information that should be disclosed to the defense in a case involving DNA analysis.

Impact Statement: The presentation intends to impact the forensic science community by deepening forensic laboratories' understanding of the need to identify evidence that is potentially favorable to the accused in the context of a case involving DNA analysis. This presentation will help bridge the gap between the laboratory and the defense community, by recommending the disclosure of specific circumstances and information that are potentially favorable to the accused. Finally, this presentation intends to promote additional discussion on what favorable evidence and *Brady* mean within the context of forensic science.

Abstract Text: Prosecutors have a duty to disclose favorable evidence to the accused.[1] This duty extends to favorable evidence known to others working on the government's behalf on a case.[2] Courts have applied this principle to extend to crime laboratories.[3-6] While the onus of disclosure falls upon prosecutors, it is clear that legal professionals often lack the training in forensic science to adequately assess lab results, much less understand the intricate technical and statistical issues involved in DNA analysis.[7] Meanwhile, laboratory personnel have the specialized training and skill to spot issues that are favorable to the accused.[8] Furthermore, only the laboratory may have the ability to develop the information in a way that can be helpful to the accused. However, laboratory personnel receive little guidance on what information is potentially exculpatory, mitigating, or may have impeachment value, and relatively low amounts of information are marked for disclosure.

A wide variety of events observed during DNA analysis are often favorable to the accused. One is a category we will call "interpretation events." These include various possible defects or inconsistencies during analysis or interpretation as well as limitations of the interpretation protocol. Some examples include instances where laboratory analysts reach non-concordant results, software reports or statistical results that are contradictory, new interpretation guidelines or software updates that are likely to affect reported results, and where samples are outside of the ranges used during the validation samples. A laboratory's interpretation protocols may not permit the interpretation of complex mixtures, may fail to properly exclude the accused from a complex mixture, may fail to compare all samples (and not just the reference sample from the accused) to all evidentiary samples, or which may simply fail to create a pathway for the defense to submit alternative hypotheses.

A second category we will call "testing events" involve unexpected or problematic data that may contradict or weaken the prosecution's theory of the case. These events include the identification of DNA profiles that are not explained by any reference profile or elimination sample, whether these profiles are full single source profiles or partial profiles.[9,10] Additionally, the serology or quantitative results might not seem to support the prosecution's theory. While the laboratory may lack the ability to investigate or answer these sorts of questions, these issues can be flagged and brought to the defense's attention. Beyond specific findings that may support a defensive theory, many testing events may raise questions regarding the reliability of the analysis, including compromised samples, personnel issues, samples requiring reanalysis, unexpected data in controls, instrument failures, non-conformities occurring during analysis, the detection of contamination near in time to the analysis, or where peak height ratios or inter-locus balance is outside of established ranges.

A final category of events we will call "undisclosed limitations," which include using potentially misleading terminology and failing to disclose important limitations of DNA analysis. Terminology, including the use of "sperm fraction" or "epithelial fraction" when no serological testing was performed or there is no linkage between source and subsource-level results or "conservative" may be misleading in the context of a case. DNA reports may understate important limitations of the results, including failing to disclose limitations of probabilistic genotyping, data modeling, presumptive screening tests, and may fail to highlight the uncertainty in low Likelihood Ratio (LR) results. While these suggestions are not meant to be a comprehensive list of the information that should be disclosed to the defense, we hope the identification of information that is potentially favorable to the accused helps add to the discussion regarding *Brady* and forensics and fosters the development of more robust disclosure policies.

References:

1. *Brady v. Maryland*, 373 U.S. 83, 87 (1963).
2. *Kyles v. Whitley*, 514 U.S. 419, 437-38 (1995).
3. *Commonwealth v. Martin*, 427 Mass. 816, 824, 696 N.E.2d 904, 909 (1998).
4. *Ex parte Chaney*, 563 S.W.3d 239, 270-71 (Tex. Crim. App. 2018).
5. *State v. Meza*, 203 Ariz. 50, 55, 50 P.3d 407, 412 (Ct. App. 2002).
6. Robert Smith, Bench Notes and Brady, *Proceedings of the American Academy of Forensic Sciences*, 75th Annual Scientific Conference. Orlando, FL. 2023.
7. Brandon Garrett, Glinda Cooper, and Quinn Beckham, Forensic Science in Legal Education, 51 *J.L. & Educ.* 1 (2022).
8. Allison Lewis, Consider the Defense: How (and When) the Defense Might Successfully Communicate with Labs… and Why Labs Should Listen, *2023 International Symposium on Human Identification* (2023).
9. OSAC 2021-S-0021: *Forensic Autosomal STR DNA Statistical Analyses – General Protocol, Protocol Verification, and Case Record Requirements*, Organization of Scientific Area Committees for Forensic Science (2021).
10. K. Slooten, Identifying Common Donors in DNA Mixtures, With Applications to Database Searches, 26 *Forensic Science International: Genetics* 40–47 (2017).

***Brady*; Disclosure; Fairness**

G17 Validation

Erin P. Forry, MS, RTI International, Research Triangle Park, MA; Melissa Taylor, BA*, National Institute of Standards and Technology, Gaithersburg, MD; Robert Ramotowski, MS*, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Objectives: After attending this presentation, attendees will learn about several select activities and reports currently being developed or recently published by the National Institute of Standards and Technology (NIST) Forensic Science Program, including scope, status, and outcomes. This presentation is one of eight that will be presented by NIST individuals.

Impact Statement: This presentation will provide the forensic science community an overview of key findings, implications, and recommendations affecting future directions and laboratory policies and procedures.

Abstract Text: Forensic science efforts at the NIST have been ongoing for the past century since Wilmer Souder's pioneering efforts in handwriting, typewriting, and firearm analysis from the 1920s to the 1950s.[1] NIST has increased its involvement in the field since a 2009 National Academy of Sciences study identified areas for improvement in forensic science.[2] Today, NIST conducts collaborative research, partners with the community to strengthen policies and practices, convenes meetings to examine issues, and explores scientific foundations of forensic methods and practices.[3] The ultimate aim of the NIST Forensic Science Program is to strengthen the scientific basis of forensic disciplines so that evidence may be appropriately collected and accurately analyzed and results effectively communicated. Through a series of short presentations, this session will provide an overview of select activities and recent NIST reports, including major findings, key takeaways, and recommendations for the forensic science community. These activities range from assessing broad strategic challenges facing the entire forensic science community to discipline-specific recommendations affecting laboratory policies and procedures as part of human factors and scientific foundation reviews.

Method validation is the process of establishing documentary evidence demonstrating a method's performance characteristics and limitations, identifying the influences that may change these characteristics, and understanding to what extent these characteristics can change. It serves as a foundational element of a robust quality assurance system, ensuring reliability, accuracy, and reproducibility. In response to a call for guidance, NIST has drafted a document outlining a set of general principles and approaches to method validation that represents its current thinking on the topic. This presentation will cover these general principles and approaches, provide information regarding upcoming NIST validation-related training efforts, and share helpful tools and references.

Presentations will also cover the use of documentary standards in legal proceedings and some new resources for officers of the court along with recent NIST efforts to assist validation studies.[4]

References:

1. https://www.nist.gov/feature-stories/who-was-detective-x; copies of Souder's notebooks are available in the NIST digital archives: https://nistdigitalarchives.contentdm.oclc.org/digital/collection/p16009coll67/search.
2. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Sci. Int. Genet.* 18: 4-20. [open access].
3. https://www.nist.gov/spo/forensic-science-program.
4. https://www.nist.gov/programs-projects/nist-forensic-science-training-program.

NIST; Forensic Science; Validation

G18 Firearm Examination Foundation Study

Theodore V. Vorburger, PhD, National Institute of Standard and Technology, Highlands Ranch, Colorado; Steven Lund, PhD*, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Objectives: After attending this presentation, attendees will learn about several select activities and reports currently being developed or recently published by the National Institute of Standards and Technology (NIST) Forensic Science Program, including scope, status, and outcomes. This presentation is one of eight that will be presented by NIST individuals.

Impact Statement: This presentation will provide the forensic science community an overview of key findings, implications, and recommendations affecting future directions and laboratory policies and procedures.

Abstract Text: Forensic science efforts at the NIST have been ongoing for the past century since Wilmer Souder's pioneering efforts in handwriting, typewriting, and firearm analysis from the 1920s to the 1950s.[1] NIST has increased its involvement in the field since a 2009 National Academy of Sciences study identified areas for improvement in forensic science.[2] Today, NIST conducts collaborative research, partners with the community to strengthen policies and practices, convenes meetings to examine issues, and explores scientific foundations of forensic methods and practices.[3] The ultimate aim of the NIST Forensic Science Program is to strengthen the scientific basis of forensic disciplines so that evidence may be appropriately collected and accurately analyzed and results effectively communicated. Through a series of short presentations, this session will provide an overview of select activities and recent NIST reports, including major findings, key takeaways, and recommendations for the forensic science community. These activities range from assessing broad strategic challenges facing the entire forensic science community to discipline-specific recommendations affecting laboratory policies and procedures as part of human factors and scientific foundation reviews.

NIST is conducting a scientific foundation review on firearm examination, a forensic technique used to compare the marks on bullets and cartridge cases under a comparison microscope with the those from a suspected firearm used in a crime.[4] A draft report will be shared for public comment when our internal review is completed. In this report, we show statistics that attempt to characterize all aspects of firearm examiner performance data from black box studies and from other proficiency tests. Those data provide an empirical basis for the foundational validity of firearm examination, but they are not sufficient to enable an assessment of validity to apply in any real-life case scenario.

Presentations will also cover the use of documentary standards in legal proceedings and some new resources for officers of the court along with recent NIST efforts to assist validation studies.[5]

References:

1. https://www.nist.gov/feature-stories/who-was-detective-x; copies of Souder's notebooks are available in the NIST digital archives: https://nistdigitalarchives.contentdm.oclc.org/digital/collection/p16009coll67/search.
2. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Sci. Int. Genet.* 18: 4-20. [open access].
3. https://www.nist.gov/spo/forensic-science-program.
4. https://www.nist.gov/spo/forensic-science-program/firearm-examination-nist-scientific-foundation-review.
5. https://www.nist.gov/programs-projects/nist-forensic-science-training-program.

NIST; Forensic Science; Validation

G19 The False Promise of Firearms Examination Validation Studies: Lay Controls, Simplistic Comparisons, and the Failure to Soundly Measure Misidentification Rates

Richard E. Gutierrez, JD, University of Illinois Chicago School of Law, Chicago, IL; Emily J. Prokesch, JD*, New York State Defender Association; Columbia Law School, New York, NY*

Learning Objectives: After attending this presentation, attendees will understand that the use of overly simplistic samples may limit the probative value and generalizability of error rate studies for the field of firearms and toolmark comparison. Attendees will learn how professional examiners in one validation study performed compared to a post-hoc lay control group of attorneys, consider how varied test conditions and bias may have influenced the results, and in doing so gain an understanding of how performance rates on different-source comparisons suggest that existing foundational literature fails to utilize sufficiently challenging samples.

Impact Statement: This presentation will impact the forensic community by demonstrating the importance of utilizing lay control groups when designing foundational research to ensure test samples are sufficiently challenging to test performance on close non-matched samples and reflect the full spectrum of case work. This is a necessary, albeit not sufficient, requirement for reported error rates to be considered an accurate measure of a method's performance. The presentation also shows the need for future research to explore the role that expertise, training, and experience play in firearms examination.

Abstract Text: Several studies have recently attempted to estimate practitioner accuracy when comparing fired ammunition. But whether this research has included sufficiently challenging comparisons dependent upon expertise for accurate conclusions regarding source remains largely unexplored in the foundational literature. This presentation builds on our recently published study, *The false promise of firearms examination validation studies: Lay controls, simplistic comparisons, and the failure to soundly measure misidentification rates*, that utilized a post hoc control group of lay people to assess whether comparison samples in one commonly cited effort to validate cartridge case comparisons, *Duez et al*., were least challenging enough to distinguish between experts and novices.[1,2] Despite lacking the kind of formalized training and experience common to professional examiners, our lay participants displayed an ability, generally, to distinguish between cartridge cases fired by the same versus different guns. And while their accuracy rates lagged substantially behind those of the original participant pool of professionals on same-source comparisons, their performance on different-source comparisons was essentially indistinguishable from that of trained examiners.

We will discuss those results and their limitations, review responses to our study from the field as well as the dialog it spurred, and ultimately suggest that our findings, coupled with a review of other foundational literature on firearms examination's accuracy, cast substantial doubt on the ability of existing false positive rate estimates to satisfy legal criteria for admissibility. Our presentation and the discussion of our study, results, and implications is situated within the broader context of assessing the quality of the foundational literature for the field of firearms and toolmark comparison, and other feature comparison methods, and considers the ways in which the validations studies of forensic disciplines could be strengthened by adhering to the standards that regulate study design for scientific research, in nearly all fields outside of forensic methods, including medical research.

References:

1. Gutierrez RE, Prokesch EJ. The false promise of firearms examination validation studies: Lay controls, simplistic comparisons, and the failure to soundly measure misidentification rates. *J. Forensic Sci*. 2024; 69: 1334-49. https://doi.org/10.1111/1556-4029.15531.
2. Duez P, Weller T, Brubaker M, Hockensmith RE, Lillien R. Development and validation of a virtual examination tool for firearm forensics. *J Forensic Sci*. 2018; 63(4): 1069–84. https://doi.org/10. 1111/1556-4029.13668.

Firearms Toolmark Comparison; Method Validation; Lay Control Groups

G20 Exploring New Approaches to Reporting Inconclusive Decisions in Firearm and Toolmark Comparison

Lynn Garcia, JD, Texas Forensic Science Commission, Austin, TX; Henry Swofford, PhD, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Objectives: After attending this presentation, attendees will learn about a report published by the Texas Forensic Science Commission that aims to implement suggestions from a May 2024 article by Swofford et al. titled "Inconclusive Decisions and Error Rates in Forensic Science," which was presented in the Jurisprudence section during last year's annual AAFS conference.[1] The authors present two distinct concepts needed for a determination of reliability—method conformance and method performance. Method conformance relates to an assessment of whether the outcome of a method is the result of the analyst's adherence to the procedures that define the method. Method performance reflects the capacity of a method to discriminate between different propositions of interest (e.g., mated and non-mated comparisons). This presentation will discuss Texas' attempt to implement the suggestions from the article in the firearm and toolmark comparison discipline.

Impact Statement: This presentation will help attorneys identify the type of questions they need to be considering with respect to error rates and inconclusive decisions; it will also offer guidance to forensic science service providers regarding a more transparent and effective method of reporting results.

Abstract Text: In the early 1990s, in the capital murder case of Nanon Williams, a firearms examiner from the now defunct Houston Crime Laboratory mischaracterized a deformed projectile recovered at autopsy as a .25 caliber bullet (consistent with the defendant's firearm). Post-conviction testing established the deformed projectile was in fact a .22 caliber bullet fired from the co-defendant's firearm. The mischaracterization was caused by several factors, including the fact that the projectile was distorted, the co-defendant's firearm was not originally submitted as evidence, the examiner did not conduct a microscopic examination, and the laboratory's overall lack of quality controls during that timeframe.

Landmark forensic science reports have called for empirical proof of the reliability and validity of Firearm And Toolmark (FATM) examinations and have specifically stressed the need for appropriate black-box studies to provide estimates of reliability. Recently, several black-box studies on the accuracy, reproducibility, and repeatability of FATM examination have been published related to bullet and cartridge case comparisons. One area of protracted disagreement concerns the best way to calculate and discuss the significance of error rates in the FATM literature, especially regarding inconclusive conclusions.

The Texas Forensic Science Commission (TFSC) agreed with authors of the referenced paper that error rates from black-box studies focusing on false positive and false negative results do not provide a suitable metric for representing FATM method performance in any specific case.[1] The Association of Firearm and Tool Mark Examiners (AFTE) Range of Conclusions provides examiners with five options: (Identification, Inconclusive [Types A, B, or C] or Elimination). It can be misleading to use false positive and false negative error rates from black-box studies as a metric of performance in a discipline that does not limit an examiner to only two choices (identification or elimination).

In the proposed new approach, Texas FATM examiners would present data for consideration using data from their own comparison method. The data would be presented in two tables referred to as a "Discriminability Table" (detailing the extent to which the method can distinguish between same source/different source) and a "Reproducibility Table" (detailing the extent to which outcomes of the method are consistently produced by different examiners). These data provide greater transparency about the method's overall performance. The authors will present the work of a statewide working group with the responsibility for implementation. They will also discuss other related recommendations from the TFSC's report. Finally, they will explore ways this approach may be extended to other forensic disciplines.

Reference:

1. https://www.sciencedirect.com/science/article/pii/S2589871X24000196?via%3Dihub.

Inconclusive; Firearms; Error Rate

G21 A Collaborative Approach to Informing the Adoption of Trustworthy AI in Forensic Science and Legal Proceedings

Melissa Taylor, BA, National Institute of Standards and Technology, Gaithersburg, MD; Dana Delger, JD, Casper, WY; Niki Osborne, PhD, National Institute of Standards and Technology, Remuera, Auckland, New Zealand; Henry Swofford, PhD, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Objectives: Attendees will learn about the transformative potential and possible challenges of using Artificial Intelligence (AI) in forensic science and legal proceedings. They will gain insights into AI-enabled applications and use cases, and understand the ethical, legal, and technical considerations needed for responsible adoption. The presentation will highlight work being undertaken by the National Institute of Standards and Technology (NIST) and industry experts to develop a framework and best practice recommendations of the responsible adoption of trustworthy AI in forensic science.

Impact Statement: This presentation will enhance forensic scientists', legal practitioners', and other criminal justice partners' understanding of the AI landscape as it relates to forensic science and legal proceedings, improving their competence and ability to understand what it means to responsibly adopt AI-enabled tools into their practice. The presentation will promote informed decision-making and performance improvements in the field.

Abstract Text: This presentation explores the transformative potential of AI in forensic science and legal proceedings, offering an examination of its impact, value, and the challenges associated with its adoption. The core proposition is that AI, when developed and implemented thoughtfully and responsibly, may have the potential to enhance the accuracy, efficiency, and reliability of forensic analysis. However, this requires a thorough understanding of AI's capabilities and limitations, as well as the establishment of robust guidelines and standards.

The National Institute of Standards and Technology (NIST) and industry experts, including forensic scientists, legal practitioners, and AI specialists, are working together to identify best practices for integrating AI tools into forensic workflows. Attendees will gain insights from these experts and what it means to responsibly adopt trustworthy AI in forensic science.

The presentation will highlight several current and potential AI applications in forensic science. For instance, AI algorithms may be able to accelerate the analysis of large datasets, such as DNA profiles, by automating repetitive tasks and identifying patterns that may not be immediately apparent to human analysts. In fingerprint and facial recognition, AI systems may be able to enhance matching accuracy and reduce error rates.

Despite these potential advantages, integrating AI into forensic science is not without significant challenge. Attendees will learn about the ethical, legal, and technical issues that must be addressed to enable AI's responsible adoption. These include concerns about algorithmic transparency, data privacy, civil liberties, and the potential for opinions informed by AI to be misinterpreted in court. The presentation will emphasize the need for continuous validation and verification of AI systems, comprehensive training for forensic and legal professionals, and the development of standardized protocols for AI implementation.

With appropriate safeguards and a collaborative approach across criminal justice partners, AI can be an asset in forensic science. The presentation will conclude with a forward-looking perspective, offering actionable steps for the successful integration of AI in the field. These steps include fostering interdisciplinary collaboration, investing in AI education and training, advocating for frameworks that support ethical and transparent AI use, and encouraging ongoing research to keep pace with technological advancements.

By enhancing the understanding of potential roles for AI in forensic science, this presentation aims to empower forensic scientists, legal practitioners, and other criminal justice partners to make informed decisions about when, how, or if to adopt AI-enabled systems into their practice.

Artificial Intelligence; Forensic Science; Technology Transition

G22 Overcoming Unscientific Opposing Arguments Using Relevant Data

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Learning Objectives: After attending this presentation, attendees will understand how reliable forensic science can overcome opposition misrepresentation of validation studies. Attendees will learn the difference between relevant and irrelevant validation data and how that differentiation can refute non-scientific arguments.

Impact Statement: This presentation will impact the forensic science and law community by showing how scientific data can refute unfounded opposition arguments. A case example teaches forensic scientists how to demonstrate scientific reliability in the face of flawed facile pseudo-scientific rhetoric.

Abstract Text: In 2020, a man was shot following an altercation. He later died from his injuries. Video surveillance linked a suspect to the murder. There were no witnesses to the shooting. Police collected the gun and magazine used in the crime. Was the suspect the killer? Could DNA evidence help?

The local crime lab tested the firearm items for DNA. Their DNA data showed at least two (gun) or three (magazine) contributors to the mixtures. A private DNA lab interpreted the data and compared it with the defendant's DNA profile. By manual inspection, the lab excluded the defendant from the major DNA contributor. However, the lab couldn't draw any conclusions regarding the minor contributors for either evidence item.

When the suspect's defense attorneys received the inconclusive DNA results, they reached out to Cybergenetics for "probabilistic" genotyping computer interpretation. Objective TrueAllele Casework analysis statistically excluded the defendant as a DNA contributor. Separated genotype components included a 6% minor contributor (gun), and a 2% minor contributor (magazine). Cybergenetics reported exclusionary Likelihood Ratio (LR) match statistics, along with exact false exclusion Error Rates (ER).[1]

The government retained an expert to review the TrueAllele results. The opposition expert's report contained many flawed arguments. A notable flaw was an incorrect description of computed ERs. Relying on this opposition report, the government filed a *Daubert* motion to challenge TrueAllele admissibility.

The opposition expert argued that TrueAllele had a high binary false exclusion error rate (LR< 1 for true contributors). For minor contributors, they described a 60% error rate for the 1-5% mixture range and an 18% error rate for the 5-10% range. They based their claim on a published validation study from a previous TrueAllele version.[2] (In the version used in the case, these hypothesized error rates would be lower at 35% and 0%, respectively.) Where was the flaw in their argument?

Error rate depends on LR.[1] That mathematical fact is given in the error rate law ER ≤ LR – exclusionary error rate can never exceed the likelihood ratio. The error rate is only meaningful relative to LR, giving the chance that other people would be excluded as strongly. But the opposing expert ignored error rate's dependence on LR.

Cybergenetics calculated and reported relevant error rates in the case.[1] However, the government incorrectly applied a cutoff of 1 for a *binary* error rate, discarding the LR value. That is not how to determine forensic DNA error rates. The relevant context is how strongly the evidence matches (or doesn't match) the suspect—the actual LR statistical support for the suspect, not some cherry-picked cutoff level.

We revisited the validation paper's LR data. We showed that the opposition's purported "error rates" entailed weak exclusionary LR values near 1 from less informative genotypes. These validation points were not relevant to the case's highly informative genotypes, which gave strong exclusionary LR values of one in 70 million (gun) or 160 billion (magazine). The prosecution's spurious argument was entirely unrelated to TrueAllele reliability or its results in the case.

Responding to the *Daubert* challenge, Cybergenetics prepared a 26-page declaration. We detailed the opposition report's flaws, refuting inapplicable arguments with science and facts. We described the TrueAllele technology's error rate, admissibility, and court acceptance. Based just on document submissions alone, without even an admissibility hearing, the judge admitted the TrueAllele results as reliable scientific evidence.

This presentation will discuss how relevant validation data can be used to persuasively rebut flawed opposing argument. The talk highlights how inapplicable validation data items are immaterial to well-founded forensic conclusions. A recent case example explains a failed opposition argument and the winning scientific response.

References:

1. Perlin MW. Efficient construction of match strength distributions for uncertain multi-locus genotypes. *Heliyon*. 4(10):e00824, 2018.
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Probabilistic Genotyping; Error Rate; Admissibility

G23 Data Transparency in Probabilistic Genotyping Validation Studies

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Learning Objectives: After attending this presentation, attendees will better understand the current landscape for accessing Probabilistic Genotyping Software (PGS) internal validation study data. Topics covered will include reasons for requesting data access, frequent hurdles encountered in gaining access, and various aspects of the data to evaluate when access is obtained.

Impact Statement: This presentation will impact the forensic science community by exploring the motivations for maintaining data secrecy by proponents of probabilistic genotyping systems and for promoting transparency by scientists and the public.

Abstract Text: Various standards and guidance documents require labs to conduct internal validation studies for new technologies or processes prior to casework use. These studies along with proficiency testing form the basis of confidence that a laboratory has demonstrated reliable use of the new method over a range of operating conditions. United States laboratories routinely release summary documents describing validation study efforts, results, and conclusions of reliability but resist disclosure of the data generated during these studies. The same standards and guidance documents require that the data be maintained for auditing and disclosure purposes. The data sets frequently amount to only 5-15GB of data—easily transferable via internet download or flash drive. The collation and transfer of these data should not be burdensome.

Arguments against disclosure of these data typically focus on a claim of genetic privacy of the study participants (who often include employees of the labs conducting these studies). Despite the use of human subjects, these studies are regularly designed with no approval from an Institutional Review Board (IRB) or the inclusion of a mechanism for informed consent of participants that would allow disclosure of their genotypes. Several laboratories have asserted that their poor experimental design would cause their sharing of the data that underlies their validation studies to constitute a criminal offense due to the necessary inclusion of this genetic information. Nearly all disclosures of internal validation study data to date that have occurred through litigation have involved protective orders that limit the ability to share those genotypes.

Public lab data can be sought under federal and state Freedom Of Information Act (FOIA) requests, though responses are likely to involve outright denials or requirements for redactions on the basis of genetic privacy. Subpoenas and case discovery more frequently result in disclosure but with the burden of protective orders. Repeat provision of the same data under protective orders requires duplication of efforts case-to-case since the data and comprehensive results cannot be publicly shared. Laboratory accountability through public access and oversight are impeded through this secrecy. A broad examination of trends and edge cases should be encouraged through public review and discussion, but disclosure in only individual cases promotes tunnel vision focused on the issues of that case.

Internal validation study data sets have proven to vary in structure and content. In some instances, disclosures include extensive laboratory documentation of data analysis processes, intermediate results, and final conclusions. In other disclosures, materials provided are mostly limited to the inputs and outputs of the PGS. Semi-automated software has been developed to extract and collate relevant data points in a way that is useful for screening validation study data for ranges of values (e.g., "diagnostics") observed under controlled conditions (e.g., a review of "factor space" described in the National Institute of Standards and Technology [NIST] draft report.)[1] While conceptually similar from lab-to-lab and study-to-study, non-trivial syntactic differences exist between laboratory data sets due to a lack of standardization for data interchange. It is presently impractical to build a singular software tool to assist independent review of all lab internal validation study data sets, even those data sets resulting from the same version of the same PGS program.

Policy decisions at the level of guidance bodies and legislatures would constitute significant progress toward transparency. Helpful policies would demand evaluation of experimental design by an institutional review board that considered the implications for human subjects that participate in the study and likely resulted in a requirement for prior informed consent for publication of participant reference profiles.

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DNA; Validation; Data

G24 AI and Black-Box Algorithms, Wrongful Conviction Risks, and the Importance of Access to Evidence

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Learning Objectives: This presentation will explore the proliferation of Artificial Intelligence (AI), specifically machine learning-based tools, in law enforcement investigations and forensics. Given these tools' growing prevalence, access to evidence and validation data in cases where they are used for investigation or prosecution is a necessary prerequisite to ensuring their validity and reliability and mitigating their susceptibility to bias. In the absence of robust evidence access or meaningful and independent validation testing, such tools risk replicating the errors of other forensic disciplines that have been implicated in wrongful convictions. After attending this presentation, attendees will understand the range of machine learning-based forensic and law enforcement tools currently in use or development, the legal hurdles to accessing information about those tools, and strategies for challenging and possibly overcoming those hurdles.

Impact Statement: This presentation will impact the forensic science community by showing how, in the absence of robust evidence access and independent verification and validation, AI-based forensic tools risk replicating patterns in more traditional forensic disciplines that have produced wrongful convictions in the past.

Abstract Text: AI, broadly defined as computer models capable of performing tasks that typically require human intelligence, is increasingly finding applications in law enforcement investigations and forensics.[1] Existing and emerging tools seek to apply machine learning models to tasks that range from facial recognition to DNA analysis to pattern-matching disciplines.[2-4] Moreover, AI-based tools that were initially developed to generate investigative leads or inform deployment decisions are now being offered as direct evidence of guilt in trials. Often, however, these tools fail to undergo either the rigorous judicial and empirical scrutiny or the robust adversarial testing necessary to vet their reliability. "Black-box" algorithms present particular challenges as the processes behind their results are necessarily opaque and difficult for humans to scrutinize or verify.

Although machine learning systems can offer the potential to improve on and mitigate human analysts' errors, subjective judgments, and cognitive biases, these systems can themselves be susceptible to coding errors, skewed training data, and mismatches between their conditions of development and deployment.[5] To assess machine learning systems, attorneys and courts need information about their training data, model parameters, error rates, and validation testing. Yet often, this information is withheld, shielded behind assumptions that it is irrelevant or unnecessary. At other times, the veneer of technological sophistication and objectivity leads courts to ignore potential flaws. Moreover, where algorithmic and AI-based systems remain in the hands of private developers rather than law enforcement, they can give rise to trade secrets claims and onerous protective orders, thus evading the discovery and disclosure requirements applicable to government actors.[6,7]

Yet robust evidence access is the first necessary step to adequate validation, bias mitigation, adversarial testing, and error assessment. As such, the Innocence Project has been urging courts to order and facilitate such evidence disclosure and scrutiny for both existing and newly developed AI-based forensic tools, whether they are offered in pretrial proceedings or at trial. Some jurisdictions have already begun to recognize the importance of evidence access as a precondition for reliability and admissibility assessments under the *Daubert* and *Frye* standards.

This presentation will examine the legal and practical barriers to meaningful evidence access and reliability testing and will outline strategies for challenging and overcoming those barriers.

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4. Alicia Carriquiry et al., Machine Learning in Forensic Applications, 16 *Significance* 29, 29-35 (2019).
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Machine Learning; Artificial Intelligence; *Daubert*

G25 Litigating AI-Generated Child Sex Abuse Material: Technical and Legal Challenges

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Learning Objectives: Attendees will learn the key legal elements of the possession of child sex abuse material and the relevant case law.

Attendees will gain a foundational understanding of AI and specifically how generative AI models create synthetic media like audio and video. These highly technical models will be explained with examples and metaphors so that attendees can easily communicate this knowledge to jurors, judges, attorneys, and others.

Attendees will understand how the nature of AI-generated media poses three distinct problems that can undermine attempts to inform the key legal elements: (1) the authentication problem where synthetic images are increasingly indistinguishable from real images; (2) the source problem where it is nearly impossible to identify the source images/audio/data used to generate artificial images (and may not even exist); and, (3) the control problem, where users are not in control of what specific images were generated and what images were used as sources.

Impact Statement: The forensic science community will undoubtedly be called upon to advise prosecutors and defense attorneys in the complex and challenging cases of AI-generated Child Sexual Abuse Materials (CSAM). However, there is very little guidance as to how the technological advancements in AI models affect the ability to forensically determine the necessary information in such a case. This presentation is one of the first to train the forensic science community on the challenges they face.

Abstract Text: "We are engaged in a race against time to protect the children of our country from the dangers of AI. Indeed, the proverbial walls of the city have already been breached. Now is the time to act."[1] This was the conclusion of a 2023 letter from 54 attorneys general from states and United States territories explaining that child CSAM generated by AI is one of the most challenging technology issues for our society. There is no question that the forensic science community will be at the center of any litigation around AI-generated CSAM.

In fact, in May 2024, the United States Department of Justice charged a Wisconsin man accused of using AI to "to create thousands of explicit images of children, marking what is potentially the first federal charge of creating child sexual abuse material applied to images produced entirely through AI."[2,3] Days later, the White House called on the tech and financial industries to voluntarily shout down the creation, spread, and monetization of AI-generated CSAM.[4]

However, there are serious technological and legal barriers that can and will undermine attempts to prove the elements of possession of CSAM beyond a reasonable doubt. This presentation will explain: (1) the Constitutional and statutory elements of CSAM-related crimes, (2) the current state of the world in AI-generated (synthetic) media, and (3) the issues that the nature of AI-generated media poses for those statutory elements.

First, the United States Supreme Court has held that prohibitions of CSAM are only constitutional under the First Amendment's Free Speech clause when the media itself depicts a real, identifiable minor.[5,6]

Second, this real, identifiable minor requirement is likely to be an insurmountable hurdle when faced with the realities of how AI generates synthetic media. Today, generating synthetic media of any kind requires almost no expertise of any kind—significantly lowering the barrier to entry from the past methods of Photoshop or physical modification of photos. Beyond the general text-to-image methods like Stable Diffusion, DALL-E, and MidJourney, there are endless numbers of "deepnudify" or "nudify" software that have the sole purpose of intaking images and generating non-consensual intimate images.[7] The most common software utilizes "diffusion models" where they create images, not by modifying existing images, but by starting from noise and moving colors and lines around until they approximate what the software was "told" to create.[8,9]

Third, the nature of diffusion models can undermine attempts to assess whether the media includes a real, identifiable minor: (1) the authentication problem where synthetic images are increasingly indistinguishable from real images; (2) the source problem where it is nearly impossible to identify the source images/audio/data used to generate artificial images (and may not even exist); and (3) the control problem, where users are not in control of what specific images were generated and what images were used as sources.[10-12]

These problems must be understood and communicated by the forensic science community who will undoubtedly be called upon to advise prosecutors and defense attorneys in these complex and challenging cases.

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6. *Ashcroft v. Free Speech* (U.S., 2002).
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Artificial Intelligence; Digital Evidence; Child Abuse

G26 A Few Common Misconceptions in AI

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Learning Objectives: The learning objectives are as follows: (1) understand the definition of AI, including its history and current use; (2) develop intuition for identifying AI misinformation; (3) develop basic AI literacy; and (4) learn how to identify positive uses for AI and apply them to the field of forensic science.

Impact Statement: It is incumbent on forensic scientists to stay abreast of the changes AI is bringing about in our world. This presentation will provide a succinct yet comprehensive overview of the state-of-the-art technologies in AI and their applications and misuse.

Abstract Text: As you know, AI has become an integral part of our daily lives. It is transforming businesses and making significant contributions in diverse fields, such as health care, finance, entertainment, transportation, and much more. It also has the potential to disrupt traditional occupations and employees in many fields.

The law governs every field of commerce and guides the justice system; it is important that members of the legal system have AI literacy so they can comprehend the legal implications surrounding AI use and adapt quickly to the evolution of technologies brought on by AI. There also is a significant and developing ethical component in the use of AI, namely whether AI will be positively transformative or an instrumentality of injustice.

The presentation takes listeners through a tour of AI, including its history through current use, and does so by dispatching with four common misconceptions about AI: (1) AI is new; (2) AI intelligence has surpassed human intelligence; (3) AI is mysterious; and (4) AI can predict the future. Participants will: (1) learn the definition of AI, including its history and current use; (2) obtain intuition for identifying AI misinformation; (3) develop basic AI literacy; and (4) learn how to identify positive uses for AI and apply them to the field of forensic science.

The presentation is not technical and is directed toward listeners of all backgrounds. It aims to demystify AI, making it accessible and understandable to those without a technical background. By engaging with this presentation, participants will gain a well-rounded understanding of AI's potential, limitations, and abuses, and obtain the requisite knowledge to leverage AI in their practice. Finally, participants will be able to give a more informed answer to the theme of the 2025 AAFS Annual Conference as it applies to AI: is it a tool for transformation or tyranny?

Artificial Intelligence; Misconceptions; Application

G27 Confronting the Confrontation Clause

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Learning Objectives: Over the past 20 years, the United States Supreme Court has decided several Sixth Amendment's Confrontation Clause issues. However, after their most recent decision in *Smith v. Arizona*, laboratories, attorneys, and judges may not be in agreement on how to properly present forensic evidence testimony.[1]

Impact Statement: This presentation will impact attendees by helping to clarify how proponents of forensic evidence testimony can comply with current Supreme Court jurisprudence.

Abstract Text: On June 21, 2024, the United States Supreme Court issued its decision in *Smith v. Arizona*.[1] The Smith case, which garnered significant attention in criminal justice and forensic science communities, represents the Court's most recent pronouncement regarding the Sixth Amendment Confrontation Cause requirements in criminal trials. The Court addresses what is sometimes called "surrogate testimony:" testimony by an expert witness who did not complete the examination(s) about which s/he is testifying.

The Court ruled 9-0 in favor of *Smith*, holding that when an expert conveys an absent laboratory analyst's statements to support their opinion, and the statements provide that support only if true, then the statements come into evidence for their truth. The Court vacated the Arizona Court of Appeals decision and remanded the case back to the state court to determine whether the out-of-court statements made by the expert were testimonial. Although the Court determined that out-of-court statements conveyed by the expert are considered for their truth and are thus hearsay, the Court did not determine whether these statements are "testimonial hearsay," thus implicating the Confrontation Clause.

But the decision has broader implications than just surrogate testimony. In certain forensic disciplines, *Smith* may alter the way evidence is offered at trial. Laboratories and prosecutors must be aware of this significant opinion and its implications in the presentation of forensic evidence. For example:

- How does this impact cold case homicides when the original medical examiner is no longer available? What can a substitute medical examiner rely upon to offer their own independent opinion?
- How does this impact "batch processing" at high throughput DNA and toxicology laboratories? Must the prosecutor call 10+ witnesses to satisfy the defendant's Confrontation Clause rights?
- Does a laboratory need to change standard operating procedures to comply with *Smith's* holding?
- How does this decision impact pattern-matching and seized drug opinions?

We will discuss these and many more questions during this session.

Reference:

1. *Smith v. Arizona*, 602 U.S. ___ (2024).

Expert Testimony; Evidence; Admissibility

G28 What Experts and Attorneys (and Hopefully Judges) Need to Know About the New Federal Rule of Evidence 702

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Learning Objectives: Attorneys and expert witnesses will understand the changed requirements for the admission of expert forensic science testimony under the recent amendments to the Federal Rule of Evidence 702. They will understand the clarification of the burden of proof at a *Daubert* hearing and how it will affect their preparation for trial.

Impact Statement: The recent amendments to Federal Rule of Evidence 702 are meant to clarify the requirements for a *Daubert* hearing and to address the failure of judges to properly perform their gatekeeping function as to forensic science evidence. The forensic science community will understand that the burden is clearly on the proponent of forensic science evidence. The community will also understand that under the revised rule the judge must decide if the reliability of the proffered evidence has been established to be "more likely than not" to the satisfaction of the judge before trial and not left to the jury

Abstract Text: Congress recently approved amendments to FRE 702 "Testimony by Expert Witnesses," meant to strengthen the judge's role as "gatekeeper" of expert testimony. In *Daubert*, it was established that the judge must evaluate the reliability of the scientific basis of expert opinions before allowing the jury to hear those opinions.[1] But many judges did not follow *Daubert* and continue to resist the judiciary's proper gatekeeping role.[2,3]

To remedy this, FRE 702 has been amended:

A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if the proponent has demonstrated to the court that it is more likely than not that:

a) the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue;
b) the testimony is based on sufficient facts or data;
c) the testimony is the product of reliable principles and methods; and
d) the expert has reliably applied expert's opinion reflects a reliable application of the principles and methods to the facts of the case.

These amendments: (1) place the burden on the *proponent* of the evidence to establish its reliability; (2) establish that reliability must be demonstrated *to the court* and not left to the jury; (3) sets the burden of proof as "*more likely than not*"; and (4) clarify that the judge must find that the expert has *reliably applied* that science.

Attorneys must anticipate a meaningful *Daubert* hearing. Attorneys are to be familiar with reliability issues. Judges may want precedent, but evidence should no longer be admitted based on precedent. Attorneys must go forward with reliability proof as the burden is on the proponent. Most proof will come from the expert witness. Experts must be prepared to discuss their findings but also to discuss the reliability of their forensic field and challenges to that reliability.

Few federal courts have discussed the amendments.[4] In civil cases, some attorneys believe they will result in more exclusion, especially in class actions.[5] In criminal cases, the biggest issue is whether states will adopt the amendments. This is not an issue in the few *Frye* states. Those that follow *Daubert* are not bound by FRE 702, although many have incorporated it's language. In Michigan, for example, the Supreme Court recently incorporated the amendments into Michigan Rule 702.[6] Whether other states will adopt these amendments is being discussed. Whether judges will become true gatekeepers is the question we have faced since 1993.

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3. Bernstein & Lasker, Defending *Daubert*: It's Time to Amend Federal Rule of Evidence 702, 57 *William & Mary L Rev* 1 (2015).
4. See, e.g., *Ozuna v. Pena*, Dist. Court, MD La., (June12, 2024); *Csaszar v. Monarch* Medical, Dist. Court, ED Pa. (March 26, 2024); *Lytle v. Nutramax Laboratories*, 99 F. 4th 557 (9th Cir. 2024); *Aecom Technical Services v. Flatiron/Aecom*, Dist. Court, D. Colo. (January 2, 2024); *Mar-Can Trans. Co. v. Local 854*, Dist. Court SD NY (March 22, 2024); *Mills v. Philadelphia*, Dist. Court ED Pa (March 22, 2024).
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***Daubert*; Evidence; Expert Testimony**

G29 The Conclusion May Not Be As It Appears: Does Your Evidence Interpretation Address the Right Question?

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Learning Objectives: Attendees will learn how to recognize and frame questions relevant to investigations in a scientifically supported manner. The audience will learn how the interpretation of scientific analyses of physical evidence can change significantly depending upon how the question being investigated is framed and understood.

Impact Statement: The audience will be introduced to the complexity and potential issues when forensic scientists attempt to interpret the significance of data derived from scientific analysis of physical evidence. The audience will be introduced to potential ways in which the value of evidence may be interpreted to properly address the question of source under investigation.

Abstract Text: Forensic investigations and intelligence operations are complex processes that often incorporate information drawn from the scientific analysis and interpretation of physical evidence. For the interpretation of the results to be non-biased, scientifically supported, and relevant to the investigation, the forensic scientist and investigator must properly frame and address the correct question(s) to advance the investigation. Stoney presented six potentially relevant questions that could be asked of the physical evidence depending on case-specific details.[1] The differences in these questions, which can be subtle, have significant impacts on how the results from scientific analyses are interpreted and presented. If the wrong question is asked, the interpretation of evidence can be erroneous and thus ultimately detrimental to the justice system. In light of the proposed sampling models for how the evidence has arisen in common-but-unknown and specific-source identification problems in Ommen and Saunders, we revisit the questions presented by Stoney with the goal of harmonizing the terminology and statistical foundations of potentially relevant questions.[1,2]

In this presentation, we use the term "Questioned item" (Q) to refer to an object that may be relevant to the event under investigation but does not have a known source (e.g., glass chip recovered from a suspect). The term "Known item" (K) is used to describe a potentially relevant item of known source (e.g., a broken window at the scene). We will describe three common tasks in forensic investigations, the various ways in which the relevant questions for these tasks may be framed, and the means to address these in a rigorous frequentist probabilistic manner. These tasks include: (1) interpretation of a comparison between a known and questioned item (K-Q comparison); (2) interpretation of a comparison between a questioned item and a database entry (e.g., Combined DNA Index System [CODIS] search hit); and (3) interpretation of a comparison between two questioned items (Q-Q comparison).

The task and the questions together determine the relevant error rates on which courts rely for determining admissibility of the forensic approach. Minor changes in the question or task can have a drastic effect on the corresponding error rates.

References:

1. Stoney, DA. Evaluation of Associative evidence: Choosing the relevant question. *Journal of the Forensic Science Society* 1984; 24: 473-482. doi:10.1016/S0015-7368(84)72326-7.
2. Ommen, DM and Saunders CP. A Problem in Forensic Science Highlighting the differences between the Bayes Factor and Likelihood Ratio. *Statist. Sci.* 32 2021; 6 (3) 344 – 359. https://doi.org/10.1214/20-STS805.

Interpretation; Evidence Evaluation; Statistics

G30 Reevaluating the Current State of Forensic Accreditation in the United States

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Learning Objectives: Attendees will understand the challenges surrounding the current systems of forensic accreditation in the United States and propose potential solutions.

Impact Statement: The forensic community will be impacted through meaningful discussion on ways to increase the quality and reliability of forensic practice through improvements to forensic accreditation.

Abstract Text: One of the core tenets associated with accreditation is that of continuous improvement. Forensic laboratory accreditation has changed dramatically since the first forensic laboratory achieved it in 1982. As it continues to evolve, it is worth constantly evaluating where the process can be improved.

Since there is no national regulatory agency for forensic practice, rightly or wrongly, the private companies that administer forensic accreditation have become the de facto regulatory agencies. Even the few states with oversight entities rely heavily if not exclusively on these accreditation providers. This creates a unique situation. There is no other example of a critical function primarily performed by the government being solely regulated by private companies operating without government oversight or accountability.

There can be times when the interests of these private companies can differ from the public's goal of increasing quality in forensic practice. Since there is no governmental oversight of forensic accreditation, it is up to the forensic community to identify vulnerabilities and develop solutions. A recent example highlights this divide.

State and local forensic laboratories must be accredited to access the National DNA databank. The initial forensic accreditation programs required that all sections of a forensic laboratory undergo the accreditation process. Subsequent providers suggested that other industries selectively accredit within their operations and offered forensic laboratories the opportunity to select which categories of testing in the laboratory would undergo accreditation. As a result, many forensic labs only accredited their DNA sections.

One of those labs was the Detroit Police Lab. In 2008, discordant firearms results triggered an audit of the lab which led to its shutdown. While part of the same laboratory, the firearms unit had not been evaluated by the accreditation process. At the subsequent news conference explaining the closure, the Wayne County Prosecutor described problems in the lab as "*…so severe as to demonstrate a systemic problem in all disciplines…*"[1]

Despite this hard lesson, the companies that provide forensic accreditation still offer forensic laboratories the option of only accrediting their DNA sections.

Instead of legislative solutions, there are steps that the forensic community can take to ensure the value of forensic accreditation in this uniquely unregulated space. Once identified, loopholes can be closed. The Federal Bureau of Investigation (FBI) makes the rules that govern the National DNA databank. To avoid a situation like Detroit from happening again, the FBI could preclude any lab systems that only accredit their DNA sections from participating in the program.

The forensic community can also learn from other industries. Automakers manufacture cars to more stringent emission standards than those set by the Federal Government. They don't want to do this. It adds cost to their bottom line. They do this because California has set a more stringent standard. The California market is too lucrative for manufacturers to lose, so the manufacturers must adopt the more stringent emission standard. It is too costly to manufacture different emission standards for different markets, so the entire country benefits.

The recently formed National Association of Forensic Science Boards (NAFSB) offers the forensic community the same pathway to improve forensic accreditation. This organization represents the few states that impose state-level requirements on forensic laboratories. If NAFSB members banded together and required accreditation vendors to include components in their accreditation programs, it could similarly force these vendors to make those changes to remain competitive. As with the automakers, it would be too costly to offer different programs of forensic accreditation and these changes could benefit the entire country.

Other similar problems facing the forensic community and potential solutions to increase the quality and reliability of forensic practice will be discussed during this presentation.

Reference:

1. Error-prone Detroit police crime lab shut down, *The Associated Press* Sept 25, 2008

Forensic Accreditation; Quality; Reliability

G31 Footwear and Tire Impression Exam Foundation Study

Kelly Sauerwein, PhD, National Institute of Standards and Technology, Gaithersburg, MD*

Learning Objectives: After attending this presentation, attendees will learn about several select activities and reports currently being developed or recently published by the National Institute of Standards and Technology (NIST) Forensic Science Program, including scope, status, and outcomes. This presentation is one of eight that will be presented by NIST individuals.

Impact Statement: This presentation will provide the forensic science community an overview of key findings, implications, and recommendations affecting future directions and laboratory policies and procedures.

Abstract Text: Forensic science efforts at the NIST have been ongoing for the past century since Wilmer Souder's pioneering efforts in handwriting, typewriting, and firearm analysis from the 1920s to the 1950s.[1] NIST has increased its involvement in the field since a 2009 National Academy of Sciences study identified areas for improvement in forensic science.[2] Today, NIST conducts collaborative research, partners with the community to strengthen policies and practices, convenes meetings to examine issues, and explores scientific foundations of forensic methods and practices.[3] The ultimate aim of the NIST Forensic Science Program is to strengthen the scientific basis of forensic disciplines so that evidence may be appropriately collected and accurately analyzed and results effectively communicated. Through a series of short presentations, this session will provide an overview of select activities and recent NIST reports, including major findings, key takeaways, and recommendations for the forensic science community. These activities range from assessing broad strategic challenges facing the entire forensic science community to discipline-specific recommendations affecting laboratory policies and procedures as part of human factors and scientific foundation reviews.

NIST is conducting a scientific foundation review on the scientific principles underlying footwear and tire impression evidence. This review includes discussion of three key topics in footwear and tire impression evidence: discriminability, reproducibility, and interpretability. Over 500 publicly available resources have been collected for this review and evaluated based on their applicability to one of these key topics.[4] This report will help promote a better appreciation of the capabilities, limitations, and knowledge gaps in footwear and tire impression evidence among the forensic science community, stakeholders, and the general public.

Presentations will also cover the use of documentary standards in legal proceedings and some new resources for officers of the court along with recent NIST efforts to assist validation studies.[5]

References:

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2. Butler, J.M. (2015) U.S. initiatives to strengthen forensic science & international standards in forensic DNA. *Forensic Sci. Int. Genet.* 18: 4-20. [open access].
3. https://www.nist.gov/spo/forensic-science-program.
4. https://www.nist.gov/spo/forensic-science-program/footwear-impression-evidence-nist-scientific-foundation-review.
5. https://www.nist.gov/programs-projects/nist-forensic-science-training-program.

NIST; Forensic Science; Validation

G32 The Accuracy of Cadaver Dogs to Detect Human Residual Odor in a Scientific Test: Implications for the Courts

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Learning Objectives: After attending this presentation, attendees will understand the results of a rigorous double-blind study that quantified the ability of Human Remains Detection (HRD) canine teams to detect residual odor from intact decedents and ignore live human scent. They will learn the relevance of canine evidence that may indicate the presence of residual odor in criminal investigations. Attendees will also learn how the integration of analytical chemistry and an understanding of canine team behavior allows better contextualization of the results for legal contexts.

Impact Statement: This presentation will impact the forensic science community by explaining results from the first study that tested whether HRD canines can detect deceased human residual odor reliably and accurately and correctly ignore residual odor from live humans, while simultaneously quantifying the odor presented to the canines using analytical chemistry techniques. Studies that have definitively linked human remains odor with canine olfactory capabilities are lacking, and courts are accepting dog handlers (both police and civilian) as expert witnesses despite the tremendous gaps in knowledge about the biological and chemical foundation for odor detection. Now having baseline knowledge of how HRD canine teams performed and insight into the basic questions surrounding "what is possible?" from an odor perspective, advancements in canine training, deployment, and investigative methods can be developed.

Abstract Text: Residual odor is odor that remains in the absence of its target. Little is known about decedent residual odor other than it is composed of perishable Volatile Organic Compounds (VOCs). During criminal acts involving homicide, residual odor is assumed to be transferred from the body to other surfaces, such as a car seat or trunk. Residual odor is not known to produce physical evidence, and no forensic tests exist to quantify it, other than canine alerts.

Canine alerts are treated in court as scientifically valid indicators of residual odor, even in the absence of physical or corroborating evidence, and without regard for the level of training, certification, or proficiency of the canine. In fact, states such as Colorado explicitly state that cadaver dog evidence requires no scientific foundation.[1] This disinterest in the scientific underpinnings of canine odor detection, including their limits of detection, is particularly problematic when no tangible evidence, such as DNA or blood, is produced from a canine alert but the alert itself is allowed to be heard as credible, scientific evidence.

The study tested a double-blind, standardized residual Odor Recognition Test (ORT) protocol for HRD canines with immediate chemistry analysis of the test samples. A total of 35 certified HRD canine teams were recruited from around the United States. Residual odor was obtained by placing cleaned gauze underneath three recently deceased donors (the Targets) and three living participants (the Distractors) for ten minutes. All 35 teams were presented with samples from the same Targets and Distractors. The ORT trials consisted of 18 clean paint cans arranged in three rows of six spaced five feet apart. Sixteen cans contained control (Blank) gauze, one can contained gauze from a Target, and one can contained a Distractor sample. Each canine team participated in three trials within a single day; the location of the Target and Distractors changed in each trial. Canine teams had no knowledge of the can contents and were given specific instruction on equipment, procedures, and reward protocols to ensure consistent data across teams. A total of 105 trials were completed over three consecutive days.

Because it is a team, the handlers determined if their canine alerted. In general, teams were unable to accurately and consistently locate the single Target sample: of 105 trials, the Target was correctly identified only 30 times (28.57% sensitivity). The Positive Predictive Value (PPV) was 0.13; that is, when a handler called an alert, it was correct only 13% of the time.

To assess if Target, Distractor ,and Blank gauze were indeed distinct, untargeted Headspace Solid-Phase Microextraction (SPME) analysis for VOCs was conducted on each gauze used in the ORT, and 1,790 unidentified spectral features were detected. Partial Least Squares Discriminant Analysis showed that the Target, Distractor, and Blank gauze produced different metabolic profiles and thus clustered by group. Together, the results demonstrate that while there are quantitative differences in chemistry between deceased residual odors, living residual odors, and blanks, the canine teams were unable to accurately distinguish them. Our preliminary results indicate that Target samples yield the least amount of statistically significant metabolites, while Distractors yield the most. This may indicate that the VOCs are more transient in deceased human odor, making it more difficult for the canines to detect reliably. These results also may indicate that canines could improve detection with appropriate training. Video and audio of the 35 teams running the ORTs will be shown to illustrate instances of canine responses during trials, handler bias, and handler misinterpretation of alert behaviors that will help contextualize the results.

Reference:

1. *Commonwealth of Virginia vs. Braulio Marcelo Castillo*, 2016 (20th Judicial Circuit Court Case CR00026450).

Cadaver Dog; Solid Phase Microextraction; Judicial

H1 Diagnostic Omission in Radiology of Odonto-Stomatological Structures: Who Is Responsible? A Case Report and Review of Jurisprudence

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Learning Objectives: After attending this presentation, participants will better understand the legal framework within which the responsibility between dentist and radiologist lies, as well as the risks associated with exceeding the limits of their profession (e.g., irregular practice of the medical profession) or omitting diagnoses or treatments (e.g., malpractice).

Impact Statement: This presentation will impact the forensic science community by providing an overview of the relationship between professional profiles of dentists and radiologists in the Italian legal framework that can be widened to most countries.

Abstract Text: The professional profile of dentists in Italy (Law 409/1985) guarantees and provides radiological services complementary to diagnostic activities. Therefore, dentists should not only have the possibility but also the competence, and consequently the responsibility, to identify and interpret potential pathological lesions on X-ray images or at least to refer the patient to a specialist for an accurate differential diagnosis. However, where is the limit of liability in the case of diagnostic omission that involves both professionals, the specialist radiologist and the dentist, particularly in cases of gross negligence?[1-3]

A case report involving the diagnostic omission of a locally invasive benign neoplasm of the upper jaw by a radiologist and a dentist will be presented. The prolonged diagnostic omission by both professionals led to the resection of half of the upper jaw and necessitated a complex surgical, implant, and prosthetic rehabilitation and reconstruction plan. Both professionals were sued for civil liability and sentenced to pay €250,000.00 in compensation, as both were held responsible. It was determined that if diagnosed and treated promptly, the neoplasm would have caused minimal damage to the bone structure and the teeth of the upper jaw. The case is framed within the context of the Italian civil legal system concerning professional liability, with the evaluative conclusions extended to the international legal framework.

References:

1. Bianchi SD, Lojacono A, Balma E. Considerazioni radiologiche sulla "malpractice" in odontoiatria [Radiological considerations of malpractice in dentistry]. *Minerva Stomato*l. 1996 Nov;45(11):541-7. Italian.
2. Weissman BJ, Serman NJ. The law and who can expose dental radiographs. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2000 Nov;90(5):663-5. doi: 10.1067/moe.2000.110086.
3. Wright B. Contemporary medico-legal dental radiology. *Aust Dent J*. 2012 Mar;57 Suppl 1:9-15. doi: 10.1111/j.1834-7819.2011.01653.x. PMID: 22376092.

Dental Malpractice in Radiology; Civil Claim; Gross Negligence

H2 Positive Identification Using Photographic Facial Superimposition in a 20-Year-Old Cold Case

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Learning Objectives: After attending this presentation, attendees will be able to utilize a technique for dental identification in cases where antemortem dental records are not available or have low evidentiary value.

Impact Statement: The method of using photographic facial superimposition is another tool used in presumed identification of remains when antemortem records are not available. In this procedure, the soft tissue outlines of an antemortem photograph of an individual are superimposed over a postmortem image of the bony landmarks of the skull: orbit of the eye, brow ridges of the eyebrows, and zygomatic process to the cheek bones and teeth to facilitate a positive identification.

Abstract Text: Adobe Photoshop has been available since 1987. Forensic odontologists have been using the software apparatus to superimpose antemortem radiographs of teeth over postmortem radiographs of teeth to help in dental identification. Another use is the superimposition of the skull of a presumed victim to an antemortem photograph of an individual and the comparison of common features of the superimposed images to formulate a positive Identification. Anthropologists have been using this technique called photographic comparison not only for deceased victims but for perpetrators of a crime linked to surveillance apparatus.

In this "cold case," a husband and a father of two went missing after telling his wife he was going to collect on a debt. A decade later, a mountain hiker unearthed his remains. According to the medical examiner's report, there was a positive dental identification. Finally, the family was able to memorialize and cremate his remains. This revelation led to the arrest of two suspects. Upon reopening this criminal case, the medical examiner reviewed the initial report and realized the antemortem dental records used to make the positive identification did not follow protocol for the chain of evidence. Also, there were three inconsistent spellings of the victim's antemortem radiographs.

The medical examiner requested a full anthropological study of the remains at the time of the autopsy. The anthropologist completed the request and in those preserved images the anthropologist took were high-resolution, full-faced, multiple-angle photographs of the skull and dentition. Those images provided the evidence needed to perform a photographic facial superimposition.

Photographic facial superimposition requires the photographer to photograph the skull in the exact three planes seen in the antemortem photograph of the individual. The antemortem photograph and the skull must be in a 1:1 ratio. This technique involves enlarging the photograph of the skull until it equals the magnification of the antemortem radiograph. A comparison of common features seen in the superimposed images are: the outline of the skull to the soft tissue of the face, the orbit to the eye, the brow ridges to the eyebrows, the zygomatic process to the cheek bones, and the teeth. By changing the transparency levels, the images will appear to be coming through one another if properly aligned.

With this technique, the forensic odontologist can properly and positively identify the remains and testify as an expert witness in a court of law.

Dental Identification; Chain of Evidence; Cold Case

H3 Optimizing Mass Fatality Operations: The Synergy of Technology and Forensic Odontology

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Learning Objectives: By attending this presentation, attendees will gain an increased understanding of the benefits and drawbacks of using recent technological advancements employed for individual human identification during mass fatality victim identification.

Impact Statement: This session intends to evaluate the relevance of several innovative scientific technologies to improve the technological skills and effectiveness of forensic odontologists conducting Disaster Victim Identification (DVI). In addition, the presentation emphasizes the significance of scientific forensic and technological collaboration to enhance DVI.

Abstract Text: **Background:** For years, the definition of mass fatality incidents has been contingent not on a fixed number of deceased individuals but on the capabilities of the local jurisdiction. This relative definition takes into account the immediate availability of local resources, including vehicles, equipment, Emergency Medical Services (EMS), hospitals, incident management teams, and forensic specialists.[1] Such collaboration facilitates the swift resolution of a mass fatality incident, enabling a rapid transition into the recovery phase. A critical aspect of the recovery phase is the identification and return of individuals and their remains to their respective loved ones. Historically, this process has been labor intensive, but recent technological advancements have expedited the identification process, which is vital given the rising number of victims in such incidents. This presentation provides a comprehensive analysis of key identification technologies employed by forensic odontologists for human identification in mass fatality scenarios: artificial intelligence, forensic photography, and DVI software.[2] It examines their application in the context of individual human identification within DVI, focusing on their capabilities, limitations, and ethical considerations. By juxtaposing their use in individual human identification, the presentation offers valuable insights into their practical applications and challenges.

Materials and Methods: Several technologies were rigorously examined. Artificial intelligence (AI) employs machine learning algorithms to analyze and interpret data. This technology significantly enhances capabilities in image recognition, pattern analysis, and predictive modeling. Advancements in this technology have facilitated the rapid and accurate identification of individuals using odontogenic evidence, including dental records and facial features.[2] Forensic photography involves capturing detailed images of evidence that supports identification and analysis processes in forensic investigations. DVI software with dental charting is designed to manage and process information related to victims in mass casualty events. WinID and DAVID are DVI software that integrate data from various sources, such as dental records and personal effects, to match missing persons with unidentified remains.[3] This integration is intended to increase efficiency and accuracy in victim identification. Each of these technologies was consistently utilized in a coroner's office by trained personnel and during the training of mass fatality response teams handling human remains.

Conclusion: This study impacts the forensic odontologist community, particularly those involved in mass fatality incidents, by evaluating the practicality of identification accuracy and efficiency through AI, forensic photography, and DVI software. It aims to streamline workflow and resource management, support continuous training and skill development, address ethical considerations, contribute to disaster preparedness and response, and guide future research and development. The integration of advanced technologies improves operational capabilities, ensuring forensic odontologists are better prepared to assist in complex disaster responses

References:

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3. Al-Amad, S. H., Clement, J. G., McCullough, M. J., Morales, A., & Hill, A. J. (2007). Evaluation of two dental identification computer systems: DAVID and WinID3. *Journal of Forensic OdontoStomatology*, 25 (1), 23-29.

Mass Disaster; Forensic Odontology; Victim Identification

H4 Recent Advances in Forensic Odontology Standards

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Learning Objectives: By attending this presentation, participants will thoroughly understand discipline-specific standards within forensic odontology. They will gain valuable insights into the latest advancements, methodologies, and best practices that define and guide this specialized area.

Impact Statement: This presentation will significantly influence the community by increasing awareness of ongoing standards development initiatives within forensic odontology. Furthermore, attendees will gain valuable knowledge about the training, tools, and resources available to support effective implementation, compliance monitoring, and a deeper understanding of the latest advancements in standards development and forensic odontology.

Abstract Text: The American Dental Association Standards Board, The Academy Standard Board, the Organization of Scientific Area Committees (OSAC) for Forensic Science, and the International Organization for Standardization are crucial in advancing forensic odontology by developing and endorsing discipline-specific standards. They accomplish this by creating, refining, and publishing standards Following ANSI approved Standards Developing Organizations (SDOs) while reviewing and endorsing these standards to ensure their quality. Once ANSI standards are met, the FSSB reviews them for possible inclusion in the OSAC Registry and serves as a central repository for these high-quality, consensus-based, and technically robust standards.

This presentation will focus on critical updates and progress in forensic odontology standards. It will begin by reviewing the OSAC process and exploring current projects undertaken by the OSAC Forensic Odontology Subcommittee. These include approving the move to the STR of a standard for the Photographic Documentation of Suspected Patterns of Dental Origin designed to establish best practices for documenting dental patterns through photography for future analysis. Another project, a standard for Education and Training in Forensic Odontology, is under review by the FSSB. It sets forth the minimum educational and competency requirements for professionals in this field to ensure they are well-equipped to perform their roles effectively. Additionally, the subcommittee is finalizing a proposed standard for Forensic Documentation of Suspected Human Abuse by Oral Health Care Professionals to improve the accuracy and documentation of abuse reports.

The presentation will also cover updates to the ADA standard board, making Forensic Odontology a distinct consensus board, allowing it to expand in scope from informatics to clinical dentistry. It will update on the status of the ANSI/ADA 1058-2010D Forensic Dental Data Set, incorporating ISO CD 20888 on forensic orodental data terminology, and ANSI/ADA 1088-2020 on human identification by comparative dental analysis and ADA/ISO Specification No. 3950 on the designation system for teeth and oral cavity areas. These updates are intended to refine and enhance current standards further.

Additionally, the presentation will highlight international developments in forensic odontology from ISO/TC 106 Dentistry and ISO/TC 272 Forensic Sciences. This includes ISO/TC 106 standards, such as ISO 5365 on tooth developmental stages, which was passed and is now moving toward US adoption, and ISO 24395 on tooth restoration classifications, also being considered for US adoption. It will also update ISO CD 21611 on vocabulary for human identification by dental evidence. ISO/TC 272 also addresses forensic data documentation, analysis, interpretation, and reporting, with implications for forensic odontology. Finally, it will discuss an update on the Portugal proposal to create a forensic odontology subcommittee at the ISO level.

Participants will gain valuable insights into these latest updates and advancements, equipping them to contribute to the evolution of forensic odontology standards and elevating the discipline's overall excellence.

Standards, Forensic Odontology, Education

H5 A Comparison of the Plaster Model and the Digital Dental Scan in the Analysis of Palatal Rugae

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Learning Objectives: After attending this presentation, attendees will better understand a novel approach to personal identification using different techniques in the analysis of palatal rugae.

Impact Statement: This presentation will impact the forensic science community by emphasizing the usefulness of different approach in analysis of palatal rugae for personal identification purposes.

Abstract Text: Palatal rugae are asymmetrical and irregular mucosal elevations located in the anterior third of the palate. Thanks to their resilience against deterioration, to their reappearing after trauma or surgical interventions, to the protection made by surrounding oral structures, palatal rugae are a useful tool in forensic odontology as a method for personal identification. This is also due to their consistent and individualistic patterns. Different techniques are well-known for the use of palatal rugae in personal identification, especially represented by qualitative methods.

This study aimed to compare the impression of the rugae obtained with the classic plaster model with the impression of the palate made with an intraoral scanner. Both impressions were compared with each other and with the photo of the real palatal rugae. The sample was composed of 19 patients (13 females, 6 males) with a mean age of 28.6 years. Two different impressions were taken from the maxillae of the subjects: a conventional impression using alginate impression material, and an optical impression using an intraoral scanner. The two impressions were compared with each other and with the photo of the palatine rugae of each enrolled subject by a software of image comparison. Parameters assessed included absolute and relative distances, perimeters, areas, shape factors, and moments. Statistical analysis was conducted considering the six parameters. No statistically significant differences were detected between the two methods of palatal rugae impression. In conclusion, both digital and traditional methods were equally reliable in capturing palatal rugae patterns. Furthermore, the use of the software facilitated accurate comparison and personal identification through the alignment of the preidentified landmarks with the aim of applying a semi-quantitative method instead of qualitative analysis. Further studies are required with a larger sample in order to enhance the speed and precision of image acquisition and comparison for broader application in personal identification.

Forensic Odontology; Dental Identification; Photography

H6 Animal Bitemarks

Megha Shah, DDS, Smile 360 Family Dentistry, Alta Loma, CA*

Learning Objectives: We have seen many cases of human bitemarks. This is a unique case involving two different bitemarks made by canines on multiple areas. Techniques to make an impression of arches and an evaluation of bitemarks are presented.

Impact Statement: Attendees will learn of a unique case of bitemarks made by two different canines, the creation of overlays, and an evaluation of non-human bitemarks.

Abstract Text: In September of 2015, odontologist services were requested by the Riverside County Sheriff's Department to analyze bitemarks made by two suspected canines on the decedent's body. Data collection was performed on two different dates. The first examination was conducted post-autopsy. About 282 .jpeg pre- and post-autopsy pictures were collected. Second visit was conducted at the Riverside County Animal Control facility where two pit bulls were being quarantined. Both canines were kept under anesthesia . Both canines' impressions were completed of the upper and lower arches with heavy and light body PVS impression material. Photographs of the anterior bites for both canines were completed. In two days, both canine impressions were poured using Exacta Temp. A total 480 .jpeg photographs of decedent pre- and post-autopsy photographs along with both of the canines' anterior dentitions and dental models were analyzed. All autopsy photographs were studied and measured to find any singular patterned injuries among multiple avulsive and/or over-lapping bites, which were consistent with unique individual characteristics from the maxillary and mandibular dentition of each dog. Inter-cuspid arch widths and all missing and/or broken teeth were measured and recorded. Patterned injuries suitable for analysis were found in four areas on the decedent body. Each of these areas could be linked to either the maxilla or the mandible of either bulldog (Hades or Tyson).

It is important to understand that in dogs, their long, fang-like canines produce prominent marks, often deep punctures or lacerations, which may not conform to the inter-canine distance of the bite, because of cuspid flare occlusally and laterally. Bite Awas on the right lower lateral back area, Bite B was on the right dorsal thigh, Bite C was on the right lateral thigh, and Bite D was on the decedent's right lateral calf area. The opinion was created based on unique and distinct individual characteristics of both Hades' and Tyson's anterior dentition and neither dog can be excluded.

Dog Bites; Accuracy; Evidence Evaluation

H7 Teeth as the Sole Identifier of Heavily Challenged Ajnala Skeletal Remains: A Multifaceted Approach

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Learning Objective: After attending this presentation, attendees will be informed about the significance of teeth in estimating the identity of heavily fragmented human remains retrieved from forensic anthropological contexts

Impact Statement: This presentation will impact the forensic community by informing attendees that in case of badly damaged and commingled human remains, teeth act as excellent forensic identifier and can help establish the biological profile of the decedents.

Abstract Text: Teeth are the hardest and most resilient structures of the human body which retain their forensic importance for longer period of time. They are the most resistant part of the human body and retain their unique features, even in the worst taphonomic degradations, biological and chemical destructions. In addition to their unique odontological, molecular and chemical characteristics; their structural anomalies, idiosyncratic occupational marks, habitual, cultural features act as valuable adjuncts in forensic identification of unknown human skeletal remains found in forensic or bioarchaeological contexts. Healthy dentition is of little use for forensic anthropological identification, compared to the teeth having subtle morphological, radiological, pathological or traumatic variabilities. Forensic identification from dental DNA is the best method as such molecular markers of identity can be conveniently extracted even from the defective or carious teeth. Dental characteristics can reveal individuality of a person even after death and thus can play a pivotal role in forensic or medico-legal death investigations by the experienced odontologists.

The recovery of human remains and contextual items from forensic or bioarchaeological contexts is akin to unearthing of a goldmine as such remains present an excellent opportunity to understand the fate of the victim/s with some degree of certainty. The exhumed remains have the potential to bring forward new information concerning the biological, cultural, historical and geographical realities of victim/s in the past. One such opportunity presented itself in the year 2014 when a textbook authored by the administrator of Amritsar in 1857 mentioned the presence of a mass burial site in an abandoned well found situated underneath a religious structure at Ajnala (Punjab, India).[1-3]

In present study, thousands of bones and teeth belonging to 282 individuals and retrieved from an abandoned well situated underneath a religious structure at Ajnala (Amritsar, India) were used for their biological profiling purposes. As the remains were exhumed non-scientifically by amateur archeologists (without any scientific inputs), the same were found heavily damaged, fragmented and commingled, presenting a tough challenge for their identification efforts. More than 9650 teeth (loose or fitted in jaws) helped the forensic anthropologists in their efforts to establish their biological identity. Except few cranial elements, clavicles and meta-tarsals, no other bone was found suitable for drawing some valid conclusions about their identity i.e., age, sex, or ethnicity attributions. The skull, long bones, pelvis and other profile-specific bones were either missing or heavily fragmented and commingled, so the teeth were considered for establishing their odontological, radiographic, chemical and molecular profile. The stable isotope analysis and ancient DNA analysis helped in establishing their identity, though aspartic acid racemization, pulp-tooth area ratio and pathological indicators played a valuable adjunct in forensic odontological identification

In this oral presentation, the multi-faceted role of teeth as sole identifier of the heavily challenged Ajnala skeletal remains will be deliberated. The detailed results will be presented in textual, tabular and graphical/diagrammatic form as an Oral presentation at AAFS 2025 Meeting to be held in Baltimore in February 2025.

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Forensic Odontology; Ajnala Skeletal Remains; Multifaceted Approach

H8 Transforming Forensic Odontology: Unlocking the Power of Artificial Intelligence (AI) and Augmented Intelligence (AuI) for Dental Identification

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Learning Objectives: By attending this presentation, participants will gain a comprehensive understanding and be updated on the latest developments in Artificial Intelligence (AI) and Augmented Intelligence (AuI) and its use in advanced identification methods in forensic odontology. They will acquire valuable insights into the methodology and cutting-edge technologies that software companies introduce to daily operations and mass disaster scenarios. Attendees will be equipped with the knowledge to enhance accuracy and efficiency in their forensic identification processes.

Impact Statement: This presentation aims to significantly impact the forensic science community by fostering a dynamic discussion on the capabilities and potential of next-generation forensic software. Attendees will be able to explore the latest advancements in AI , AuI, and visual technologies, contributing their insights and proposals to enhance the identification process further.

Abstract Text: In the evolving field of forensic science, integrating advanced AI and AuI as well as advanced visual tools, will revolutionize forensic odontology. Incorporating these tools will push the boundaries of accuracy and efficiency in the identification process. The presentation will cover the fundamentals of AI and AuI, exploring their definitions, key differences, and implications. AI refers to the capacity of machines to simulate human intelligence and perform tasks such as learning, reasoning, and problem-solving. In contrast, AuI emphasizes enhancing human intelligence through the collaboration of human and artificial intelligence, leveraging the strengths to achieve superior outcomes. This foundational understanding will set the stage for discussing the critical role of these technologies in transforming various fields, particularly forensic odontology.

The presentation will focus on developing AI-driven dental identification software, driven by the urgent need for rapid and accurate victim identification following the October 7 attacks. This event highlighted the shortcomings of traditional software packages like WinID, Plass DVI, and UDIM, which rely on restoration-based methods. Due to the widespread use of fluoride, which has led to a decline in restorations, these systems faced challenges with ranking and, therefore, assisting in identification. In response, significant advancements in AI within forensic science have greatly improved the efficiency, accuracy, and reliability of dental identification processes. This shift underscores the need to adopt new technologies and methods, making AI-driven solutions increasingly vital to addressing these challenges.

The presentation will discuss how AI and AuI have catalyzed a paradigm shift in forensic odontology. This shift is characterized by integrating sophisticated algorithms and machine learning techniques, revolutionizing traditional practices, and setting new standards for accuracy and speed. Attendees will gain insights into the latest software developments in the field, showcasing how innovative AI tools are being implemented to solve real-world challenges in forensic identification. This update will highlight ongoing research on current software applications and future directions for AI and AuI in forensic odontology.

Forensic Odontology; Artificial Intelligence; Mass Disaster

H9 Research Dynamics in the Application of Mandibular Radiometric Dimensions for Sex and Age Estimation Using Different Radiological Modalities: A Bibliometric Analysis

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Learning Objectives: After attending this presentation, the attendees will be informed about the current trends in forensic odontological research based on mandibular dimensions measured on different radiological modalities, their advantages, limitations, and future probabilities through bibliometric and content analysis of the available information on the topic.

Impact Statement: This presentation will impact the forensic community by informing attendees about the current dynamics of research in use of mandibular radiometric dimensions in forensic odontological, which will help in prioritizing their research interests in the field

Abstract Text: The mandible is the largest and strongest bone of human cranial skeleton, most commonly used for age, sex, and ethnicity estimations from its dimensions or the teeth contained therein it. It significantly contributes to identification of the living or the victims of mass disaster incidents from where mutilated or severely damaged human remains are retrieved.[1] Its exploitable shape and size, patterned growth indicators, ease of its imaging, dense and compact nature, and being free of any overlying bony structure makes its features and the mandible itself as the preferable cranial element for providing vital information about sex, age, and ancestry of unknown human remains.[2] The systematic and bibliometric review provides the most accurate, reliable, and validated information about any particular method or technique. By critically evaluating methodologies, sample sizes, and results, bibliometric review can highlight the gaps for future research and assess the reliability and validity of current studies. Such analyses aid forensic practitioners in making informed decisions to ensure the quality and credibility of forensic analyses needed for legal as well as humanitarian contexts.[3] The present review synthesized a comprehensive, systematic, and bibliometric review of mandibular radiometric parameters used in forensic sex and age estimations to highlight current research dynamics, identifying consistent patterns, discrepancies, and under-researched areas and the future directions in the field.

Out of 175 articles listed in a scientific database search of PubMed, Google Scholar, Web of Science, and Scopus, 55 full-text articles were considered for bibliometric review in the present study. The articles selected were extracted in a systematic manner, considering the authorship, year of publication, country, and population assessed, the mandibular parameters, radiological modality used, sample size, age range of the subjects, etc. using the search strategy of the combinations of keywords and the Boolean operators. The analysis revealed that 60% studies (n=33) used radiographic mandibular parameters for sex estimation, 38.2 % (n=21) for both sex and age estimation, and only one study considered only age estimation. Orthopantogram (OPG) was reported as the most commonly used imaging modality in clinical practice; 69.1% (n=38)of articles used panoramic radiographs, 21.8% (n=12) od articles utilized Cone Beam Computed Tomography/Computed Tomography (CBCT/CT) scans, and only 4 articles were based on cephalometric scans for forensic sex and/or age estimations. Only 50% of studies reported accuracy percentages of the sex or age estimates and, out of 22 such studies, 72.2% of the studies reported an accuracy percentage above 70%. Mandibular ramus height was reported as the most reliable sex and sex-estimating parameter, followed by other mandibular dimensions like gonial angle, coronoid height, ramus breadth, and bigonial breadth.[4-12] The co-authorship and keyword network analysis reflected the collaboration dynamics, key themes, research hotspots, and the intellectual structure of the field, which will help researchers and policymakers in understanding the landscape of the research efforts and directing future possibilities. The detailed results will be presented in textual, tabular, and graphical/diagrammatic form.

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Forensic Odontology; Mandibular Radiographic Parameters; Bibliometric Review

H10 From Inside to Outside or Vice Versa? An *In Vitro* Study on the Deposition of Substances of Abuse in Dental Tissues

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WITHDRAWN

H11 An Evaluation of Degradation Pattern of Different RNA Types and Gene Expression in Human Dental Pulp Tissue Under Varied Temperatures and Storage Time Intervals

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Learning Objectives: The objective of this study is to know the pattern of RIN and gene expression and how Postmortem Interval (PMI) is determined using these patterns.

Impact Statement: The finding of this study provides the basis of further research and development of bigger grids to determine PMI more accurately.

Abstract Text: PMI is defined as the length of time elapsed since death. Determination of PMI is essential for the investigating forces. With recent advancements in technology, molecular profiling analysis is becoming increasingly important.[1,2] For decades, many methods have been developed to determine the PMI.[3] With tremendous changes happening in the field of microbiology, stability of various components like DNA and RNA in postmortem conditions are now explored for precision in PMI estimation. RNA is much more labile in nature and sensitive to degradation in a tissue-specific manner.[4] There are conflicting reports on how the PMI affects RNA integrity, but several studies in different mammals have shown that RNA can remain largely intact even for considerable time periods, when samples remain properly stored.[5-13]

In the present study, we have analyzed the RNA degradation pattern and gene expression (messenger RNA: HIF1α, GAPDH, β- Actin, micro RNA: MIR 143, MIR 125, ribosomal RNA: 18s RNA) under varied temperature ranges (-20, -10, 0, 4, 20, 37°C) and time intervals (1, 5, 30,180, 365 days) to estimate the PMI from the dental pulp tissue. The results of RNA degradation show us a degradation pattern. However, the results of 180 days and 1 year RIN were found below 2, and therefore were considered degraded and were not found suitable for further experimentation. Similarly, in the gene expression analysis using quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR), different genes HIF1α, GAPDH, β-Actin show a pattern of expression in the terms of fold change as compared to the control. The other endogenous biomolecular markers ,including MIR 125, MIR 143, and 18S RNA, have shown more stability, but the pattern is also clearly visible in their expression. These patterns were further analyzed separately at the time of validation which show that RIN no and genes HIF1α, GAPDH, and β-Actin show more accuracy whereas MicroRNA: MIR 143, MIR 125, and ribosomal RNA:18S RNA have shown significantly less accuracy.

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Postmortem Interval; RNA Degradation; Dental Pulp Tissue

H12 Teeth as Indicators of the Late Postmortem Interval (PMI): A Method Based on Histomorphological Changes of Dental Pulps and the Integrity of the Odontoblastic DNA

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WITHDRAWN

H13 Advancements in Forensic Odontology: Enhancing Medicolegal Death Investigations Through Technology and Collaboration

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Learning Objectives: After attending this presentation, participants will gain a deeper understanding of the evolving field of forensic odontology within the context of Medicolegal Death Investigations (MDIs).

Impact Statement: This paper examines the evolving role of forensic odontologists in contemporary medicolegal death investigations, emphasizing how advancements in technology and methodology have influenced the contributions and implications for forensic dental experts and investigators within the medicolegal community.

Abstract Text: **Background:** MDI encompasses the interpretation of evidence for the victim's family and the legal system, including civil and criminal jurisdictions. The investigator may be a coroner, medical examiner, or an MDI who interacts with many areas of forensic expertise.[1] Forensic odontology has long been an indispensable component of MDIs, serving as a specialized branch of dental sciences. This discipline establishes a critical nexus between dentistry and the legal system, offering essential support to investigators in identifying individuals, conducting anthropological age assessments, analyzing human bitemarks, and addressing mass disaster incidents.[2] The field has experienced advancements due to the integration of cutting-edge dental and medicolegal technologies, including Postmortem Computed Tomography (PMCT) scans, digital photography, and intraoral scanners.[3]

PMCT scans provide detailed internal images of the body, enabling comprehensive examinations without the need for invasive procedures. Digital photography delivers high-resolution images that are invaluable for documentation and subsequent analysis. Intraoral scanners capture precise impressions of dental tissues, which are critical for individual identification and bitemark analysis. Collectively, these technological innovations have revolutionized forensic investigations by providing more reliable and detailed data, thereby enhancing the overall investigative process.[4]

Materials and Methods: This research provides a thorough analysis of the evolving relationship between MDI and forensic odontology. It examines historical and contemporary case studies, authentic medicolegal cases, and advancements in technology related to the collection and interpretation of dental evidence. The study employs and demonstrates several advanced technologies that have enhanced the precision and efficacy of forensic investigations. These technologies include PMCT scans, digital photography, and intraoral scanners. Each tool was meticulously selected for its recent significant contributions to the accuracy and effectiveness of forensic analyses.

Furthermore, in-depth discussions were conducted with practicing specialists, including forensic odontologists, medicolegal death investigators, forensic pathologists, and forensic DNA specialists, to gather a diverse range of perspectives and insights. These comprehensive dialogs not only facilitated the exchange of expert knowledge and practical experiences but also illuminated various challenges and advancements within the field. By engaging with these professionals, the study was able to incorporate a multifaceted understanding of contemporary forensic dental practices, thereby enriching the overall quality and depth of the research findings.

Conclusion: This presentation aims to make an impact on the forensic community by elucidating recent advancements in investigative technologies and methodologies. It underscores the specialized contributions of forensic odontologists to MDIs, highlighting their indispensable role. Moreover, it emphasizes the importance of interdisciplinary collaboration and continuous professional development within the domain of forensic investigations. By highlighting innovative tools and techniques that augment forensic investigations, the presentation reinforces the invaluable role of forensic odontologists and underscores the necessity for ongoing professional growth and collaboration to advance the field of forensic science.

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Medicolegal Death Investigation; Forensic Odontology; Dental Identification

H14 The Use of Self-Portrait Photographs (Selfies) for Dental Identification: A Pilot Study

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Learning Objectives: After attending this presentation, attendees will have an opportunity to evaluate the potential of self-portrait photographs (selfies) in dental identification of unknown subjects.

Impact Statement: This presentation will impact the forensic science community by analyzing the possible use of selfies as non-clinical images for dental comparison and identification of unknown individuals.

Abstract Text: Along with the DNA and friction ridge analysis, comparing ante- and postmortem dental records is considered one of the three primary identifiers in the human remains' identification process.[1] In situations involving a large number of casualties, dental identification can be effective when other methods, such as genetic comparisons, are not feasible due to poor sample quality. An example of this was seen after the devastating tsunami in Thailand in 2004.[2] However, access to reliable and high-quality antemortem data is critical to the successful odontological identification of unknown bodies. In addition, the unavailability of clinical antemortem data can affect the process of identifying victims of mass fatalities, such as natural catastrophes, aviation disasters, terrorist attacks, and migrant disasters. Considering the popularity of self-portrait photographs (selfies) taken with smart phones held at arm's length, this image source could be identified as possible antemortem non-clinical data in dental comparison.

This research explores the possible use of selfies as an alternative or supplementary form of antemortem data in identifying human remains with an application in Disaster Victim Identification (DVI).

Thirteen volunteers, six males and seven females, between the ages of 20 and 40, have been recruited. Their dentition has been photographed under laboratory light and with professional equipment, as in a postmortem examination. The volunteers provided five selfies from their social media accounts, cropped to eliminate possible identification marks (e.g., facial hair) that could lead to identification bias.

The maxillary incisal outline of each selfie has been traced and compared by superimposition with the outline from the "postmortem" photographs to investigate the efficiency of selfie images in dental identifications. In addition, the casts of the volunteers' maxillary teeth were obtained and photographed. The incisal outlines traced from the casts were compared with those from selfies and teeth images.

A classification was introduced to assess the compatibility between the three sets of images and to distinguish between positive, possible, and negative identifications. The results revealed that in 61.5% of the cases, it was possible to establish a positive identification using at least one of the five selfies provided. In the remaining 38.5% of the cases, selfies suggested a possible identification. The comparison of the incisal outlines traced from plaster models and the dental pictures of the volunteers resulted in a 100% positive identification, indicating that the outlines from the cast provide enough detail for comparison, even in the form of digital images. This result suggests the advantage of sharing them for a prompt response in international DVI cases. In addition, the study discusses possible limitations of this visual comparison as an identification tool, including the average age range of people who take selfies and the behavioral patterns in shooting, posting, and editing selfie images between genders.

In conclusion, while almost 37% of incisal outlines traced from selfies were not suitable as a basis for positive identification due to poor angles and low resolutions of images, it is important to note that future advancements in camera lenses and sensors are likely to make non-clinical images even more helpful in dental comparisons in both single and multiple casualty incidents.

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Dental Identification; Anthropology; Photography

H15 Forensic Odontology in Mass Disaster Victim Identification: The Surfside Condominium Collapse Case Study

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Learning Objectives: This presentation aims to detail the methods and challenges encountered during the forensic odontological identification of victims in the Surfside condominium collapse, highlighting the integration of technology and collaborative efforts in a mass disaster scenario.

Impact Statement: Out of the 98 victims, a significant number were identified through dental records. The multi-agency collaboration and use of advanced technology facilitated a swift and reliable identification process. Challenges included the condition of the remains, the availability of antemortem records, and the need for deployment of forensic odontologists to the disaster site. Also, the incident highlighted critical flaws in the South Florida building code that contributed to the collapse.

Abstract Text: The Surfside condominium collapse in June 2021 was a tragic event that resulted in the loss of 98 lives, making it tied with the third-deadliest non-deliberate structural engineering failure in United states history. Forensic odontologists played an important role in the identification process of the victims, utilizing dental records to provide accurate and timely identifications. The identification process involved extensive multi-agency cooperation between Miami-Dade Fire Rescue Department, Miami-Dade Police Department, the Federal Bureau of Investigation (FBI), the Miami-Dade Medical Examiner Department, and the University of Florida Forensic Anthropology Department. After the effort transitioned from rescue to recovery, advanced forensic odontology techniques, including digital radiography and 3D scanning, were employed to enhance the accuracy of identifications despite the complex conditions of the remains, in cooperation with the University of Florida Forensic Anthropology Department.

The Surfside collapse case underscored the importance of preparedness and for the need for standardized protocols in forensic odontology for mass disaster victim identification. The use of technology proved to be a double-edged sword; while it enhanced the identification process, it also posed challenges in terms of data management and the need for specialized training. This presentation will significantly contribute to the forensic science community by showcasing the important role of forensic odontology in one of the largest building collapse disasters in United States history.

The multi-agency cooperation and advanced identification techniques employed during the Surfside condominium collapse will serve as a model for improving disaster response protocols and enhancing inter-agency collaboration. By addressing the operational and structural challenges encountered, this presentation aims to foster discussions on policy improvements and the necessity for robust building codes to prevent future tragedies. Ultimately, attendees will be better equipped to handle similar mass disaster situations, thereby improving public safety and the efficacy of forensic investigations. This serves as a wakeup call for the need to inspect and update building structures in South Florida.

Dental Identification; Decomposition; Fragments

H16 A Machine Learning-Assisted Combination of Skeletal and Dental Methods for Age Estimation in Subadults

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WITHDRAWN

H17 Can Standard Forensic Processing Practices Alter Dental Age Estimation Markers?

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Learning Objective: Attendees will acquire a proper understanding of the effects of high and low temperatures on adult dental age markers.

Impact Statement: This presentation will impact the forensic science community by providing pioneer information about the effects of high and low temperature exposure on dental age markers.

Abstract Text: Age estimation is one of the main components of the biological profile reconstruction of unidentified remains, narrowing down the list of potential candidates for the identity.

Dental age estimation (DAE) methods based on tooth development are the standard for subadult age estimation, offering higher accuracy and precision than the osteological methods. The reason behind this is the tide genetic regulation of teeth formation and development.[1] On the contrary, the process of aging is far less predictable, therefore, dental age estimation methods in adults present wider age ranges. Several post-formation changes have been explored for age estimation purposes such as dental wear, root resorption, cementum apposition, dental coloration, periodontal recession, secondary dentine apposition and root translucency.[2]

Secondary dentin is formed after the tooth completion, and it starts in the coronal portion of the pulp and progresses through the root canal towards the apex. As a consequence of the accumulation of secondary dentin, the pulp cavity progressively reduces its size, which can be observed in radiographic images.[3,4]

Root translucency is produced by the increase in peritubular dentin deposits of hydroxyapatite, which changes the refractive index of root dentin. This physiological change is evident from 20 years of age, and it starts in the dentin closest to the apex and it progressively extends towards the crown.[4,5,6]

Unidentified human remains are analyzed by forensic practitioners with the goal of establishing their identity and determine the cause and manner of death. During the analyses, the remains are analyzed by pathologists, forensic anthropologists, and forensic odontologists. They are often exposed to high temperatures during forensic body processing, and they are kept at low temperatures at the morgue until their final deposition. Forensic odontologists generally analyze the remains once they have been processed by the forensic anthropologists, while they are kept at low temperatures at the morgue. However, there is lack of evidence what the effects of high and low temperatures are on the dental age markers used in forensic cases.

The aim of the present study is to stablish the effects that high and low temperature may have on root translucency and pulp size measurements.

Forty (40) upper and lower extracted permanent canines were exposed to hot water at 52ºC and froze at -4ºC, during different time periods. Translucency and anterior pulp area were measured prior and after the different treatments.

The results showed that root translucency was affected by both treatments, experiencing an increase in dispersion in high temperatures, and a significant decrease over time in low temperatures. Pulp dimensions were affected by high temperatures, showing a significant decrease over time, but there were no significant effects detected in low temperatures.

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Forensic Odontology; Age Estimation; Teeth

I1 A Decomposition Scoring System for Indoor Death Scenes

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Learning Objectives: Attendees can expect to acquire an understanding of the decomposition processes associated with human remains located indoors.

Impact Statement: This presentation will impact the forensic science community by contributing to the development of a method for the quantitative analysis of indoor decomposition.

Abstract Text: Decomposition scores are widely used to assess postmortem changes in research and medicolegal settings.[1-3]. A number of decomposition scoring systems exist and they all function similarly; gross postmortem changes are assigned a numerical value.[1-3] Decomposition scores complement qualitative descriptions by facilitating the quantitative analysis of taphonomy, including the calculation of error rates and development of formulae to estimate Postmortem Interval (PMI).[1,4] Yet an indoor scoring system has yet to be developed, even though most decomposition cases are located indoors. Instead, scoring systems developed from outdoor studies are used. This is potentially problematic because indoor decomposition can differ significantly from outdoor decomposition.

A decomposition scoring system, hereinafter referred to as Indoor Taphonomy Score (ITS), was developed and used to quantify decomposition at 217 decomposition cases in the city and county of Honolulu, HI. Seventy-two of these cases were of particular interest because they had known, reliable PMIs and shared three additional characteristics: decedents were adults without physical trauma located indoors. Decomposition was analyzed retrospectively using scene photographs and assigning a numerical value (0–10) to the whole body based on gross postmortem changes such as livor mortis and marbling. Spearman correlation coefficient (r) and linear regression were used to investigate the relationship between ITS and PMI. Cases were also grouped to investigate the relationships between decomposition and clothing (fully clothed, partially clothed, nude), insect activity (present, absent), location of decedent (bathtub, bed/sofa, chair, floor), and season of investigation (spring, summer, autumn, winter). Research was approved by Chaminade University of Honolulu Institutional Review Board (IRB00007927).

PMI of decedents ranged from zero to ten days. The majority (75%) of decedents were male. Decedent age ranged from 20–91 years with a mean of 60 years. Mean height and weight equaled 174cm and 79kg, respectively. Most decedents (60%) were partially clothed, yet several were fully clothed (22%) or nude (18%). Manner of death also varied and included Natural (69%), Accident (17%), Suicide (4%), and Undetermined/Pending (10%). Cases were investigated in Spring (18%), Summer (21%), Autumn (29%), and Winter (32%). Insect activity was observed at 26% of scenes.

ITS ranged from 0–6 with a mean of 3. ITS increased from 1.4 ± 0.3 on day 0 to 4.3 ± 0.5 on day 6 before equaling approximately 4.0 from day 7 to day 10. Both Spearman correlation ($r = 0.749$, $P < 0.001$) and linear regression ($R^2 = 0.516$) detected a significant relationship between ITS and PMI. Significant effects of clothing, location, and season were not observed. However, the ITS of decedents colonized by insects tended to be greater than without insects even though insect activity was not observed prior to 3 days postmortem.

Our results show that ITS increased steadily for the initial six days postmortem. Decomposition scores stabilized after this time. This pattern of decomposition, rapid change followed by slower change, has been observed in many other decomposition studies and possibly demonstrates the ability of ITS to detect changes in the rate of gross postmortem change.[5] We conclude these findings merit additional research to increase the number of cases and identify informative trends in indoor decomposition throughout the city and county of Honolulu. For example, a consistent three-day delay in insect colonization would be helpful to cases relying on entomological evidence to estimate PMI. Ultimately, these results demonstrate the importance of establishing a system to facilitate quantitative analysis of indoor decomposition.

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Medicolegal Death Investigation; Taphonomy; Built Environment

I2 Temperature Variation in Marquette, Michigan, in Relation to Protein Degradation Patterns for Postmortem Interval Determination

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Learning Objectives: After attending this presentation, attendees will understand how temperature variation in a specific climate (Marquette, MI) affects the rate of postmortem human skeletal muscle degradation.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of studying environmental factors influencing protein degradation and its implications for estimating the Postmortem Interval (PMI).

Abstract Text: PMI estimation is a crucial aspect of forensic work because determining the PMI can provide valuable investigation information. Current methods to estimate PMI have limitations, such as the timeframe within which they are effective or selective to certain circumstances surrounding the death. In the past few years, researchers from the University of Salzburg, Austria, proposed that the observation of protein degradation in human skeletal muscle could be used as a novel approach for PMI estimation due to specific proteins degrading in a predictable and time-dependent fashion.[1] Regardless of the method being used, PMI estimation is most accurate through early and active stages of decomposition and becomes less reliable as decomposition progresses into the more advanced, later stages. To further understand the applicability of protein degradation as a method for estimating PMI, research into the intrinsic and extrinsic factors that affect the rate of protein degradation is critical. This study, inspired by the group at the University of Salzburg, seeks to establish how Marquette, MI, temperatures in early spring and summer at the Northern Michigan University (NMU) Forensic Research Outdoor Station (FROST) affect the patterns of degradation in protein markers β-actin, desmin, and cardiac troponin-t (cTnT).

To conduct this study, muscle samples were collected from the thighs of two human donors during separate ten-day controlled (laboratory) and environmental (outdoor) studies. The controlled study involved three temperature-controlled environments in a biochemistry laboratory at NMU, while the environmental study was conducted at FROST, in which one donor was studied during the early spring and the second was studied during the early summer. The protein from the collected muscle samples was extracted, BCA assayed for protein concentration, and then western blotted using primary antibodies directed against β-actin, desmin, and cTnT. Ambient temperatures at FROST were collected continuously using the onsite weather station at FROST, and local temperatures were collected using a handheld infrared thermometer. Current results show that the degradation patterns for two out of the three proteins tested are characteristic for certain times since death. Analysis of the western blots shows slow or no degradation in colder temperatures and faster degradation in hotter temperatures. Data from this project will be used further to develop protein degradation as an application of PMI estimation and as a reference for further protein degradation marker research.

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Postmortem Interval; Protein Degradation; Temperature

I3 An Analysis of the Sequencing Quality of Next-Generation Sequencing for the Entire Mitochondrial Genome in Decomposed Human Samples

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Learning Objectives: Attendees will: (1) understand the impact of decomposition on DNA and mitochondrial DNA (mtDNA) profiling: Attendees will learn how decomposition affects DNA integrity, and how (mtDNA) serves as a reliable alternative in cases of severe DNA degradation; (2) explore Next Generation Sequencing (NGS) methods for forensic mtDNA analysis: Attendees will gain insight into the use of NGS panels for analyzing mtDNA from human remains, and how this advanced technology enhances forensic identification; (3) identify optimal tissue types for mtDNA analysis in decomposed bodies: Attendees will learn that some types of soft tissues, such as uterine and aortic tissues, are suitable for mtDNA analysis substituting hard tissues, especially when traditional sources like blood are unavailable; and (4) assess the reliability of mtDNA sequencing in various stages of decomposition: Attendees will evaluate the success rates of mtDNA retrieval from different soft tissues at varying decomposition levels, emphasizing that even highly degraded tissues can yield complete or near-complete mtDNA sequences.

Impact Statement: This presentation will enhance the forensic science community's ability to perform genetic identification in cases with severely decomposed human remains by demonstrating the effectiveness of mtDNA analysis using soft tissues and NGS panels. By identifying alternative soft tissues for reliable mtDNA retrieval, the findings will streamline forensic processes, improve the accuracy of postmortem identification, and optimize resource utilization in challenging forensic cases.

Abstract Text: **Background:** The decomposition of the human body causes significant DNA damage, which poses challenges for DNA profiling techniques.[1-7] MtDNA has been used as an alternative and complementary approach to genetic identification for many years, especially in cases where DNA degradation has occurred.[8-12] Hard human tissues, such as bones, nails, and teeth, consistently provide DNA suitable for identification even after severe decomposition, but DNA extraction requires a complex and time-consuming process. Some studies attempted to use soft tissues or swabs of decomposed human bodies; however, they had limitations such as a small number of samples or cases, a narrow selection of soft tissues, and a sole focus on Short Tandem Repeat (STR) results, disregarding mtDNA sequencing outcomes.[13-21] This study seeks to investigate the potential of soft tissues for mtDNA analysis in human remains, taking advantage of advancements in forensic genetics, such as the NGS panel method.

Methods: A total of 292 samples were collected from 30 autopsy cases. Out of these, 290 samples were used for mtDNA analysis. The analysis was carried out using a commercial NGS panel that targets the entire mitochondrial genome. Two hundred thirty-nine samples were at different stages of decomposition and the remaining were not decomposed. The samples included nine types of soft tissues: heart, liver, kidney, lung, brain, pectoralis muscle, iliacus muscle, aorta, and uterus. Additionally, rib cartilage and blood samples were also included.

Results: A confirmation of over 90% of the entire mtDNA sequence was achieved in 49.6% of decomposed samples. This percentage increased to 78.7% when specifically focusing on hypervariable regions. Surprisingly, even highly decomposed soft tissues yielded 95-100% of the mtDNA sequence, which is comparable to the results obtained from rib cartilages. In situations where blood samples, which are the most reliable, were not available, it was recommended to use uterine tissue in females or aortic tissue in individuals without a uterus. Interestingly, no significant correlation was found between the quality of mtDNA sequencing and the total body score or the degradation index of nuclear DNA.

Conclusion: The NGS panel method was able to successfully obtain most of the mtDNA sequences, even from highly decomposed human soft tissues. This indicates that the decomposition should not discourage genetic testing. To streamline the processing of hard tissues in forensic genetics practices, it could be beneficial to use uterine or aortic tissues as alternatives, or blood if available. This would save both time and effort.

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Mitochondrial DNA; Next Generation Sequencing; Decomposition

I4 The Effect of Decomposition on Recovery of DNA Evidence and Its Subsequent Impact on Identification

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Learning Objectives: This presentation will illustrate the difference in DNA recovery success in fresh versus decomposed remains and provide attendees with the best storage conditions for biological evidence. Additionally, attendees will gain an awareness of the importance that collecting evidence as soon as possible has in regard to identification and will understand the impact of hot, dry climates on DNA recovery.

Impact Statement: Understanding the impact of decomposition on the success of DNA profiling will enhance the success rate of DNA recovery, enabling the identification of the optimal time to collect biological specimens for DNA testing, which can then be implemented in practice. In turn, this advancement will facilitate the identification of a greater number of decedents, for the successful sequencing of DNA extracted from unidentified remains or the surrounding soil holds immense significance in establishing an accurate DNA profile for the deceased and promptly returning their remains to their families. The results from this project benefit not only forensic researchers but crime scene investigators, laboratory technicians, medical examiners, and the larger forensic science community; therefore, this project is interdisciplinary in nature and provides reliable, measurable outcomes in several forensic disciplines, shaping practices and methodologies for future forensic investigations.

Abstract Text: As decomposition of carrion progresses in external environments, DNA evidence becomes progressively more challenging to extract and amplify successfully, primarily due to degradation.[1] Additionally, climates characterized by higher temperatures and lower humidity, such as those found in regions like Arizona, will expedite the decomposition process, consequently leading to the degradation of DNA. With degraded DNA, large regions of DNA are difficult to successfully sequence, and this factor can make it difficult to identify evidentiary materials, such as remains, insects, and bodily fluids.[2] Therefore, it is imperative that body tissues and any other biological evidence be either immediately sampled for DNA analysis or preserved immediately for subsequent DNA analysis to ensure the proper identification of the deceased. Additionally, if the remains were found on soil, collecting the soil beneath the remains may provide reliable DNA profiles.[3] The success of DNA extraction amplicons and subsequent profiling is contingent upon validated standard operating procedures applicable to various forensic disciplines, reliant upon the nature of the evidence. Most decomposition studies involve porcine remains due to the similarity in skin and composition.[4]

The primary objective of this project was to investigate the decomposition of porcine remains in Phoenix, AZ, to determine whether various environments had an impact on the recovery and amplification of DNA from these remains. We aimed to: (1) examine the effect of different time frames on the success rate of DNA recovery and amplification; (2) examine the effect of DNA recovery and amplification from porcine remains in several simulated Arizona environments; and (3) determine the optimum preservatives for preservation of DNA recovered from decomposed porcine remains and insect specimens. Various tissue samples were collected from pig remains that had undergone different treatment intervals for a two-week period and ambient temperature was recorded. Given their significant role in the decomposition process, various insect specimens were also collected at the same intervals to identify the species involved. Subsequently, both pig and insect samples underwent distinct preservation treatments, varying in temperature and duration. DNA was extracted from both the pig tissue and blow fly specimens using a modified Qiagen DNeasy method and quantified using QuBit fluorimetry. A 650bp mitochondrial DNA barcoding region was amplified using Polymerase Chain Reaction (PCR) to determine the sample success rate and integrity of the barcoding region. A subset of successful amplicons was sequenced using Sanger sequencing at Arizona State University and inputted onto Blastn for insect species assignment validation. DNA quantification and success rate of amplicon products were analyzed using statistical software with time and temperature as variables. Results found that both time frame and temperature had a significant effect on the recovery and success rate of the barcoding region. DNA amplicons obtained from pig tissue and from soil varied in significance, with tissue samples having a better recovery and amplification success rate. Regarding insect activity and decomposition, results, as expected, showed that insect activity increased significantly in the early stages of decomposition.

Given Arizona's consistently high temperatures throughout most of the year, future research will investigate how extending sampling intervals beyond two weeks impacts decomposition rates, measurable DNA recovery, and successful DNA amplification.

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DNA; Decomposition; Identification

I5 Immersion Deaths in Seoul, a City With a River: The Implication of Decomposition in the Diagnosis of Drowning

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Learning Objectives: Attendees will identify the characteristics of immersion deaths that occurred in Seoul, Republic of Korea, which were compared with other global cities with a large river running through the center. Most of the cases were suicidal drownings found in the Hangang River. Attendees will identify the influence of decomposition on autopsy findings of drowned bodies: fluid in the sphenoid sinus, petromastoid hemorrhages, and liquid in the stomach and duodenum were not altered by decomposition.

Impact Statement: This study was conducted in a large city with a river in the Republic of Korea. It should be noted that the study results do not represent the overall picture of drowning in the country. Common autopsy findings that have been used for diagnosing drowning were reviewed considering the impact of decomposition. The results of this study would help manage immersion deaths in a city with a river and diagnose drowning in decomposed bodies.

Abstract Text: Drowning refers to the process of respiratory impairment caused by submersion in water, and it is a significant cause of death worldwide. Diagnosing drowning through a systematic classification of all abnormal autopsy findings is crucial, as various circumstances, from natural death to homicide, are possible in water. However, diagnosing drowning remains a challenging issue in forensic pathology as it requires the exclusion of all other possible scenarios, especially in decomposed bodies. Therefore, this study aimed to analyze immersion bodies found in Seoul, a city notable for its major river. It presents an overview of drowning cases and provides information on the diagnosis of drowning in decomposed bodies.

Data were collected and analyzed from immersion bodies autopsied at medical examiner's office at Seoul National University College of Medicine from April 2016 to October 2023, examining the observed autopsy findings and their correlation with decomposition. Most immersion bodies were discovered in the Hangang River, with drowning suicides being the most common cases. For suicides, mental illness was the most common underlying disease. The more decomposed the body, the more likely it was that the manner of death was undetermined, with single-person households being the most common. Drowning findings affected by decomposition included foam in the airway, emphysema aquosum, aortic hemolytic staining, and positive diatom test. Drowning findings that were not affected by decomposition included fluid in the sphenoid sinus, hemorrhages in the petromastoid bone, and liquid in the stomach and duodenum. This study has some limitations, as it was based on a limited number of autopsy cases performed at a single medical examiner's office covering a restricted area of Seoul. However, a substantial proportion of decomposed bodies were included, providing valuable information on the diagnosis of drowning in decomposed immersion bodies. Understanding the factors that influence drowning findings is crucial for making accurate diagnoses. Additionally, further research is needed to advance the diagnosis of drowning.

Drowning; Postmortem Diagnosis; Forensic Pathology

I6 Animal Predation Versus Scavenging in Medicolegal Death Investigations

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Learning Objectives: After attending this presentation, attendees will understand investigative distinctions and autopsy findings between deaths caused by animal predation and deaths where scavenging is present but not related to the cause of death.

Impact Statement: This presentation will impact the forensic science community by highlighting the importance of recognition between the types of animal activity in death investigations and how reliably documenting the animal involvement can assist in the determination of the cause and manner of death.

Abstract Text: Predation involves an active attack by an animal on another living organism, resulting in their death.[1] Causes for attacks include hunger, territorial, protecting their young, being in pain, and provocation from others.[2] Predation can occur as an attack from a single predator or from a multiple in a pack-like setting. Evidence of predation typically includes bite marks, claw marks, and puncture wounds. In cases of predation, there may be signs of a struggle or defensive wounds on the decedent along with puncture wounds and tearing in the extremities, head, and neck. The predator may still be present at the scene.

Scavenging occurs when an animal—including insects—feeds on the remains of the decedent after they have died from other causes. Evidence of scavenging includes the absence of defensive wounds or signs of struggle and scavenger marks such as gnawing, tearing, or consumption of soft tissues.[3] Multiple animals can feed on the same individual over time, leaving different types of patterns, and dispersal of remains.[3] Scavenging can occur outdoors—where wild or stray domestic animals may have undisturbed access to the body; or indoors—where domestic animals such as dogs and cats have access to their deceased owners, as well as intruding animals, such as rats.[4] Animals often feed on locations of exposed tissue through injury, destroying information as well as creating new injuries. Postmortem injuries typically do not have a vital reaction and occur on parts of the body that are accessible to the scavenger. Decedents found in a water environment are also exposed to aquatic life, including fish, turtles, and alligators.

Techniques including the postmortem interval can help delineate between predation and scavenging and signs present during the investigation that indicate pre- or postmortem injuries. Decomposition makes this determination challenging. Factors such as environmental conditions and the presence of other scavengers can interfere with the initial interpretation of the level of animal involvement but can be amended during the examination.[3]

Several cases were reviewed from the Harris County Institute of Forensic Sciences, Houston, TX, to highlight the common types of predation and scavenging observed in our casework and challenges encountered.

- Alligators: A 63-year-old woman was found dead on the bank of a bayou, next to a 12-foot alligator. Both upper extremities and various organs were recovered from the alligator's stomach. There were crushing injuries, punctures, lacerations, abrasions, and contusions. The lower extremities of an unknown man were found with multiple alligators. Bilateral legs, hips, and partial spine were located.
- Dogs: A 44-year-old and 66-year-old woman were found dead with multiple wounds of the head and extremities after reported dog attacks. Two 52-year-old men found dead in their separate locked residences, with their dogs. Empty boxes of dog food were found, and both living and dead dogs were noted. Both decedents had exposed bone, with absence of vital reaction and no evidence of hemorrhage at the scene.
- Aquatic activity: A 68-year-old man was found floating prone in a bayou and a 27-year-old man was found floating prone in a river. Both had evidence of postmortem activity, including absence of the right ear and regions of the lips.
- - Rodents: A 61-year-old man was found dead in his home with bleeding circular skin defects on the face and genitalia.

By carefully considering the factors that are present on scene and employing a combination of observational and forensic methods, investigative personal can interpret deaths that are caused by animal predation or when scavenging is merely present. Documentation at the scene of animals observed, and the assistance of a forensic entomologist and anthropologist can be invaluable.

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Animal; Predation; Scavenging

I7 The Changes in Cuticular Hydrocarbon Profiles of a Single Blow Fly Species as a Function of Developmental Stage and Preservation Method

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Learning Objectives: Attendees of this presentation will learn about the changes in Cuticular Hydrocarbon (CHC) profiles across the developmental stages of laboratory-reared *Lucilia silvarum* (Meigen, 1826) (Diptera: Calliphoridae) specimens and the influence of preservation method on the CHC profile.

Impact Statement: This presentation will impact the forensic science community by further demonstrating the potential of CHC profiles to provide an objective method for Postmortem Interval (PMI) determination. Specifically, this work demonstrates that differences in profiles can be used to distinguish developmental stages of a single blow fly species. Further, differences in profiles as based on preservation method are apparent that may affect the ability to distinguish among developmental stages.

Abstract Text: Blowflies (Diptera: Calliphoridae) present at death scenes are often collected and evaluated morphologically to determine developmental stage, which is subsequently used to estimate a minimum PMI. In recent years, several researchers have demonstrated the utility of CHC profiles for age determination.[1] These hydrocarbons are present on the exterior surface of the specimens and have the potential to provide a more objective method for PMI determination. However, many of these studies focus on a single life stage (e.g., larvae, adults, puparium) and use different methods to preserve collected specimens, extract the hydrocarbons, and analyze the resulting extracts.[2] As such, the objective of this work was to evaluate differences in cuticular hydrocarbons across developmental stages of a single blow fly species and among preservation methods using the same extraction and analysis methods.

Eggs were first collected from a decomposition study and reared to adulthood for species identification based on morphological evaluation using an identification key[3]. Most adults were provisionally identified as *Lucillia silvarum* (*L. silvarum*) and were used for a rearing study. The adults laid eggs that were reared in cages containing pig liver as a nutrition source for the larvae. Specimens of 1st, 2nd, and 3rd instar larvae were collected, along with empty puparial cases, and adult flies, and stored at -20°C until extraction. A statistically optimized method was used to extract cuticular hydrocarbons from each specimen, and the resulting extracts were analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). The total ion chromatograms were normalized and differences in CHC profiles were evaluated based on identity and relative abundance of compounds present.

Differences in CHC profiles as a function of developmental stage were apparent. For example, *n*-octane (C8) was present in first and second instar larvae, only present in some 3rd instar larvae, and not present in the empty puparial casings or adults. Similarly, *n*-eicosane (C21) was present in all 1st, 2nd, and 3rd instar larvae, inconsistently present in puparial casings, and absent in adults. Additionally, relative abundance differences in some hydrocarbons show potential to distinguish among larval stages. For example, *n*-nonacosane (C29) was at least six times more abundant in 3rd instars compared to 1st and 2nd instar larvae. These, and other differences, illustrate the changes in CHC profiles across developmental stages of *L. silvarum* within a single generation.

To evaluate the influence of preservation method on CHC profiles, excess 3rd instar larvae from the rearing study were collected and stored in 70%, 80%, and 95% ethanol solutions. Excess adult flies from the rearing study were also collected and stored in 70% and 90% ethanol solutions. After 7 days, these samples were extracted and analyzed and the resulting CHC profiles were compared to those collected from specimens stored at -20°C. Overall, specimens preserved in 90% and 95% ethanol showed higher relative abundance of cuticular hydrocarbons compared to those preserved in 70% and 80% ethanol solutions. Further, specimens stored at -20°C showed higher abundance of cholesterol than specimens stored in ethanol.

A field study is currently being conducted in which larvae are collected from decomposing swine carcasses. Specimens are separated based on instar and preserved using three different methods: dry frozen at -20°C, suffocated in 95% EtOH, and killed by immersion in hot water, then stored in 95% ethanol. Cuticular hydrocarbon profiles will be generated from the larvae and compared to the profiles generated from the laboratory-based studies described above. Overall, this presentation will discuss differences in CHC profiles across developmental stages of *L. silvarum* as a function of preservation method and provide a comparison of profiles between laboratory reared and field-collected specimens.

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GC/MS; Cuticular Hydrocarbons; Blow Fly Developmental Stage

I8 Disguising Decomposition: Attempted Concealment and the Insects Associated With Remains in Criminal Investigations

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Learning Objectives: Attendees will learn about the various modes of concealment of remains performed by perpetrators in a variety of case reports and the application of forensic entomology for investigative purposes.

Impact Statement: The information provided in this presentation will impact the forensic science community by enhancing the ability of forensic practitioners to recognize the impact of concealment methods on the decomposition of remains and how insect evidence can assist with investigative timelines.

Abstract Text: Criminal investigations present a complicated scenario that may involve a variety of forensic practitioners. Contributing to the intricacies of this process, a variety of concealment methods may be employed by perpetrators in an effort to hide remains, eliminate potential evidence, or diminish the odors of decomposition. The method of concealment or alteration of the remains can impact the rate of decomposition and the invertebrate scavengers that can access this resource.[1]

In forensic entomology, a great deal of information can be gained from the proper collection, identification, and analysis of insect evidence that can provide a minimum Time Of Colonization (TOC), which represents the amount of time that insects have been developing on the remains.[2] The TOC can be used to provide a minimum Postmortem Interval (mPMI), or time between death and discovery of the remains, especially if insects can access the remains and begin to deposit eggs.

Several recent cases involving concealed remains in the Midwestern United States have requested the use of forensic entomology to assist with investigative timelines. The scenes included both indoor and outdoor habitats with varied concealment methods, including contractor-grade plastic bags, vehicles, furniture, and debris piles. Despite various attempts at disguising the remains, diverse communities of insects arrived, deposited eggs, and utilized the remains as a resource. Many of the insects associated with concealed remains were common species of blow flies (Diptera: Calliphoridae) and several families of forensically relevant beetles.

This presentation will provide an overview of forensically relevant insects associated with concealed remains and will discuss a report in which forensic pathology and forensic entomology were the two fields that yielded the most valuable information in this investigation. The information provided in this case study serves as an example of a complex investigation and further supports the use of forensic entomology in criminal proceedings.

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Entomology; Decomposition; Casework

I9 A Developmental and Genomic Study to Strengthen Forensic Entomology Baseline Data

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Learning Objectives: Attendees will understand the principles of how developmental data in forensic entomology plays a fundamental role in Postmortem Interval (PMI) estimation from insect specimens. Attendees will understand the importance of how more research is needed on particular blow fly species of forensic importance that are present in the United States.

Impact Statement: This presentation will provide an understanding of how developmental research is still needed in forensic entomology. By demonstrating the applicability of data to calculate minimum PMIs, this presentation will show fundamental data that is beneficial to the forensic entomology community.

Abstract Text: Forensically important blow fly development data is limited for populations of many species across the United States. This data is critically needed for a forensic entomologist to accurately estimate a minimum PMI estimation from insect evidence collected at a crime scene. While there are developmental datasets available for some species of interest, there are some forensically important species that have no development data available.[1] Further, it has been shown that developmental differences exist among populations of the same species; however, the methodology used to determine these differences is not consistent and the associated error with the datasets has not been determined.[2] Without determining associated error, there is a possibility of over- or under-estimating a minimum PMI. Typically, developmental data for a forensic species of blow fly will be used from the geographically closest and most climatically similar published development data and implemented on the evidence, meaning that the datasets used are sometimes from a different temperature, region, or country.

This research aimed to examine the developmental and genomic differences among two geographically separated populations of the forensically important blow fly, *Calliphora coloradensis* Hough (Diptera: Calliphoridae) across the United States. This species is a predominantly carrion-feeding insect in the United States.[3] Developmental time of each immature life stage was recorded at two different temperatures, 25°C and 30°C, in controlled conditions across a sampling period to extrapolate developmental differences and life-history traits exhibited. Several generations of each population were sampled. Statistical analysis was performed to determine mean development times across life stages and any significant differences between population, generation, and temperature. In addition to developmental data, total DNA was extracted using a spin-column technique. Both nuclear and mitochondrial DNA barcoding regions of approximately 650bp were amplified using blow fly specific primers and sequenced using standardized protocols. Barcoding consensus sequences were analyzed for Single Nucleotide Polymorphisms (SNPs) and phylogenetic analysis performed using software. The results revealed a statistically significant difference in total developmental time between the two distinct populations. The Arizona blow fly population exhibited differences of approximately 15-18 hours slower in total developmental time compared to the Californian blow fly population and is hypothesized to be due to differences in thermal preferences. The pupal stage of *C. coloradensis* was found to be statistically significant when compared to similar species collected in the region. No statistical difference was found between generations of the species in either population. Genomic data showed similarity in both nuclear and mitochondrial DNA barcoding regions between the two blow fly populations and shows feasibility for use of the region for identification purposes. The availability of this data will allow forensic entomology evidence to be incorporated in more investigations across the United States and is critically needed for the United States due to the absence of accurate developmental data for this forensic species.

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Entomology; DNA; Postmortem Interval

I10 A Rare Case of Penetrating Atherosclerotic Ulcer Rupture in the Ascending Aorta With Cardiac Tamponade

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WITHDRAWN

I11 Bulletproof Heart: Defying the Odds With an Embedded Remote Projectile

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Learning Objectives: After attending this presentation, attendees will gain insights into the implications of retained projectiles within the myocardium and the possibility of long-term stability in rare cases.

Impact Statement: This presentation will impact the forensic science community by enhancing forensic professionals' understanding of retained projectiles and improve their ability to recognize and analyze similar injuries in forensic contexts. This will contribute to better-informed legal decisions and enhance the overall quality of forensic investigations and reports.

Abstract Text: In 2022, the National Safety Council estimated that the probability of death from a gunshot wound in the United States was 1 in 89.[1] Gunshot wounds to the heart are particularly severe, often resulting in high mortality rates due to the potential for life-threatening damage. The impact of cardiac injury depends on factors such as bullet design, firearm type, projectile velocity, and trajectory.[2,3]

This report details the case of a 64-year-old Black male who was examined postmortem at the District of Columbia Office of the Chief Medical Examiner after sustaining a gunshot wound to the torso. Postmortem radiographs indicated radiopaque changes in the abdomen and chest, consistent with the presence of projectiles.

The examination identified an acute penetrating gunshot wound involving the rectum, left common iliac artery, large bowel, mesentery, and small bowel and skeletal muscles and soft tissues of the left abdomen where a copper-colored projectile was recovered. The myocardium was noted as red-brown and firm, with no significant lesions except for an oxidized, gray-colored projectile embedded in fibrotic tissue. This remote projectile was lodged in the right ventricular wall without surrounding hemorrhage.

Survival following the occurrence of projectiles embedded in the myocardium is rare but noteworthy. Such injuries can significantly alter cardiac physiology through electrophysiological and mechanical disruptions. Electrophysiologically, associated inflammation, and fibrosis can interfere with conduction pathways, potentially causing arrhythmias. Mechanically, obstruction of atrial and ventricular chambers can result in hemodynamic instability and reduced cardiac output.[4]

Despite the projectile, the decedent remained hemodynamically stable and asymptomatic for years. The cause of death was determined to be a "Gunshot wound to the torso," with the retained projectile not contributing to the fatal outcome.

This case challenges conventional perspectives on managing cardiac gunshot wounds and their implications. The unexpectedly favorable long-term outcome highlights the need to reassess forensic approaches to retained intracardiac bullets, offering valuable insights for clinical and forensic practice.

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GSW; Autopsy; Cardiac

I12 Severe Acute Myocardial Injury Associated With Heat-Related Illness

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the autopsy, laboratory, and pathological findings associated with heat-related illness and its lethal complications.

Impact Statement: This presentation will impact the forensic science community by enriching the literature with the present case report. The authors will discuss all the findings and available criteria for making a proper diagnosis when doubting heat-related deaths.

Abstract Text: The term heat illness refers to incapacitating conditions directly related to a rise in body temperature, such as heatstroke, as well as to milder disorders such as heat exhaustion, head syncope, heat cramps, and heat rash.[1] Heat-related illnesses are with seasonal peaks and are a serious worldwide problem with the warming climate.[2]

Heat stroke is a serious and life-threatening condition defined as a core body temperature > 40.0°C (and by a central nervous system abnormalities failure of proper maintenance of thermoregulation due to the high core body temperature ensuing from either exposure to high environmental temperatures or strenuous exercise.[3,4] Heat stroke may cause multiorgan dysfunction syndromes. People are susceptible to cardiovascular complications of heat stroke, including arrhythmias, myocardial ischemia, heart failure, shock, and sudden death, especially those with pre-existing impaired cardiovascular functions.[5,6]

A homeless White man with an unknown identity, aged approximately 40- to 45-years-old, was found dead in the city center on a hot summer day, with temperatures exceeding 35°C during the day. At the autopsy, it was noted that the deceased was wearing multiple layers of clothes, which was inadequate clothing for the weather and ambient temperature, although typical for people experiencing homelessness, who most of the time wear all of their possessions (e.g., clothes). Externally, there were no visible signs of traumatic injuries over the body. Internally, the most pronounced autopsy finding was congestion of the internal organs, severe brain and lung edema, multiple petechiae, and hemorrhages of serosal membranes of the internal organs. The heart was not enlarged, and the coronary vessels were without atherosclerotic plaques. The toxicology analyses were negative. The most significant laboratory findings were the highly elevated creatine kinase levels in blood, urine, and vitreous humor, as well as the MB fraction of creatine phosphokinase in the blood. Tissue samples for further histopathological evaluation were taken; the last were stained with hematoxylin and eosin. Additionally, the heart tissue immunohistochemistry was performed, confirming the myocardial impairment of diffuse cardiac myocytolysis. The cause of death was attributed to acute cardiovascular and respiratory failure associated with heat-related illness.

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Heat Stroke; Heat-Related Illness; Sudden Death

I13 Unlocking the Mystery Behind Sudden Cardiac Death: A Case Series

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Learning Objectives: Through this presentation, attendees will learn about the most important forensic steps in a multidisciplinary approach to studying Sudden Cardiac Death (SCD), illustrated through the examination of four main cases. Integrating perspectives from forensic pathology, cardiology, and genetics is fundamental for an in-depth understanding of SCD. This multidisciplinary and multi-specialist strategy not only improves the accuracy of medico-legal investigations, but also contributes to the development of more effective prevention and intervention strategies.

Impact Statement: This presentation aims to provide a better understanding of the crucial role of autopsies in investigating cases of SCD. Autopsies provide essential data to determine the precise cause of death, identify potential genetic factors, and uncover the underlying pathophysiological mechanisms involved.

Abstract Text: A sudden and unexpected death due to a sudden cardiac event in a person without pre-existing heart disease is known as Sudden Cardiac Death (SDC); it is a global public health issue; it can often be diagnosed postmortem, as an autopsy finding. Frequently, traditional autopsies lead to negative results, and, in the case of Sudden Unexplained Deaths (SUDs), a cardiac cause can only be discovered through genetic analysis, carrying out what is known as a "Molecular Autopsy." This retrospective study aims to highlight the importance of a complete medico-legal investigation, to discover the exact cause of death in all those SUD cases. Analyzing a dataset of 380 complete autopsy reports performed during five years (from 2019 to 2023), we selected 49 autopsies with a cardiac origin as the cause of death. Based on our exclusion criteria, all cases related to drug intoxication or poisoning were excluded; moreover, we excluded all cases involving people who underwent any type of major surgery and people with any prior diagnosis of cardiac pathology. Therefore, we identified four cases of SCDs; these had been individuated by adopting further investigations, such as postmortem imaging, and toxicological, molecular, histological, and immunohistochemical analysis, which had a pivotal role in the diagnosis. SCDs are a very important global health issue and it is extremely necessary to understand the precise causes behind a sudden cardiac death to identify genetic and familiar predispositions to SCD. This means that understanding the conditions underlying these cases of SCD can prevent misdiagnoses among surviving relatives, potentially saving their lives through appropriate screening and medical interventions.

This approach requires a collaboration between many professional figures, including forensic pathologists, geneticists, and clinicians to make a complete evaluation of the case and a subsequent appropriate follow-up. Our study emphasizes the crucial role of autopsies in understanding cases of SCD; indeed, autopsies provide essential data to determine the precise cause of death, identify potential genetic factors, and discover the underlying pathophysiological mechanisms of death. Furthermore, a multidisciplinary approach, integrating the perspectives of forensic pathology, cardiology and genetics, is crucial for an in-depth study of SCD.

Forensic Pathology; Sudden Cardiac Death; Autopsy

I14 An Assessment of Myocardial Ischemia Using Coronary Postmortem Computed Tomography Angiography Based on the Voronoi Algorithm: A Case Report

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Learning Objectives: Attendees will learn how to apply the Voronoi algorithm to assess myocardial ischemia using Postmortem Computed Tomography Angiography (PMCTA). Specifically, they will gain insights into the process of identifying and quantifying perfusion volumes and ischemic areas within the myocardium. This presentation will also cover the practical steps of analyzing cardiac perfusion areas and understanding the correlation between PMCTA findings and autopsy results. By the end of the session, attendees will be able to evaluate the effectiveness of combining PMCTA with the Voronoi algorithm for non-invasive visualization and quantification of myocardial ischemic regions and understand its potential applications in forensic and clinical settings.

Impact Statement: This presentation introduces an innovative method to assess myocardial ischemia using PMCTA combined with the Voronoi algorithm, which will significantly impact forensic science. This non-invasive approach enhances the accuracy of diagnosing coronary artery conditions postmortem and has potential for broader clinical applications. By sharing this technique, the presentation aims to inspire further research and contribute to advancements in forensic medicine and clinical diagnostics.

Abstract Text: PMCTA is widely recognized in forensic medicine for its effectiveness in diagnosing hemorrhages in trauma cases, owing to its detailed examination of the vascular system.[1] Selective coronary PMCTA is particularly useful for assessing the degree of coronary artery stenosis and occlusion, and it can reveal characteristic changes associated with anomalies, such as anomalous aortic origins of coronary arteries and hypertrophic obstructive cardiomyopathy.[2,3] This case report demonstrates the use of the Voronoi algorithm to assess myocardial ischemia using coronary PMCTA. A male in his 70s was found unconscious in a car after colliding with a traffic light pole. Despite medical interventions, including pericardial drainage and cardiopulmonary resuscitation, the patient died two hours later. PMCTA revealed significant filling defects in the Left Anterior Descending artery (LAD), consistent with plaque rupture and narrowing observed during autopsy. The cause of death in this case was likely cardiac tamponade due to cardiac rupture secondary to myocardial infarction resulting from LAD stenosis. Cardiac perfusion areas were analyzed using the Voronoi algorithm, demonstrating a total myocardial volume of 151.9mL in the left ventricle. Perfusion volumes were calculated as 92.9mL (61.2%) for the LAD, 34.2mL (22.5%) for the left circumflex artery, and 24.9mL (16.4%) for the right coronary artery. The predicted ischemic volume distal to the LAD stenosis was estimated to be 49.8mL (32.8%). Furthermore, the ischemic areas observed during autopsy macroscopically corresponded well with the predicted ischemic regions.

This case highlights that combining PMCTA with the Voronoi algorithm provides an accurate method for assessing myocardial ischemic areas, offering a non-invasive approach to visualize and quantify perfusion and ischemic regions. Future prospects for this method include its potential utility in accurately estimating perfusion territories and ischemic regions in cases involving anomalous coronary artery origin or course. Accumulating additional case studies would be beneficial for further validation and for enhancing broader clinical applicability.

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Computed Tomography; 3D Technology; Forensic Imaging

I15 Broken Heart Syndrome: Can an Argument Kill?

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Learning Objectives: The aim of this study is to emphasize the crucial role of a comprehensive and precise autopsy examination as well as the evaluation of circumstantial data, for an accurate postmortem diagnosis in cases of sudden death.

Impact Statement: This presentation impacts the forensic community by elucidating the diagnostic challenges and medicolegal methods applied in postmortem examinations in cases of sudden cardiac death following intense emotional or physical stress. By highlighting the critical histological features and the importance of thorough medical history evaluations, this work aims to enhance the accuracy of Takotsubo Syndrome (TTS) diagnoses. Additionally, it provides forensic data that can inform the development of improved diagnostic protocols and preventive measures, ultimately contributing to better management and understanding of this condition in both clinical and forensic contexts

Abstract Text: TTS, also known as stress cardiomyopathy, neurocardiogenic injury, or broken heart syndrome, is a rare condition characterized by temporary left ventricular dysfunction resulting in acute heart failure, without significant obstruction of the coronary arteries.[1-3] This condition carries a significant risk of adverse events. Although generally benign, TTS can have unfavorable outcomes, with a mortality risk of up to 17% due to acute hemodynamic instability and consequent arrhythmic complications. The exact cause of this condition is not yet fully understood, but it is believed that the activation of the sympathetic system and the consequent release of catecholamines, triggered by intense physical or emotional events (such as the loss of a loved one, situations of domestic violence, or significant emotional stress), may play a significant role in its pathogenesis.[4-6] Previous studies have suggested that TTS is often preceded by triggering stress factors.

In cases of sudden cardiac death, it is advisable to conduct a thorough medical history examination and a precise evaluation of autopsy results, as well as postmortem toxicological and/or genetic analyses, to exclude other possible causes and reach a diagnosis of this syndrome. Diagnosing TTS during an autopsy is complex as it usually lacks clear macro or microscopic signs. The histologic appearance of neurogenic cardiomyopathy is non-specific; however, recent literature reviews have highlighted some recurrent, though not pathognomonic, microscopic features of TTS: (1) the presence of Contraction Band Necrosis (CBN); and (2) inflammatory alterations (e.g., interstitial infiltrates of mononuclear lymphocytes and macrophages), typically in absence of the typical findings of acute myocardial infarction.[2] In the initial phase of neurocardiogenic injury, diffuse hypereosinophilic myocytes, often accompanied by CBN, are frequently observed and are surrounded by leukocytes, evolving into coagulative myocytolysis/CBN. This process develops rapidly. In the acute stage, it can be confused with viral myocarditis, which, however, does not display hypereosinophilic myocytes or CBN.[2,4-8]

The discussed case involves the death of a 66-year-old woman. According to information provided by the Judicial Police, the woman, who had hypertension and hypercholesterolemia, was involved in a heated argument with her daughter, both verbal and physical, one evening in October 2022, during which she fell violently to the ground. As a result of the fall, she was taken to the emergency room, where health care providers noted minor contusions on her lower limbs and discharged her with a two-day prognosis. The next morning, the woman's body was found lifeless and lying on the bed in her home. Five days later, an autopsy was performed to determine the cause of death. The external and autopsy examination ruled out any traumatic injuries on the woman's body and organs. Histological examinations of the heart revealed the presence of perivascular and interstitial fibrosis, myofiber breakup, waviness, interfascicular edema, foci of interstitial lymphocyte infiltrates, and CBN; as already highlighted and supported by the literature, the latter is a characteristic marker of cardiac damage due to sudden sympathetic stimulation, leading to a catecholamine surge. Samples taken from the coronary arteries showed the presence of intimal-medial hyperplasia and patent lumen. Toxicological investigations ruled out the presence of ethyl alcohol, benzodiazepines, and psychotropic substances in the blood. These findings, and especially the presence of CBN, allowed for the classification of the death as sudden cardiac death, attributable to an arrhythmic event caused by acute hemodynamic instability, preceded by an intense stressful event represented by the violent family argument.

In conclusion, diagnosing this condition is challenging for doctors and pathologists as it requires identifying distinctive signs that can differentiate it from other cardiac pathologies. To overcome the current challenges, further studies are needed to identify valid practical approaches in the postmortem diagnosis of TTS.

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Sudden Death; Postmortem Diagnosis; Takotsubo Syndrome

I16 The Impact of a Cardiac Surgery Scar on Heart Rupture Following a Fall From Height: A Case Report

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Learning Objectives: Attendees will be able to identify key injury patterns associated with high falls, understand the role of post-surgical scarring in exacerbating cardiac injuries, and evaluate the significance of toxicological findings in determining the circumstances surrounding a fall. These objectives not only help attendees gauge the relevance of the material but also offer a clear framework to assess the educational value of the session. This structured approach ensures focused content delivery and measurable learning success.

Impact Statement: This presentation will impact the forensic science community by offering new insights into the complex injury patterns associated with fatal falls, particularly in cases involving individuals with previous cardiac surgery. The detailed analysis of this unique case—highlighting the role of fibrotic cardiac adhesions in exacerbating blunt trauma injuries—provides valuable information for forensic pathologists when interpreting similar fall cases. Additionally, the discussion of how intoxication contributed to the fatal outcome underscores the importance of comprehensive toxicological analysis. By enhancing the understanding of post-surgical cardiac vulnerabilities in trauma, this case can inform future forensic investigations and autopsy protocols.

Abstract Text: **Introduction:** Falls from heights represent a significant cause of injuries, disabilities, and deaths worldwide, primarily due to suicides, workplace accidents, or substance abuse effects, and are rarely associated with homicidal dynamics. According to the 2021 World Health Organization (WHO) statistics, falls rank as the second-leading cause of unintentional death on a global scale (following traffic injuries), with approximately 684,000 fatal falls occurring annually.[1] Falls can be classified as pure, if the injuries are due to ground impact, or mixed, if the body hits obstacles during the fall. Injuries vary based on the height, point of impact, and the surface struck. Severe internal injuries, such as multiple bone fractures and organ ruptures, are common. Blunt cardiac injuries, with an incidence between 5% and 50% in fall cases, can be fatal.[2] Studies show that cardiac injuries from falls over 6 meters are significant, with greater severity in falls over 15 meters.[3,4]

Case Report: We present a case of death from a 13-meter fall of a 29-year-old man who had undergone cardiac surgery several years earlier to correct a transposition of the great vessels. Surveillance cameras recording the area showed the man, in an advanced state of intoxication, autonomously approaching the fall point.

The autopsy (along with liver lacerations, lung contusions, multiple rib fractures and a left hemothorax of 2,000mL) revealed extensive cardiac rupture, with avulsion of the right anterior wall of the heart. The heart laceration showed dark red margins and involved the whole length of the organ, originating from the lateral edge of the right atrium, extending towards the adjacent ventricle and the cardiac apex, and then ascending along the interventricular septum towards the aortic ostium. The well-healed surgical scar suggested that fibrotic adhesion of the heart to the pericardium contributed to the severity of the injury. As a confirm, microscopic examination was conducted with traditional Hematoxylin and Eosin (H&E) staining and revealed marked thickening of the pericardium with strong adhesions to the underlying epicardium, the overlying parasternal intercostal striated muscle, and retrosternal fat. In addition, both myocardial and pericardial tissue samples showed extensive interstitial and perivascular hemorrhages. Finally, toxicological investigations on peripheral blood confirmed the cause of the psychomotor impairment observed shortly before the fall (blood alcohol level >2.58g/L).

Discussion: Blunt cardiac rupture usually occurs due to vertical deceleration and compression between the sternum and the spine, with a higher likelihood of injuries to the right heart.[5] Our case shows a peculiar and atypical pattern of injury, characterized by avulsion of the atrial and ventricular walls of the heart. It may be explained as a stretching mechanism, caused by the traction exerted from the retrosternal adhesions during the expansion phase of the chest cage, following blunt traumatic cardiac rupture due to impact with the ground.

In conclusion, the severity and the peculiar features of the injuries in our case require further studies to better understand the impact of post-surgical scarring processes on injuries resulting from falls.

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Heart Rupture; Fall From Height; Cardiac Surgery

I17 The Medicolegal Implications of Atrial Fibrillation Ablation Complications and Their Management

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Learning Objectives: The aim of this presentation is to compare fatal and non-fatal cases with the existing literature, to clarify critical moments in the procedure and the management of complications. We find that the key factors leading to complications included procedural errors, delays in recognizing and addressing complications, and inadequate post-operative monitoring.

Impact Statement: This presentation aims to drive significant advancements in forensic science by introducing innovative techniques, promoting interdisciplinary collaboration, and addressing ethical and legal challenges. These improvements will not only enhance the quality and efficiency of forensic investigations but also ensure that the forensic science community remains a crucial pillar in the pursuit of justice.

Abstract Text: Atrial and ventricular ablation, predominantly performed using radiofrequency energy, has become a primary treatment for Atrial Fibrillation (AF) and ventricular arrhythmia, particularly due to the limitations of antiarrhythmic drugs.[1] This procedure involves inserting catheters into the right atrium via the femoral vein, guided by fluoroscopy, intracardiac echocardiography, and various mapping systems.[1] The objective is to create transmural heat injury to cardiac tissue, leading to scar formation that isolates abnormal electrical impulses. Although cardiac ablation offers therapeutic benefits, it is associated with significant complications (1%-5.3%) and an in-hospital mortality rate of approximately 0.03%-1.48%.[2] Complications from radiofrequency ablation can be immediate, such as pulmonary artery perforation and cardiac tamponade, or they can develop later, such as esophageal fistula.[2-4] The detection and confirmation of these complications, as well as the evaluation of procedural correctness, often rely on autopsy findings.[5] According to Bindu Challa et al., employing Letulle techniques during autopsies can help examine and preserve the relationships between organs to various extents.[6] These autopsy techniques are essential for understanding the extent and nature of complications resulting from AF ablation procedures.[7] Given the severity and potentially fatal outcomes of these complications, the medicolegal implications are substantial. Proper documentation, timely diagnosis, and effective management of complications are crucial to mitigating legal risks and enhancing patient safety. The need for meticulous procedural protocols and diligent post-operative care cannot be overstated, as failing to address complications can lead to serious legal consequences for medical practitioners and institutions.[8]

We present seven forensic cases selected from our case series over the past five years, involving complications such as atrial and pulmonary artery perforation, hemopericardium, coronary dissection, liver laceration, and hemoperitoneum. These cases highlight specific aspects of complication management that have undergone medicolegal evaluation. By comparing our fatal and non-fatal cases with the existing literature, we confirm the importance of continuing to follow the guidelines on the subject. In the analysis of our case series, we demonstrate that the key factors leading to fatal complications include not only procedural complications but, above all, delays in post-operative recognition and failure to manage them appropriately.[9] In cases where the complication was recognized or intraoperative error occurred immediately, patient harm was reduced. In two cases where recognition of the complication occurred more than 30 minutes later and no ultrasound guidance was used, severe or fatal injuries occurred. Our results support the findings in the literature and emphasize the importance of adhering to the guidelines on this topic to avoid fatal errors. To mitigate these risks, it is essential for physicians to strictly follow procedural guidelines and maintain a high index of suspicion for potential complications. By understanding and addressing the factors that still contribute to these complications, clinicians can improve patient safety and reduce legal risks.

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Atrial Fibrillation; Ablation Procedure; Cardiac Surgery

I18 A Case of Suicide by Sodium Nitrite and Dimethyl Sulfoxide Ingestion

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Learning Objectives: This presentation will inform attendees of the uses of sodium nitrite and Dimethyl Sulfoxide (DMSO), the mechanism of sodium nitrite intoxication, and its increasing use in suicide.

Impact Statement: This presentation will impact the forensic science community by raising awareness of the increasing rate of sodium nitrite intoxication as a cause of suicidal death and the associated autopsy and investigative findings.

Abstract Text: Sodium nitrite is a yellow-white, odorless, water-soluble crystalline powder. It is commonly used as a food preservative and prescribed as an antidote to cyanide poisoning. When acutely ingested in large amounts, sodium nitrite induces methemoglobinemia, hypoxia, hypotension, arrhythmias and can lead to death unless treated with methylene blue.[1,2] Easily accessible online, sodium nitrite has been recommended on online forums as an effective method of suicide.[3] Poison control centers in the United States reported that fatalities from sodium nitrite intoxication increased 166 percent in 2021 compared to 2018.[4]

DMSO is a clear, odorless solvent used primarily in chemical synthesis and pharmaceutical manufacturing. Due to its chemical properties, DMSO dissolves both polar and non-polar molecules. DMSO also penetrates cell membranes without damaging them and is used to increase penetrance of poorly soluble topical drugs. At low concentrations, DMSO exhibits analgesic, vasodilation, anti-coagulation, and anti-inflammatory properties. Some reported side effects are headaches, skin irritation, and itching. The toxicity of DMSO alone is thought to be low. However, due to the penetrance of DMSO, it may increase the effects of co-administered substances.[5]

Described here is a sudden death of a 28-year-old man with history of an eating disorder. He was found supine on the bathroom floor of a hotel room with apparent vomitus around the mouth. Nearby, there were containers of sodium nitrite and DMSO and a measuring cup filled with a thick clear substance and a spoon. The decedent's computer found at the scene showed a recent internet search of "How to kill yourself with Dimethyl Sulfoxide?" The investigative findings were consistent with deliberate and self-inflicted ingestion of sodium nitrite dissolved in DMSO.

Autopsy examination revealed signs of methemoglobinemia. There was blue-grey cyanosis of the face, neck, lips, and fingernail beds. The thoracic and abdominal viscera and blood were red-purple discolored. Marked pulmonary edema was present. The stomach contained approximately 30ml of light tan-red liquid. Postmortem blood toxicology revealed elevated concentrations of methemoglobin (42%) and dimethyl sulfoxide (36mg/dl). Death was attributed to toxic effects of sodium nitrite and DMSO. Manner of death was suicide.

This case highlights the investigative and autopsy findings of a suicidal death by sodium nitrite and DMSO e ingestion, which is an increasing method of suicide in the United States.

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Suicide; Autopsy; Nitrate

I19 Fatal Occupational Exposure to Hydrogen Sulfide: A Case Report Emphasizing the Need for Differential Diagnosis

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Learning Objectives: This presentation will cover: (1) Identification of Hydrogen Sulfide Risks: Recognize hydrogen sulfide as a significant hazard in occupational settings, particularly in confined spaces like wastewater treatment pits; (2) Understanding Toxic Effects: Learn about the toxic effects of hydrogen sulfide, including its symptoms and physical manifestations observed in autopsy findings and toxicological analysis; (3) Importance of Accurate Measurement: understand the significance of precise and timely measurement of hazardous gas levels and how discrepancies between scene and blood measurements can impact the interpretation of exposure; and (4) Differential Diagnosis: Emphasize the importance of considering multiple hazardous substances and maintaining a high level of suspicion in diagnosing toxic exposure cases.

Impact Statement: This presentation will: (1) Raise Awareness: Highlights hydrogen sulfide risks, prompting forensic professionals to consider this hazard in similar cases; (2) Promote Accuracy: Emphasizes the importance of precise gas measurement and interpretation, improving forensic practices; (3) Enhance Diagnosis: Stresses the need for differential diagnosis when multiple hazardous substances are involved, leading to more accurate determinations; (4) Advocate for Safety: Encourages better safety protocols in industrial environments to prevent similar fatalities; and (5) Provide Educational Value: Serves as a real-world example for training and case discussions, illustrating the complexities of toxic exposure.

Abstract Text: Introduction: Hydrogen sulfide (H_2S) is a colorless gas with a higher density than oxygen.[1] It has a strong odor of rotten eggs and is a naturally occurring product of decaying organic matter in anaerobic environments, geothermal and volcanic processes, crude oil and natural gas, and endogenous enzymatic and non-enzymatic processes in mammals.[2,3] Occupational exposure to H_2S primarily occurs in environments involving petroleum, natural gas, soil, sewer gas, and various industrial chemical reactions.[1]

Case Report: We present the case of a 34-year-old male technician at a water plant in El Paso, TX, who was found unconscious at the bottom of a wastewater treatment pit while attempting to drain it. A few minutes after opening a valve to allow incoming water to reach the drainage pump, he was discovered unresponsive, face down, floating in the water by a coworker. Emergency services were called promptly, but despite the swift arrival of rescue teams and resuscitative efforts, he was declared dead at the scene.

Levels of hydrogen cyanide recorded at the tank were 56ppm, exceeding the Immediately Dangerous to Life or Health concentration (IDLH) of 50ppm established by the National Institute for Occupational Safety and Health (NIOSH).[4] Hydrogen sulfide levels were recorded at 18ppm (IDLH 100ppm).[2,5] It is noteworthy that these measurements were taken after several hours of tank ventilation. Autopsy findings from the El Paso County Office of the Medical Examiner revealed fixed red livor, facial abrasions, and pulmonary parenchyma with moderate to marked amounts of blood and frothy fluid. Toxicology analysis identified thiosulfate levels of 14µg/mL in femoral blood (normal levels 0.3µg/mL).[6] Cyanide levels were below reference values. The cause of death was certified as toxic effects of hydrogen sulfide with possible drowning, and the manner of death was certified as accidental.

Discussion: Hydrogen sulfide is one of the leading causes of workplace gas inhalation deaths in the United States, second only to carbon monoxide, with the vast majority of these incidents occurring in confined spaces.[7] Although hydrogen cyanide levels exceeded the IDLH threshold, blood tests were negative for cyanide and positive for hydrogen sulfide. Conversely, while hydrogen sulfide levels recorded at the scene were below the IDLH threshold, they were still high in femoral blood analysis, suggesting that exposure levels at the time of the incident could have been higher. Overexposure to hydrogen sulfide can cause several central nervous system symptoms, such as dizziness, nausea, headache, and abrupt physical collapse, which could have contributed to drowning.[1] This case underscores the importance of maintaining a high level of suspicion when multiple hazardous substances are involved and highlights the need for comprehensive safety protocols that address various dangerous compounds in industrial environments.

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Hydrogen Sulfide; Occupational Incidents; Diagnosis

I20 Synthetic Cannabinoids and Sudden Death

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Learning Objectives: After attending this presentation, attendees will have a better understanding of synthetic cannabinoids and how they can cause or contribute to death.

Impact Statement: This presentation will impact the forensic science community by illustrating cases in which synthetic cannabinoids caused or contributed to non-traumatic death, including cases where a pre-existing medical condition was exacerbated by synthetic cannabinoid use. Identifying potential risk factors for adverse effects may lead to public health strategies designed to reduce mortality in affected populations.

Abstract Text: Synthetic cannabinoids, commonly referred to as "Spice" or "K2," emerged as early as 2004 on the internet and other retail outlets. They are marketed as herbal incense and upon smoking the substance, produce similar effects to cannabis. While synthetic cannabinoids have nearly identical chemical structures to Tetrahydrocannabinol (THC), the active component of marijuana, they are believed to have a higher affinity for the CB_1 and CB_2 receptors. Synthetic cannabinoids may be used as an alternative to cannabis due to their lower price, increased accessibility, and difficulty of detection on routine urine drug screens.

Synthetic cannabinoid short-term side effects may include psychotic symptoms, cognitive impairments, tachycardia, cough, seizures, hyperthermia, and acute kidney injury.[1] Longer-term effects include an increased risk of developing psychotic, anxiety and mood disorders, cardiovascular disease, and pulmonary complications.[1]

We present two cases in which synthetic cannabinoids caused or contributed to death: (1) XLR-11 [1-(5-fluoropentyl)-1H-indol-3-yl](2,2,3,3-tetramethylcyclopropyl)methanone; and (2) 5F-AB-PFUPPYCA N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-3-(4-fluorophenyl)-1H-pyrazole-5-carboxamide.

Case 1: A 19-year-old male with a history of asthma and synthetic marijuana abuse was found incapacitated in his residence after experiencing an asthmatic attack. Emergency medical personnel were called, but resuscitative efforts failed. The patient was known to be using synthetic marijuana almost daily for the past three years and had been intubated for respiratory failure four years before his death due to exacerbations of his asthma. Microscopic examination revealed patchy interstitial pneumonitis and the characteristic changes associated with acute and chronic asthmatic bronchitis. The decedent's postmortem blood tested positive for XLR-11 with a level of 0.21ng/mL. XLR-11 is a fluorinated synthetic cannabinoid, and it is known that fluorides can induce inflammation within the lungs.[2] With a history of long-term, significant XLR-11 abuse, the XLR-11 exposure was deemed to be contributory to death, which was primarily ascribed to acute and chronic asthmatic bronchitis.

Case 2: A 60-year-old male was found suffering from apparent heatstroke outdoors. Emergency Medical Services (EMS) recorded an elevated body temperature of 105.1°F, started cooling protocols, and began to transport him to an Emergency Department (ED), but he was arrested while en route. All subsequent resuscitative efforts were to no avail. The outside temperature was elevated at an average temperature of 91.3°F and a high of 101°F. At autopsy, external findings revealed small abrasions and contusions on the legs. Internal findings were unremarkable. Initial toxicology analysis revealed no positive findings; however, further analysis revealed 5F-AB-PFUPPY-CA in the decedent's blood and urine. Medical literature has previously reported hyperthermia associated with synthetic cannabinoid use.[3,4] Thus, the cause of death was ruled as "complications of hyperthermia exacerbated by synthetic cannabinoid use."

Synthetic cannabinoid use may cause or contribute to death in a variety of ways. These cases elucidate the potential dangers of both acute and chronic synthetic cannabinoid use and highlight the importance of considering synthetic cannabinoids in cases where symptoms and side effects align, and initial toxicology testing returns negative.

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Synthetic Marijuana; Synthetic Cannabinoids; Psychoactive Substances

I21 Heat Shock Proteins (HSPs) Expression in Cocaine-Related Deaths: A Systematic Review

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the involvement of HSPs in cocaine abuse-related deaths.

Impact Statement: This presentation will have an impact on the forensic science community by providing promising avenues for the use of HSP levels as a valuable tool in cocaine-related deaths, leading to more precise diagnoses and ultimately lowering the chances of misdiagnoses.

Abstract Text: Investigating deaths that occurred without external trauma can be a difficult task. In such cases, it is imperative to consider all possible scenarios. Asphyxia and various forms of intoxication should be taken into account, including acute and chronic cocaine abuse.[1] HSPs are naturally occurring proteins produced following exposure to harmful stressors in different organs.[2] Psychostimulants, including cocaine, have been shown to alter brain HSP levels similarly to other stressors.[3] Specifically, cocaine induces changes in the neuroendocrine system of the brain, affecting HSP expression; however, there is still limited information on the specific levels of each HSP involved.[4] This systematic review aims to comprehensively collect and analyze existing literature data regarding the relationship between HSPs and cocaine abuse to investigate whether HSPs can be utilized as forensic markers for accurately diagnosing cocaine-related deaths.

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline, the Boolean search methodology was used to search the PubMed and Scopus databases to find relevant studies that have been published between January 1, 1992, and April 1, 2024, using the keywords "heat shock protein" and "cocaine" combined with the Boolean operator "AND." From this first research were excluded articles "not in English" and "not full text." Afterward, in the screening phase, based on the abstract reading, only studies concerning HSP involvement in cocaine administration/assumption were assessed for eligibility (ten articles).

The data gathered pertained to both human and murine species. The majority of the analyzed articles revealed an elevation in HSP25, HSP27, HSP60, HSP70, HSP72, and HSP73 levels in the brain, cerebellum, heart, liver, and kidney, thus indicating cocaine-induced stress in such organs.

The detailed understanding of HSP responses to cocaine exposure presents promising avenues for forensic medicine. One potential hypothesis is that the levels and distribution of specific HSPs, such as HSP27 and HSP70, could serve as biomarkers for determining the extent and type of cellular stress involved in cocaine use. In addition, in the liver, cocaine exposure leads to selective induction of HSP25 and HSP70i, particularly in cells with metabolite binding capacity. In conclusion, in cases where cocaine use is only suspected, the presence of elevated HSP levels in the brain, cerebellum, heart, liver, and kidney could provide supporting evidence of cocaine-induced stress, even if the drug is not directly detectable in the bloodstream. This could be particularly useful in scenarios involving delayed death following cocaine use where traditional toxicology screens might fail to detect the drug or in cases of withdrawal.

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Autopsy; Cocaine; HSP

I22 Accidental Vehicular Carbon Monoxide Fatalities: A Ten-Year Review

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Learning Objectives: After attending this presentation, attendees will better understand the complexities of investigating deaths and determining the cause and manner of death resulting from accidental Carbon Monoxide (CO) toxicity associated with vehicles.

Impact Statement: This presentation will impact the forensic science community by providing insight into aspects of investigation and pathology of CO deaths. This will also impact public safety by bringing awareness of uncommon situations that can be hazardous.

Abstract Text: CO is a toxic, colorless, odorless gas that forms due to the incomplete combustion of carbon-containing fuels. The gas can be formed from motor vehicles, small engines, stoves, lanterns, grills, fireplaces, gas ranges, or furnaces. Death from CO poisoning is usually associated with acute high-level exposure that causes asphyxia due to the preferential saturation of hemoglobin by CO compared to oxygen. Accidental CO poisoning has been recognized as a cause of death for centuries.[1] According to the Center for Disease Control, during 2010–2015, a total of 2,244 deaths resulted from unintentional CO poisoning in the United States, with the highest numbers of deaths each year occurring in the winter months.[2] It is not uncommon to see intentional deaths where the source of CO is vehicle exhaust fumes in an enclosed space. However, unintentional deaths resulting from vehicle exhaust fumes is unusual and could be missed without thorough scene investigation and pathological examination of the decedent.

For investigators approaching a scene for a death in a vehicle, some of the questions that should be answered include whether the keys are in the ignition, if the vehicle can be started, the gas level of the vehicle, whether the doors and windows were open or closed, if there was damage to the vehicle, and how the decedent was positioned in the vehicle.

The electronic database of the Harris County Institute of Forensic Sciences, Houston, TX, was searched for all accidental carbon monoxide fatalities between 2013 and 2023, excluding those related to a collision with resultant vehicular fire. This resulted in 24 cases. The manner of death in all cases was Accident. The cause of death was CO toxicity in all cases, with other factors in part one of the death certificate in seven cases: combined with illicit or prescription drugs, or ethanol (n=6); or cardiovascular disease (n=1).

Three deaths were related to a storm: two companion deaths occurred during Winter Storm Uri (2021) due to one of the decedent's charging their phone in a running vehicle in the enclosed garage attached to the residence; and one death during the 2016 Tax Day Flood due to the vehicle exhaust being submerged in flood waters causing buildup of CO in the vehicle. Three cases were related to defective components of vehicles, including one with a missing catalytic converter. Eight cases occurred where the vehicle was in an outdoor environment, such as a driveway, and 16 occurred in an enclosed space, such as a garage. One death occurred due to the lighting of charcoal within the vehicle as a heat source.

Autopsy examinations showed a discernible bright cherry-red discoloration of the skin and viscera in all but four cases. One decedent had decompositional changes; and one had a survival interval of 13 hours. The carboxyhemoglobin level of postmortem blood samples in scene deaths ranged from 42% to greater than 60% saturation, except in the decomposed body, which was 21%. Illicit drugs detected included cocaine, methamphetamine, and fentanyl.

It is critical when a death occurs inside a vehicle, whether indoors or outdoors, that CO toxicity is a consideration. Before a vehicle is released back to a family member, consideration should be given for previously unknown malfunctions (for example, a small leak in the exhaust system can lead to a buildup of CO inside the vehicle). The external appearance of the body with cherry-red discoloration may prompt postmortem toxicological testing for carboxyhemoglobin.

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Carbon Monoxide; Motor Vehicle; Scene Investigation

I23 A Survival Analysis Based on a Forensic Investigation of Motorcycle Road Traffic Accidents in the Athens Metropolitan Area During 2021–2023

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Learning Objectives: After this presentation, the attendees will be better acquainted with the characteristics of motorcycle-related incidents and with factors affecting the survival of victims.

Impact Statement: This presentation will impact the forensic science community by presenting data relevant to motorcycle accident-related deaths.

Abstract Text: **Introduction:** Road Traffic Accidents (RTA) are among the top five causes of all-cause mortality globally.[1] Motorcycle accidents, specifically, have grown to become a significant problem worldwide.[2] Various protective measures, mainly the use of a helmet, have been proposed to reduce fatal outcomes of motorcycle accidents.[3] Most victims are described to be mostly males of a young age, some of lower socio-economic status.[4-8] Cranial and spinal injuries are often described as common injuries in motorcycle-related accidents.[9,10] Thoracic injuries and lower extremity fractures are also frequently described.[11-15] Alcohol use before the accident has vastly been described as a major predisposing factor.[16]

Materials and Methods: Cases of motorcycle-related RTAs, examined during 2021-2023, were included in the study sample (94 cases in total). Data was collected from the database of the Department of Forensic Medicine and Toxicology (DFMT), of the School of Medicine, NKUA. The DFMT jurisdiction area comprises the southeast, the northeast Attica region, and Evoia island, in which resides approximately 10% of the entire Greek population.

Anonymized demographics, medical and social history information, place of accident, distance to the hospital, time required to arrive at the hospital were collected. Both external and internal injuries observed during the postmortem examination were grouped in anatomical regions and were recorded. Toxicological analysis results were collected as well. A retrospective cohort study was conducted to analyze survival data, with survival time measured in days until death.

The primary endpoint was survival time, calculated as the number of days from the study's initiation to the date of death. Kaplan-Meier survival curves were generated to estimate the survival function for the cohort and were stratified by relevant categorical variables, allowing visualization of survival probabilities over time. For all variables, a univariate Cox proportional hazards regression model was used to evaluate the impact of each variable on survival time. Hazard Ratios (HR) with 95% Confidence Intervals (CI) were reported to quantify the relationship between continuous variables and the risk of death. Statistical analyses were performed using Stata (version 16). A p-value of less than 0.05 was considered statistically significant.

Results: The analysis revealed several critical findings. Cervical injuries were associated with the worse outcomes, with a median survival time of 0.066 days compared to 0.081 days for those without, and an HR of 1.62 (95% CI: 1.04-2.67, p = 0.047). Thoracic injuries significantly reduced survival time to 0.07 days from 5.85 days, with an HR of 2.15 (95% CI: 1.3-3.56, p = 0.003). Abdominal injuries also showed a significant effect, with a median survival time of 0.068 days versus 0.079 days for those without, and an HR of 1.79 (95% CI: 1.16-2.77, p = 0.009). Additionally, multiple site injuries were associated with a median survival time of 0.066 days compared to 0.2 days for single-site or double-site injuries, with an HR of 1.94 (95% CI: 1.24-3.02, p = 0.004). Positive toxicological screening for substances affecting the Central Nervous System (CNS) demonstrated a significant impact on survival, with positive cases having a median survival time of 0.055 days versus 0.073 days for negative cases, and an HR of 1.85 (95% CI: 1.12-3.05, p = 0.017). Alcohol presence in toxicology significantly impacted survival, with those testing positive having a median survival time of 0.05 days versus 0.07 days for those negative, and an HR of 1.74 (95% CI: 1.12-3.11, p = 0.041).

Conclusions: Multiple site injuries, cervical, thoracic and abdominal injuries appear to significantly affect the survival of victims. Alcohol and other CNS-affecting substances positive toxicological screening also appear to heavily impact the survival.

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Accident; Motorcycle; Autopsy

I24 The Forensic Utility of Botany in Railway Accident Cases

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Learning Objectives: After attending this presentation, attendees will understand the importance of forensic botany in railway accident cases.

Impact Statement: This presentation will impact the forensic science community by demonstrating how forensic botany is fundamental in a multidisciplinary evaluation for the resolution of these particular forensic cases.

Abstract Text: **Introduction:** Forensic botany is a heterogeneous discipline that encompasses many aspects of plant sciences, particularly taxonomy, field botany, anatomy, and ecology. Internationally, there is a significant opportunity to expand the application of forensic botany in criminal investigations. In civil proceedings, forensic botany may, for example, be called into question in commercial disputes such as accidental contamination of goods. Despite the potential, there are barriers to the broader application of forensic botany in criminal cases; there is a widespread need to improve the efficiency of botanical trace identification. This could be addressed in part by embracing innovations in image recognition and accessing the huge number of specimens and images housed in natural history collections around the world. Furthermore, recent advances in DNA sequencing technologies, and the expansion of environmental DNA (eDNA) and forensic ecogenomics, offer opportunities to more rapidly provide species-level identifications.[1]

The case: The case we report concerns a railway accident in which a boy was sitting on the ground near the train tracks and was run over. The victim was found at a point very distant from the point of impact and the body was dismembered into several parts with the head completely decapitated from the spine. During the investigations, the point of impact and therefore possible liability of the railway company for failure to control was not understood. An on-site investigation of the scene was carried out with forensic botanical analysis and external and internal examination of the body. Each botanical element on the victim was compared to the elements highlighted at the scene.

Discussion: Forensic botany undoubtedly represents an important source of useful and valuable information in the investigation of a variety of different forensic scenarios. This discipline can offer great potential in discovering the location of the victim, the duration of his stay in a specific environment, and the sequence of events that may have taken place in a given scene. Forensic botany, however, can actually assist the forensic pathologist or investigators only if the collection of botanical evidence has been carried out by forensic experts or on-site personnel who have at least a minimal amount of training in implementing sampling protocols, accurate recording techniques, and in the collection of environmental data regarding a specific discovery location.[2]

In the reported case, among the elements of forensic interest, various botanical elements were found such as shrubs and small leaves which were collected and analyzed macroscopically and microscopically. From the analysis of the latter, it emerged that they were compatible with those taken from a specific point on the tracks adjacent to an unfenced area. The analysis of that point revealed other biological traces belonging to the victim that located the victim in that point at the time of impact, allowing the case to be resolved and allowing the railway company to be defined as not being responsible.

Only if these preliminary on-site steps are carried out correctly can the collection, identification, and classification of botanical evidence be presented to an investigative body or in court. The discipline of forensic botany should be considered in any scenario that may potentially present botanical evidence. The information that can be provided, even if sometimes only circumstantial, can certainly shed light on many of the classic investigative questions. It is a fact that to this day, botanical evidence has been increasingly presented during court proceedings and has become a widely debated topic. For this reason, awareness of the potential that this discipline can provide in a wide variety of scenarios must increase among representatives of the forensic scientific community.

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Forensic Science; Botany; Railway

I25 A Differential Diagnosis Between Acute Mechanical Asphyxia and Fatal Arrhythmia During Law Enforcement Restraint

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Learning Objectives: After attending this presentation, participants will understand key points of the differential diagnosis between the acute mechanical asphyxia and fatal arrhythmia during law enforcement arrests, with a particular focus on the available forensic methodologies.

Impact Statement: This presentation will have an impact on the forensic community by stimulating debate on the controversial use of the term "excited delirium" and the possible pathophysiological mechanisms underlying deaths that have occurred during restraint by law enforcement.

Abstract Text: A 31-year-old man entered a store in a clear state of psychophysical agitation and attempted to purchase alcoholic beverages. The store owner, noting the conditions of the man and suspecting the banknote was counterfeit, refused to sell the beverages. This decision caused the man to become increasingly agitated and threatening, prompting the store owner to alert the police. The responding officers attempted to identify the man, who resisted violently, running and struggling. Additional officers and medical personnel were called to help immobilize and calm the individual. During the immobilization, with the man in a prone position and an officer kneeling on him without exerting pressure on the torso, the subject began experiencing dyspnea. Immediate resuscitation efforts by the medical personnel present were unsuccessful, and the man was declared dead. Postmortem findings revealed only epicardial petechiae. Histology showed signs of myofiber break-up, myocardial fiber stretching, nuclear elongation, and contraction band necrosis. Toxicology tests were positive for cocaine, benzoylecgonine, ethanol, and cocaethylene. Immunohistochemical methods, such as the HIF-1 alpha factor, were employed to rule out mechanical asphyxia and were negative on pulmonary histological preparations.

After a complex court proceeding, the forensic pathologist appointed as expert of the judge, concluded that the death was not due to acute mechanical asphyxia but to a malignant arrhythmia induced by psychomotor agitation from cocaine and alcohol in a state of psychophysical agitation.

This case demonstrates the necessity of integrating multiple data points through a multidisciplinary approach, as different pathophysiological mechanisms may be involved in deaths under similar circumstances. In fact, the term "Excited Delirium" is currently being intensely debated in the scientific community.[1,2] In the past, many deaths that occurred immediately after or during periods of restraint by law enforcement were attributed to this pathological entity, which has never been accepted by the psychiatric community. Today, many American societies and states have taken a stand on this issue by rejecting the use of terms like "Excited Delirium" in such deaths. For instance, the National Association of Medical Examiners (NAME) stated in 2023 that terms like "Excited Delirium" are not endorsed or recognized by classifications such as the World Health Organization (WHO), *International Classification of Disease, 10th edition, Clinical Modification* (*ICD-10*), and *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*), advocating instead for identifying the underlying cause of death.[3] Nevertheless, despite the strong and definitive stance of the American scientific community, a residual debate regarding this type of death persists globally.[4,5] Some authors have proposed a change in terminology, suggesting the term "hyperactive delirium" to shift the focus of the controversy from the underlying pathophysiology to the language used to describe it.[6] Therefore, it is incumbent upon the global scientific community in forensic pathology to take further steps to discuss and raise awareness of this issue, encouraging the use of novel technologies and a multidisciplinary approach in cases where the cause of death may be attributed to a complex chain of causative factors.

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Acute Mechanical Asphyxia; Excited Delirium; Arrhythmia

I26 Five Types of Hepatic Lacerations: Can You See the Difference?

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Learning Objectives: Hepatic lacerations are not all the same. Attendees will learn to discriminate between the various causes and effects coming from hepatic laceration onset. By using histological hematoxylin-eosin and immunohistochemical technique based on an antibody antihuman Glycophorin A and anti-KL1 mono-clonal mouse antibody (cytokeratin 1 clone KL1) staining, they will be able to obtain more crucial information to establish the origin of lesions and their role in determining death.

Impact Statement: This paper aims to bring attention to hepatic lacerations and their differences in cause, time of onset, and role in death occurrence. Post-autoptic histological (hematoxylin-eosin staining) and immunohistochemical (antibody antihuman Glycophorin A staining; anti-KL1 mono-clonal mouse antibody) techniques should be combined to achieve a correct framing of each case.

Abstract Text: Are hepatic lacerations all the same? Can we develop a discriminating method to distinguish between spontaneous and traumatic lacerations? The authors aim to arouse readers' curiosity about hepatic lacerations and their differences, providing some elements to achieve a correct framing of each case.

Hepatic lacerations can be found in several traumatic scenarios. They are common in traffic accidents; for example, the impact of the car on a wall or in falling from a height. These traumatic accidents have in common the impact on a blunt surface and the effect of rapid deceleration.[1,2]

But, what about spontaneous hepatic ruptures? These rare conditions occur without any external cause and manifest with a spontaneous internal hemorrhage, caused by a breach inside the parenchyma.[3] They usually affect individuals suffering from systemic or hepatic pathologies such as: hepatocarcinoma, liver metastasis, polyarteritis nodosa, hepatic peliosis, amyloidosis, etc.[4-9]

In both cases (spontaneous or traumatic), an acute condition is established, which can swiftly lead to a severe hemorrhagic shock and death.

Does a common predisposing condition exist? Often, an underlying chronic or acute liver condition, which impairs the normal liver structure, is found. By presenting a series of different autopsies conducted on individuals presenting hepatic lacerations, the authors aim to highlight both differences and similarities among them. Histochemical, and immunohistochemical methods, allowed response to different questions concerning the primary cause of lacerations, time of death, and corollary of subject's pathologies, when existing.

The authors present five different cases of hepatic lacerations encountered in individuals who died from five different causes (traumatic or not): traumatic hepatic laceration due to car impact on a wall; traumatic hepatic laceration from a height falling, in a drug-addicted man; spontaneous hepatic laceration in a man affected by a systemic miliary tuberculosis; traumatic hepatic laceration after falling from a height in a young and healthy subject; and fire-caused hepatic laceration in a carbonized subject. Autopsies have been carried no more than three days after the death.

Histological studies were performed by using hematoxylin-eosin staining. This experimental study aims to investigate a monoclonal antibody, anti-human Glycophorin A (anti-GPA) (the most predominant of glycophorins, some of the erythrocyte transmembrane proteins) and anti-KL1 mono-clonal mouse antibody (cytokeratin 1, clone KL1) immunohistochemical staining (Cytokeratins are proteins of intermediate filaments containing keratin located at the level of the intracytoplasmic cytoskeleton of the epithelial cells) in order to evaluate the vitality of the liver lesions. In fact, according to literature, anti-GPA staining has been tested in several soft tissues (retina, larynx), but never on the liver, resulting to be positive in case of vitality of the lesion; also, anti KL1 staining has demonstrated to be useful for liver staining in corpses from 1 to 3 days after death.[10-12] Our results showed to be positive in both cases (GPA and KL1), showing that immunohistochemistry could be useful in discriminating between the different hepatic lesions we could find.

By highlighting the presence of anti-GPA and anti-KL1, it is possible to highlight the vitality and the age of the lacerations. Therefore, both techniques (histological and immunohistochemical) should be combined to achieve a complete and reliable information about the time and cause of death.

In case of hepatic rupture, post-autoptic histological, histochemical and immunohistochemical techniques must be carried on to determine: the type of laceration, pre-existing hepatic conditions, the time and the real cause of death, and discriminate which role the hepatic laceration had in causing death.

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Hepatic Laceration; Histochemical; Immunohistochemical

I27 "Terminal Burrowing" and Paradoxical Undressing: A Case Series Representing Lethal Hypothermia

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the death scene investigation, autopsy, and pathological and toxicological findings associated with cases of lethal hypothermia.

Impact Statement: This presentation will impact the forensic science community by examining in detail a phenomenon that, although known, is underreported in the literature. It will provide valuable and helpful information in managing similar cases.

Abstract Text: Hypothermia occurs when the body temperature is 35°C or less. Three grades depend on the measured body temperature: mild – temperature between 35-32°C; moderate – 32-28°C; and severe – below 28°C.[1,2] Hypothermia occurs in individuals exposed to extreme environmental conditions but can also occur in temperate climates, indoors or outdoors.[3] The identification of hypothermia in the forensic pathology practice is always a challenging task due to unspecific, inconsistent, or even negative autopsy findings. There are cases where no morphological signs are present, and the diagnosis must be based on excluding other possible causes of death and circumstantial evidence.[1,4] The death scene in cases of lethal hypothermia may raise suspicions of a crime due to phenomena such as terminal burrowing and paradoxical undressing.[3] The last one refers to the situations in which the bodies were found partly or completely naked, with most commonly the undressing to start with the lower half of the body. Terminal burrowing, also known as "hide and die" syndrome, refers to a situation in which the deceased person has burrowed their way to the area of perceived cover, putting furniture or other household items on top of themselves, hiding under a bed, behind a wardrobe, on a shelf, etc.[5,6]

We are presenting and describing in detail three cases of lethal hypothermia in which the syndromes mentioned above and phenomena were present, which at first led to the wrong conclusions that the cases were homicides. We are going to compare the information from the death scene (indoor or outdoor), autopsy findings, and toxicology reports in cases of lethal hypothermia. The study aims to raise awareness about the possible wrong interpretation and conclusions concerning cases of lethal hypothermia and to highlight the importance of a multi-disciplinary approach when analyzing information to determine a likely sequence of events.

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Hypothermia; Paradoxical Undressing; Terminal Burrowing

I28 An 18-Year Review of Decedents Recovered From the Mississippi River and Associated Tributaries at the West Tennessee Regional Forensic Center

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WITHDRAWN

I29 Overkill: A Case Series Analysis by Detailed Body Examination, Crime Scene Investigations, and Criminological Characterization

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Learning Objectives: The attendees will recognize the importance of adopting a multidisciplinary methodological approach in analyzing and characterizing a crime in which the forensic pathologist plays a central role. The study demonstrates how the forensic pathologist interprets and integrates the crime's circumstances, the crime scene's appearance, and the autopsy findings to define the offense.

Impact Statement: This study will significantly impact the forensic community, highlighting the crucial role of the forensic pathologist in classifying these crimes and supporting the entire judicial system.

Abstract Text: Overkill is a form of homicide in which victims suffer a disproportionate number of injuries. The murderer's will is to kill the victim with cruelty, repeatedly striking the body, unable to restrain his homicidal impulse. The injury pattern is generally multifocal and brutal, with extensive contamination of the crime scene. According to the literature, corpses have a higher number of wounds than the number of fatal wounds.[1] The forensic pathologist must carefully evaluate this complex scenario to assist and direct forensic investigations. The autoptic examination is essential to reconstructing the dynamics of the events through the description of the number, orientation, and position of the wounds. This helps to identify the presence of one or more assaulters or any defensive actions by the victim.

The aim of this retrospective study is to analyze the cases of "overkill" observed by the Departments of Forensic Medicine of Foggia and Rome-Sapienza between 1999 and 2024. From every necroscopic report, the authors analyzed the sex of both the killer and the victim, the crime scene, the reason for the homicide, the identity of the killer, the type of weapon, the number of wounds, and the cause of death.

A total of 24 cases of overkill have been selected. The victims were 15 males and 9 females, while the killers were females in only one case. In 6 cases, the murderer was the victim's partner; in 4 cases, the son; in 1 case, the father; in another, a nephew; in one, the roommate; in 4 cases, an acquaintance; in 2, a neighbor; while in 5 cases an unknown person. The reason was passionate in 8 cases, economical in 3, due to domestic disputes in 4, while in single cases, it was racial, work-related problems, due to psychiatric issues, and gambling disputes; in the other cases, it was not possible to identify a clear motive. The crime scenes were the victim's home (10 cases), condominium areas (3 cases), and outdoor areas (6 cases), while in single cases, the neighbor's house, a parking lot, the victim's workplace, a train station, and a service station. In one case, 76 lesions were detected on the corpse; in 9 cases, between 12 and 20; in 4 cases, between 21 and 30; in 2 cases, between 32 and 39; less than 10 in 9 cases. The identified causes of death were hemorrhagic shock (13 cases), cranial trauma (8 cases), myocardial infarction (1 case), and cardiorespiratory distress (2 cases). In 3 cases, multiple tools caused the injuries. In 15 cases, the weapon used was a sharp instrument; in 2 cases, a firearm; and in 7 cases, a blunt instrument. This study highlights how this type of crime involves individuals with many types of relationships. A correct forensic methodological approach allows us to correctly identify and classify such crimes. According to the Federal Bureau of Investigation's (FBI's) assertions, the detailed examination of the body and the crime scene is essential to outline the crime's context and assist in judicial investigations.

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Forensic; Overkill; Crime Scene Investigation

I30 Understanding the Phenomenon of Overkill: An Analysis of Eight Cases From Southern Italy

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Learning Objectives: After attending this presentation, attendees will gain an understanding about the phenomenon of overkill by analyzing the profile of both perpetrators and victims, focusing on biological aspects, relationship between offenders and offended, motives, weapons mostly used, type of injuries, cause of death, and psychiatric disorders.

Impact Statement: This presentation is set to make a significant contribution to the forensic community by sharing data from eight overkill cases from reports of the autopsies performed in Campania, Italy, from 2017 to 2023.

Abstract Text: "Overkill" represents a particular type of homicide where the number of inflicted injuries greatly exceeds the number of the fatal ones or necessary to cause one's death.[1] In forensic literature there is no generally accepted definition of overkilling. Different authors drew attention to distinct aspects of this phenomenon, emphasizing the number, distribution, and severity of wounds inflicted or focusing on the emotional element of the crime and the relationship between victim and offender.[2-4]

According to the Italian Institute of Statistics (ISTAT), Italy, a country with a population of 59 million, shows one of the lowest homicide rates in Europe with less than 400 homicides per year.[5] Among the other European countries, a general decrease in the homicide rate has been observed in 2021 compared to the previous years, varying from 1 per 100,000 population in Western Europe to 4.3 in Eastern Europe. On the other hand, Northern America has seen an increase in the homicide rate over the past decade, especially since the onset of the COVID-19 pandemic.[6] The absence of national and international epidemiological data about overkilling shows an underestimation of the phenomenon that requires a further investigation, involving a multidisciplinary approach.

Data from eight overkill cases from autopsy reports performed in Campania region (Italy) from 2017 to 2023 were collected to analyze the characteristics of both perpetrators and victims, focusing on their biological profile (age and gender), motives, modus operandi, type of relationship, number and type of injuries, cause of death, and mental health issues.

Results: The victims were mostly males (five in total) versus three females aged between 30 and 92 years (mean age 60.87 years). The crime scene was mostly represented by the victim's house (five cases), followed by vehicles (two cases). Only one case occurred outdoors in a public area. A shotgun was used in one case where the body of the victim was later set on fire. Sharp force injuries occurred in five cases with a minimum of 10 to 100 stab wounds. Blunt force trauma occurred in three victims: the offender used a stone in one case, a pressing iron in a second case, and in the third case, the victim had been hit with a heavy pan after being stabbed more than 30 times. Sharp force injuries were mostly located in the neck, chest, and face; blunt force injuries were mainly observed in the head and face of the victims. More than one weapon was used in two cases; several stab wounds related to two different knives were reported in one case whereas several blunt force injuries along with stab wounds were found in another victim. All the perpetrators were males and had a familial relationship with the victim in two out of eight cases. The killer was the husband in one case and the son and the victim's girlfriend's ex-husband in one case each. In four cases, a victim's friend or acquaintance was involved and in one case the perpetrator was the caregiver. Motives varied from familial fights (one case), jealousy (one case), trivial reasons (two cases), drugs (one case), dispute over money (two cases), and depression (one case).

Crimes involving overkill can be challenging to identify and therefore should be faced considering multiple aspects in order to understand the perpetrator's motives, the emotional element of the crime, and their psychological background while committing the homicide. The current analysis is set to provide valuable insights into the different factors that characterize these heinous crimes.

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Overkilling; Violent Crime; Homicide

I31 Sudden Deaths in Athletes: The Role of Autopsy in a Literature Review

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Learning Objectives: After attending this presentation, attendees will understand the role of autopsy in assessing the causes of sudden deaths in athletes, with the aim to implement screening and prevention methods and to reduce its risk.

Impact Statement: This presentation will impact the forensic science community as it discusses the postmortem findings associated with the phenomenon of sudden deaths in young athletes.

Abstract Text: **Introduction:** Sudden Cardiac Death (SCD) is defined as a natural cardiac death that occurs suddenly, within a few minutes after the onset of symptoms, preceded by a sudden loss of consciousness in subjects with or without a known pre-existing heart disease. This event, therefore, shows unpredictability occurring in apparently healthy people. More specifically, the Sudden Death from Sport (SDS) has a direct relationship (cause-effect) with the sporting activity.

The incidence of these events in the general population is about 10% of annual cardiac deaths, and it is even less in the sports population. SDS, in fact, does not exceed 1-2% of the global number of all sudden deaths. The athletes most affected attend low-level competition (80%), being amateurs. This is also because there are fewer medical checks and, in general, less accuracy for this category. The frequency of SDS is also higher in official competitions (79%) than in training (21%). It is estimated that 1-3/100,000 young, apparently healthy athletes die suddenly during exercise. Males are affected up to 10 times more than females. Basketball and football players in the United States are at greatest risk, while footballers are in Europe.

Materials and Methods: A literature review was carried out. The search engines Pubmed NCBI and Scopus were used by two independent operators. The following keywords were used: "SCD and autopsy" AND "athletes." The research involved the analysis of the role of postmortem investigations and autopsy findings in cases of sudden deaths in athletes. The articles were first selected by title and abstract, then full text was read according to relevance (i.e., the description of autopsy findings in these cases). The authors included articles of the timeline (2004-2024). Papers that were not in the English language or not about the theme were excluded.

Discussion: Competitive sports activity is associated with an increased risk of SCD in adolescents and young adults with clinically silent cardiovascular disorders. While in middle-aged/elderly athletes, atherosclerotic coronary disease represents the vast majority of SCD, in young athletes, the spectrum of substrates is wider and includes hereditary structural heart disease (cardiomyopathies) and congenital heart disease (abnormal origin of coronary arteries). The most common mechanism of cardiac arrest during sports activity is sudden Ventricular Fibrillation (VF) as a result of an underlying cardiovascular disease.[1-3]

A considerable percentage of sports-related SCD victims have no evidence of structural heart disease at autopsy and the cause of their cardiac arrest is often related to primary electrical heart condition; that is, hereditary defects of cardiac ion channels (channelopathies) including long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, and Brugada syndrome. A study, the Federation Internationale de Football Association (FIFA) Sudden Death Report (FIFA-SDR), was commissioned by FIFA and conducted by Saarland University and published in 2020.[4] The report recorded deaths worldwide attributed to sudden cardiac arrest or other unexplained sudden death while playing (or immediately after playing) soccer during the period from 2014 to 2018.There were 617 cases over the five-year period. In most cases where an autopsy was performed, the cause of death was coronary artery disease. Sometimes the use of exogenous substances can also act as a trigger in pre-existing disease conditions.

Conclusions: The autopsy, as well as toxicology and the study of cardiac markers, are essential to understand the causes of death and prevent them.[5] In particular, we emphasize the investigation of undiagnosed pathologies during fitness testing and the use of drugs or other performance-enhancing drugs. Based on the results obtained, screening methods can be implemented in order to look for silent cardiac pathologies early and reduce mortality in young athletes through more accurate and frequent toxicological screenings.

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Forensic Science; Sudden Cardiac Death; Athletes

I32 Fatalities Caused by Elapids and Viperids: A Critical Analysis of Cases Coming to a Tertiary Care Center

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Learning Objectives: After attending this presentation, the attendees will know the fatalities caused by elapids and viperids in persons engaged in or cohabiting in places where snakes exist.

Impact Statement: This presentation will impact the forensic science community by imparting knowledge of the danger to persons prying in the snakes' vicinity, farms, or even workplaces or homes. We'll discuss the manner of causation of death, probability of survival, correlation of snake bites with survival, if any, etc.

Abstract Text: In Asian countries like India, there are 216 species of snakes, out of which 52 species are reported to be poisonous. The most common, important, and deadly are the "Big 4 snakes" (cobra, krait, viper and saw scale viper).[1,2] A snakebite is an important and serious medical problem in many parts of the world, especially in South Asian countries.[1-4] It causes great suffering to the families whose bread earner or beloved one is lost.

The present study was carried out in our tertiary care center to find the relationship of the elapid and viperid bites with fatalities. The cases were studied for a period of seven years to have substantial number of cases to ascertain any inference.

A total of 128 cases related to snake bites were found in the given period. Most of the fatalities caused were due to viperine bite (46.8%), followed by cobra (15.6%), and krait (10.9%). Also, in considerable number of cases the biting culprit remained unknown (26.5%). We also studied the seasonal variation, distribution of cases according to gender, and place of occurrence. The incidence of bite with the survival period was also studied.

We concluded that of all the fatalities, the viperine bite was most common. However, comparing bite fatalities, it was found that the cobra and krait didn't give as much time to survive as the viperine bite. Such a study is important for future studies and also to educate about the preventive aspect in such cases, which might help in curbing the occurrence of such incidences.

This presentation will deal with the age and sex distribution of the human fatalities in correspondence with the elapid and viperid bites. The probable reason behind the victims being involved in snakebite encounters will be sought out. The medicolegal death investigator should become more familiar with such type of incidents of victims being involved in snakebites and manner of deaths caused by it. As people in general and many tourists do visit the countryside or places which have prevalence of such cases, they must be aware of this so as to take the necessary precautions.

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Toxicity; Autopsy; Cause of Death

I33 Suicide Analysis in India: Causes, Trends, and Forensic Implications

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Learning Objectives: After attending this presentation, attendees will understand the alarming rise in suicide rates in India, the primary causes driving these tragedies, and the demographic and socio-economic profiles of the victims. They will gain insights into the urgent need for targeted interventions and comprehensive strategies to address this critical issue.

Impact Statement: This presentation will impact the forensic science community by providing a detailed analysis of suicide trends, causes, and demographic factors in India, thereby highlighting critical areas for intervention. It will inform forensic experts about the prevalent methods and circumstances surrounding suicides, enhancing their ability to contribute to preventive measures and policy development.

Abstract Text: Every instance of suicide is an individual calamity that prematurely terminates a life and deeply affects families, friends, and communities. Annually, about 100,000 individuals in India engage in suicide, motivated by a range of factors, including professional or career difficulties, social isolation, abuse, violence, familial conflicts, mental illnesses, addiction, financial setbacks, and severe physical pain. The National Crime Records Bureau (NCRB) gathers information about suicides from police records in order to comprehend and tackle this matter.

India had a total of 170,924 suicides in 2022, indicating a 4.2% rise compared to the previous year. The suicide rate, determined by extrapolating population statistics for non-census years and using Census 2011 data, experienced a 3.3% increase in 2022 as compared to 2021. Maharashtra recorded the greatest incidence of suicides, with a total of 22,746 cases, followed by Tamil Nadu with 19,834 cases, Madhya Pradesh with 15,386 cases, Karnataka with 13,606 cases, and West Bengal with 12,669 cases. These five states comprised 49.3% of the overall suicide rate in the nation. Conversely, Uttar Pradesh, the state with the highest population, has a comparatively lower rate of suicides (4.8%).

The primary factors contributing to suicides in 2022 were family issues and illness, which accounted for 31.7% and 18.4% of instances, respectively. Additional notable factors contributing to suicide rates were substance abuse or alcohol dependency (6.8%), marital problems (4.8%), extramarital affairs (4.5%), financial difficulties such as bankruptcy or debt (4.1%), joblessness (1.9%), academic failure (1.2%), issues related to one's profession or career (1.2%), the loss of a loved one (1.2%), and conflicts over property (1.1%).

With respect to the occupational position of individuals who died by suicide, housewives accounted for 52.5% of female victims and 14.8% of all suicides. The proportion of government employees was 1.3%, private sector employees accounted for 6.7%, and public sector employees made up 1.6%; 7.6% of suicides were committed by students, whereas unemployed individuals accounted for 9.2% of suicides; 11.4% of victims belonged to the self-employed category. Within the agriculture industry, a total of 11,290 individuals took their own lives, consisting of 5,207 farmers or cultivators and 6,083 agricultural laborers. This accounts for 6.6% of all suicides.

An analysis of marital status revealed that 67.0% of individuals who died by suicide were married, 24.6% were single, and smaller proportions were widowed, divorced, or separated. In terms of economic status, 64.3% of those who died by suicide had an annual income of less than ₹1 lakh, while 30.7% had an income ranging from ₹1 lakh to ₹5 lakh. In terms of education, the largest group of victims had completed matriculation or secondary level education (23.9%), followed by those with middle-level education (18.0%), higher secondary education (15.9%), primary education (14.5%), and illiteracy (11.5%). Just 5.2% of the victims possessed a bachelor's degree or higher.

The suicide techniques exhibited diversity, with hanging accounting for 58.2% and poisoning for 25.4% of cases, making them the predominant means. Additional techniques encompassed submersion (5.0%) and encountering moving cars or trains (2.9%). Between 2021 and 2022, there was a rise in the occurrence of hanging and poisoning, while the incidence of fire-related self-immolation and contact with electric wires had a minor decline.

This data highlights the urgent need for targeted interventions to address the diverse and complex factors contributing to suicides in India. Comprehensive strategies involving mental health support, financial assistance, and community engagement are essential to mitigate this pressing issue.

Suicide; Intervention Strategies; NCRB

I34 An Autopsy of a Patient With Schizophrenia and Compulsive Eating: A Case Report Highlighting Choking Risks in Psychiatric Patients

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WITHDRAWN

I35 A Forensic Analysis of a Carbonization in a Complex Case of Dubious Suicide

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Learning Objectives: After attending this presentation, attendees will understand the complexity of suicides due to fire and the use of accelerating fuels such as gasoline.

Impact Statement: This presentation will impact the forensic science community by demonstrating the importance of accurate collection of evidence with comparison of all investigative hypotheses.

Abstract Text: **Introduction:** Suicide represents a global health emergency. The reconstruction of the dynamics of a suicide can prove very complicated, such as in suicides occurring with the use of fuels. They produce flames at very high temperatures, with consequently low evidence at the scene.

Case Report: We report the case of a burned man that was found sitting in a car on the driver's side located on an isolated road at night. The car was completely burned out with the driver's door open. The investigators hypothesized that the man may have been killed and burned after death. The alternative hypothesis was an accidental car explosion, while trying to go to the hospital or to meet another person.

Materials and Methods: A scene investigation was performed. Gasoline combustion levels in the car were measured at each seat. A postmortem 3D Multislice Computed Tomography (CT) scan with autopsy was performed. Toxicological and genetic investigations on cadaver fluids were also carried out. Investigators collected videos and testimonies from family members, using a psychological autopsy method, and conducted investigations on the man's phone and on witnesses to the event. The geographical route of the car was studied.

Results: The car appeared completely burned, as if accelerant fuel had been used. Higher levels of gasoline combustion were found on the driver's side than the other car sites. The 3D Multislice CT showed no retained bullets. The skull was totally skeletonized with total loss of physiognomy and it was more burned than other sites, like the thorax or abdomen. Examination of the airway at autopsy revealed soot within the glottis and the upper and lower airways, proving the man had breathed the fumes. The brain was still preserved, even if burned, and did not show traumatic injuries on gross analysis. The heart did not show any pathological sign. Toxicological investigations proved a blood percentage of carboxyhemoglobin (HbCO) of 13% and excluded other substances in blood. Genetic investigations proved the identity of an elderly man who lived alone. Psychological autopsy showed that the man, shortly before his death, had spoken to his family and appeared healthy, even if in recent months he had suffered from an obsessive-compulsive disorder. Investigative data found a video of the last night showing the car burning with very high flames with the man in the car.

The analysis of the car and of the head burn trauma, the absence of other injuries at autopsy and the data collected allowed us to conclude that the man sprinkled his head with petrol. The HbCO levels proved that he died shortly after the fire due to the fire injuries. Furthermore, the head was completely burned compared to other anatomical sites and traces of petrol were found only on the driver's seat.

The hypothesis of a homicide was excluded due to the absence of injuries at autopsy and CT and due to the absence of evidence from the investigative data. The hypothesis of illness was excluded because the autopsy did not reveal any acute disease and the street traveled by the subject was totally isolated. The hypothesis of an accident was excluded because the evidence of gasoline only on the driver site was incompatible and because the man would have had time to escape from the car.

Discussion: Most car suicides occur through Carbon Monoxide (CO) inhalation.[1] Car suicides by gasoline are rare. In these cases, despite the difficulties caused by the use of fuels, an accurate collection of evidence is essential. This evidence must be compared with all the hypotheses and possible methods so as to allow the case to be resolved through a process of exclusion.

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Forensic Science; Fire; Gasoline

I36 May–Thurner Syndrome: A Case Report of an Undiagnosed Left Iliac Vein Thrombosis

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Learning Objectives: The aim of this presentation is to highlight the importance of investigating the iliac vein tract. Ordinarily, in the case of suspected deep vein thrombosis, the physicians investigate the vein anatomically situated in the leg. With this case report, we underline the importance of considering also a evaluation of the iliac tract in the cases where the classic investigations result negative but the symptomatology is related to a condition of thrombosis.

Impact Statement: This presentation will significantly impact the forensic science community by raising awareness of May-Thurner Syndrome (MTS) as an underdiagnosed condition with potentially fatal consequences, particularly in cases of unexplained venous thrombosis and sudden death. By highlighting a case where MTS was only identified postmortem, it emphasizes the importance of considering MTS in forensic investigations, especially when conventional vascular assessments fail to provide answers. The findings underscore the need for forensic experts to utilize advanced imaging techniques and maintain a high index of suspicion for deep venous conditions like MTS, which can alter the outcomes of death investigations and improve the accuracy of cause-of-death determinations.

Abstract Text: **Introduction:** MTS, also known as ilio-caval compression syndrome, iliac vein compression syndrome, or Cockett syndrome, is a pro-thrombotic condition resulting from compression of the ilio-caval vein against the vertebral column, most commonly at the level of the fifth lumbar vertebra, by the right common iliac artery.[1] May and Thurner, through studies conducted on cadavers, noted that this variant is present in 22% of cases.[2] Autopsy studies suggest a prevalence of 14-32%, though it is often underestimated. Additionally, many studies suggest that this condition is more prevalent in females.[3]

Case Report: We present a case of death associated with MTS in a 63-year-old female patient who presented to the Emergency Room (ER) with complaints of lower limb pain and venous leg ulcers. Her clinical history included Crohn's disease that could be related to skin lesions. Lower limb ulcers are associated with pathological venous conditions in 80-90% of cases.[4] During her hospital stay, specific diagnostic tests were conducted to exclude vascular conditions and diabetes. An eco-color Doppler of the lower limb was negative, and biochemical investigations showed no signs of diabetes, with low glucose levels. Furthermore, to rule out vascular abnormalities related with the possibility of pulmonary embolism, the patient did not exhibit any thoracic symptoms such as chest pain or dyspnea typically associated with this condition. The physicians initially suspected a condition related to vasculitis, but tests for this were negative. During hospitalization, the patient's condition worsened following a fall without evidence of trauma, and she died from cardiogenic shock. Autopsy findings revealed thrombotic formation in the left iliac vein and an embolus in the inferior vena cava, disrupting venous return to the heart and causing cardiogenic shock.

Discussion: MTS is a potentially lethal condition if not diagnosed in a timely manner. However, as indicated by the clinical course of the reported case, diagnosing thrombosis in the iliac vein is particularly challenging due to its deep anatomical location. While Doppler ultrasound is the gold standard for evaluating the lower limbs, assessing the ilio-caval region requires the use of multi-detector computed tomography venography or magnetic resonance venography, both of which have a sensitivity and specificity of 95% for diagnosing MTS. It is also important to exclude other potential conditions associated with iliac compression, such as uterine leiomyoma, aortoiliac aneurysm, retroperitoneal fibrosis, and osteophytes. MTS is commonly associated with thrombosis in the iliac vein, as compression by the iliac artery and the vertebral column alters blood flow, especially on the left side. In the literature, there are other case reports linking this syndrome to severe complications, including fatal iliac vein rupture, which can lead to hemorrhagic shock.[5,6]

In conclusion, this case study suggests that a more in-depth evaluation is necessary for patients presenting with ulcers and lower limb pain, particularly when no vascular abnormalities are detected in the lower limbs. Typically, the diagnostic process involves examining the lower limbs with Doppler ultrasound. However, if the results are negative, a thorough evaluation of the iliac vein may be crucial for diagnosis, given that MTS is often underdiagnosed and potentially lethal.

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Thrombosis; May Thurner Syndrome; Lower Limb Pain

I37 The Eyewear Suicide: A Rare Case of Suicide Caused by the Inhalation of Homemade Hydrogen Sulfide

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Learning Objectives: The objective of this report is to emphasize the importance of implementing enhanced safety measures and raising public awareness to prevent incidents involving commonly used household products. In such cases, judicial inspections, autopsy, and toxicological evaluations must be conducted with precision to avoid serious errors. This report aims to highlight these critical aspects of forensic practice to prevent significant miscarriages of justice.

Impact Statement: The autopsy must be meticulously conducted to ensure no evidence is overlooked, and comprehensive toxicological analysis should be performed using a wide range of biological substrates, including vitreous humor, urine, brain, lungs, and liver, as in the presented case. We want to bring the attention to the importance of conducting a thorough and flawless judicial inspection as we did. When approaching such cases, the forensic pathologist must carefully consider homicidal, suicidal, and accidental causes of death.

Abstract Text: Hydrogen sulfide (H_2S), a rapidly acting and highly toxic gas, is produced and used in the chemical industry. Numerous acute poisonings and fatalities have been reported from exposure to hydrogen sulfide in industrial settings, sewers, wells, and septic tanks.[1] Suicidal H_2S poisoning is infrequently encountered in medical examiner practice; most H_2S deaths that have been reported occurred in association with accidental industrial exposure.[2]

The first cases of suicide by H_2S inhalation were reported in Japan in 2007. This method involves mixing common household chemicals, such as hydrochloric acid (found in commercial pool cleaners) and lime sulfur (calcium polysulfide, commonly found in pesticides), to generate H_2S gas, which is then inhaled in an enclosed space. Literature on these rare suicide cases often notes that vehicles are a frequent choice for the enclosed environment. Victims are sometimes found wearing protective eyewear to shield their conjunctivae from the irritating effects of H_2S.[3]

In this study, we report a unique case of H_2S poisoning that occurred in an enclosed shower cabin. In April 2024, a 24-year-old man was discovered on the floor of a shower cabin in the dormitory room of the military air force station where he was employed. A strong chemical odor was noted during the judicial inspection. The deceased was found in a supine position, fully clothed, and wearing protective eyewear. A bucket containing a whitish liquid with yellow droplets on the surface was found near his feet. Inside the shower, empty containers of hydrochloric acid and calcium polysulfide were found on the sill of a closed window.

External examination revealed extensive purple hypostasis on the posterior and dependent areas, as well as on the anterior areas of the head, neck, shoulders, and upper limbs, along with hemorrhagic petechiae. Additional findings included green-gray discoloration of the facial skin and labial mucous membranes, conjunctival hyperemia, blood oozing from the nostrils and mouth, and small punctate burns indicative of corrosive spatter on the forearms and fingers.

Autopsy findings included enlarged lungs with hemorrhagic petechiae on the surface and foamy blood material in the airways. Hemorrhagic petechiae were also present on the cardiac surface. Microscopically, massive pulmonary hemorrhagic edema was observed. Postmortem toxicological analysis of multiple biological samples confirmed H_2S poisoning, with asphyxiation identified as the cause of death. Sulfide analysis was conducted on blood samples from the vena cava, as well as samples from the vitreous humor, urine, brain, lungs, and liver, using the Gas Chromatograph/Mass Spectrometry (GC/MS) method. The results revealed a significant presence of sulfides in all samples, except for the vitreous humor and urine. These findings confirmed acute exposure to H_2S, providing a clear explanation for the cause of death.

This case report illustrates the fatal risk of H_2S poisoning from mixing household chemicals in confined spaces. Key forensic findings confirmed death by asphyxiation. Enhanced safety measures and public awareness are essential to prevent such incidents.

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Toxicology; Hydrogen Sulfide; Suicide

I38 The Strange Case of Cable Ties: Suicide or Murder?

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Learning Objectives: This case report will help the attendees: (1) learn about a rare self strangulation method, where the mechanism of asphyxia is predominantly vascular; and (2) discriminate between homicide and suicide when the crime scene is unclear.

Impact Statement: Self strangulation is rare and sometimes achieved by unusual and fanciful means. This presentation will impact the forensic science community by showing a self-strangulation method rarely reported in scientific literature.

Abstract Text: In the countryside, the body of a 43-year-old man was found lying on his back with his arms stretched out. The ground at both sides of his head was stained with blood running from his mouth. Two separate ligatures were secured around his neck. Was it suicide or murder? The crime scene was suspicious, so the prosecutor requested an autopsy to investigate the cause of death.

At a closer inspection, we discovered that each ligature was made of two black plastic cable ties interlocked to each other and tied tightly around the neck. The uppermost cable tie was right above the laryngeal incisure of the thyroid cartilage, the lowermost about 2 centimeters lower; the skin in between the loops was congested and edematous; the face was cyanotic.

Before removing the cable ties, a laryngoscopy was performed in order to assess the patency and conditions of the upper airways. The laryngoscope could easily pass through the glottis into the trachea, but we couldn't see much of the mucosa because of intraluminal blood. The cable ties were then cut, showing two ligature marks, both running horizontally and continuously around the entire circumference of the neck. The grooved pattern of the cable tie was imprinted on the skin. Could it be murder?

No suicide note was found at the crime scene; the deceased had no clinical diagnosis of depression, but he was dealing with both personal and financial problems and had recently expressed suicidal intentions to his partner, as witnessed by chat transcriptions. The external examination showed no evidence of significant traumatic injuries that could justify his death; there was no evidence of scratches or fingernail marks on the neck, meaning that the victim didn't attempt to get free of the ligature; neither were there defense wounds or bruises on the hands and fingers that could record the attempt of the victim to defend himself from an assault.

The autopsy findings were: brain congestion; hemorrhagic infiltration of the soft tissues and muscles of the neck; hemorrhagic infiltration of the thyroid gland; congestion of the laterocervical lynph nodes bilaterally; distraction of the left thyroid ligament; hemorrhagic spots on the tongue and laryngeal tissues. Neither the hyoid bone nor the thyroid horns were fractured. Could it be suicide?

Self-strangulation is possible, yet not common.[1] Only a few cases of self ligature by plastic cable ties have been described in literature.[2-4] Cable ties are produced in many different sizes, lengths, and colors and can easily be found at any hardware store. They have a peculiar self-locking mechanism: after they are locked, they can only be tightened further, but it is impossible to loosen them; also, they can hardly be cut with ordinary scissors. What if it was suicide? How did he do it?

A reasonable reconstruction is that the cable ties could have been interlocked to each other loosely to form a circle, then wore across the head to the neck; finally, the loose ends could have been pulled hard by hands at the same time, determining the constriction of the neck.

The glottis being patent, we hypothesized that the asphyxial mechanism was predominantly vascular: when the loose ends of cable ties are pulled hard, the self-locking mechanism maintains a high, constant pressure on the neck, even after the loss of consciousness and muscle tone, compressing simultaneously both the carotid artery and the jugular vein bilaterally.[2].Our medicolegal diagnosis was suicide by self strangulation.

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Suicide; Self Strangulation; Cable Tie

I39 Death by Self-Inflicted Captive Bolt Device

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Learning Objectives: After attending this presentation, attendees will be provided the foundational knowledge of the use of and how captive bolt devices work.

Impact Statement: This presentation will impact the forensic science community by comparing injury patterns of a captive bolt device to other firearms-related cases.

Abstract Text: A captive bolt device is used to inflict a forceful strike on the forehead with the bolt to induce unconsciousness.[1] These devices are used for stunning animals such as cattle prior to slaughter. When used, the bolt penetrates the skull of the animal, enters the cranium, and damages the cerebrum and part of the cerebellum. After the initial impact, spring tension causes the bolt to recoil back into the barrel. The device must be placed perpendicular to the skull surface for maximum striking power. Angling the device will reduce the ability of the captive bolt to penetrate the brain. It has the same effect on the animal as a firearm with a live bullet.[2]

In Washington, DC, a 54-year-old male completed suicide using a captive bolt device. The decedent had a history of depression and left a suicide note. The suicide note indicated he was diagnosed with early onset Alzheimer's. The decedent drove to an alley and used the weapon inside his vehicle. The captive bolt device was found on the passenger seat. The decedent was transported to the hospital, but eventually was pronounced deceased. An autopsy was later performed at the DC Office of the Chief Medical Examiner.

Wounds inflicted by captive-bolt guns have physical characteristics that are similar to wounds made by other firearms such as the size and shape, but others that are unique lack abraded margins and soot deposition.[3]

This presentation is intended to provide participants with the foundational knowledge of the use of and how captive bolt devices work, as well as comparing injury patterns to other firearms-related cases. In cases where the entrance wound has sharp edges, without abraded margins, no exit and/or radiographic or autopsy evidence of a retained bullet, one should suspect the use of a captive-bolt device.

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Suicide; Head Trauma; Firearms

I40 Suicide by Knitting Needle—An Ambiguous Method of Self-Inflicting Fatal Heart Injuries: A Case Report

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Learning Objectives: Attendees of the presentation will receive important information about a rare case of suicide by knitting needle, about which there is very little scientific literature. They will be able to learn, discuss, and debate with the author about the methods applied in studying the cause of death as well as observe how the study of the heart and the search for exogenous substances in body fluids are performed.

Impact Statement: The forensic science community will gain expertise regarding the integrated working method and instrumental techniques used as well as particular injuries not described in the literature as a cause of death.

Abstract Text: This case report presents an 88-year-old Caucasian man who self-inflicted 50 stab wounds using a knitting needle. His body was discovered in the bathtub by a family member. Emergency services were alerted, declared the death, and law enforcement officers assessed the scene, ruling out any external involvement in the man's death.

A full autopsy was performed to accurately determine the cause of death. Samples from major organs and thoracic skin were collected for immunohistopathological analysis, and biological fluid samples underwent toxicological testing. The heart was fixed in formalin, imaged with a Computed Tomography (CT) scan for 3D reconstruction of the lesions, and then dissected.

The external examination revealed 32 wounds to the chest, 12 wounds to the left inguinal region, and 6 wounds to the right inguinal region. Injuries in the groin area were confined to the skin and subcutaneous adipose tissue. Most thoracic injuries were superficial, affecting only the skin and subcutaneous tissues, with a few penetrating the pectoralis major, serratus anterior, and intercostal muscles. The pericardial cavity contained a small amount of blood, while the left pleural cavity held 900cc of blood and a 10-gram clot. Notably, 21 of the thoracic wounds penetrated the heart. The CT scan's 3D reconstruction revealed the trajectories of the self-inflicted wounds, facilitating a detailed examination of the heart. Toxicological tests were completely negative, indicating the man was not under the influence of drugs or painkillers at the time of the self-injurious act.

There are no documented cases in scientific literature of suicide by stabbing the heart with a knitting needle. This case report illustrates the importance of teamwork in reconstructing the cause and method of death, offering insights into the unusual and numerous wounds located in difficult-to-reach areas. The findings suggest that the man was not experiencing an altered mental state due to drugs at the time of the incident, despite the peculiar nature and location of the wounds.

Suicide; Heart; Death Investigation

I41 A Decade of "Omics" in Forensic Science From Lab to Court: Where Are We Standing, Where Are We Going?

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Learning Objectives: Attendees will learn about new omics methods useful to estimate Postmortem Interval (PMI) and about the gap existing between forensic research and implementation of the findings to real caseworks. A discussion will be prompted on the basis of a survey presented to forensic practitioners, legal professionals, researchers and academics, law enforcements agencies, regulatory agencies, and policy makers to determine the future directions to pursue and integrate these new molecular approaches effectively into legal proceedings.

Impact Statement: This presentation will impact the forensic science community by exploring future directions to implement omics analyses effectively into legal proceedings.

Abstract Text: Over the past decade, omics disciplines have gained popularity in forensic research, due to their potential to improve personal identification, determine cause and manner of death, and estimate time since death. With the recent technological advancements and the availability of genetic sequencers and mass spectrometers in forensic laboratories, techniques such as epigenomics, metagenomics and metabarcoding, transcriptomics, proteomics, metabolomics and lipidomics, and their integration in multi-omics research started to be applied to forensic research in a growing manner.

The rising interest and advancements in the intersection of omics technologies and forensic science are reflected in the recent substantial increase in related publications.[1] For example, the use of metabarcoding and metagenomics for estimating the PMI has seen notable progress since the first papers on the topic emerged in 2013. Today, there are over 40 original research publications on this subject, with 50% of these being published since 2020. Similarly, over 20 research papers were published on the use of proteomics for improving PMI estimation from 2017 onward, and more than 15 on the use of metabolomics for the same forensic application since 2015. More widely, multi-omics publications have more than doubled from 2022 to 2023 compared to the previous decade, demonstrating an increasing focus on applying these technologies to complex biological questions, also including forensic problems.

The current stage of development of these disciplines, however, is not yet fully reflected in daily practice, as omics technologies have been accepted as evidence in court, as investigative support, only on limited occasions and are not yet widely integrated into forensic procedures.[2,3] The challenges of validation, standardization, and gaining acceptance within the scientific community are significant barriers to creating legal precedents that could pave the way for their future use in legal proceedings.

From a researcher's perspective, several barriers hinder the application of new disciplines to real-world scenarios. One significant challenge is the limited communication between researchers and practitioners, compounded by the fact that science and law often speak different languages. This distance raises reluctance to adopt new techniques, as there is a preference for sticking with established methods, also to obtain investigative leads, and a lack of understanding about the advantages of new techniques. This is coupled with the absence or difficulty of implementing standardized operating procedures and with the scarcity of personnel trained in these new analyses and instrumental availability. Finally, there are constraints on resources that can be allocated to these analyses, especially when results are not guaranteed.

To gather a comprehensive understanding of the barriers and challenges preventing the application of omics disciplines in court, a survey will be conducted among forensic practitioners (forensic biologists and forensic pathologists), legal professionals (lawyers and judges), researchers and academics (both in omics and in forensic disciplines), law enforcements agencies, regulatory agencies and policy makers both in the United States and abroad. This presentation aims to inform attendees on the (rare) precedent use of omics analyses in court, to present the results of the survey, and to stimulate a constructive debate on the position of omics research in forensic science, focusing on the progress made over the past decade, the current standing of these technologies from lab to court, and the future directions we should pursue to integrate them effectively into legal proceedings.

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Omics; Postmortem Interval; Admissibility

I42 The Impact of Heroin on Thanatomicrobiome and the Postmortem Interval

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Learning Objectives: After attending this presentation, attendees will be familiar with how to use 16S ribosomal RNA sequencing to identify the thanatomicrobiome and postmortem microbiome of the liver and prostate samples from Italian cadavers. Attendees will also understand molecular biology techniques to assess microbial diversity after death.

Impact Statement: This presentation will have an impact on the forensic science community by presenting new insights into thanatomicrobiome signatures in drug overdose cases involving heroin. This new knowledge will contribute to developing improved methodologies and accuracy when determining the time and cause of death. Additionally, the identification of specific microbes present in liver and prostate issues may provide a potential connection between drug abuse and microbial activity during the putrefaction.

Abstract Text: The addictive opioid drug heroin represents a significant public health crisis. The study of the postmortem microbiome has its roots in forensic science, microbial ecology, and microbiology.[1] It emerged as an innovative approach to understanding the ecological changes within and on a deceased body during putrefaction. Thanatomicrobiome, or the postmortem microbiome, has been investigated as an informative microbial biomarker of the time and location of human death.[2,3]

In this study, the role of the gut microbiome, which will be examined in the postmortem liver and prostate samples, as a potential mediator between ten natural deaths and ten accidental deaths (drug overdose) among criminal cases from the University of Pavia's Morgue in Italy, with a focus on substance use disorders, particularly heroin abuse. We hypothesized that postmortem samples of a similar cause of death would reveal similar microbial taxonomic relationships. We compared microbial diversity profiles using amplicon-based sequencing of the 16S ribosomal RNA gene V3 and V4 hypervariable regions. Postmortem DNA was extracted, and universal primers (341F/805R) were used to profile conjoined V3-V4 hypervariable regions of the 16S rRNA gene to generate amplicon sizes of approximately 465 base pairs. Analysis of Similarities (ANOSIM) is typically used for taxa-in-examples data; the p-value was 0.039, and the microbial communities in the comparison groups differ statistically significantly. The most abundant phyla were Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes across all sampling sites. Firmicutes and Actinobacteria have gram (+) cell walls and are involved in the metabolism of carbohydrates by consuming oxygen and reducing the redox potential in the gut environment. Proteobacteria and Actinobacteria, a significant phylum of gram-negative bacteria, are crucial in preparing the gut for colonization through the stringent anaerobes essential for gut function.

The findings reveal a significant association between substance use disorders and shifts in the gut microbiome composition. This study contributes to the growing understanding of the intricate relationship between gut microbiota and substance abuse, highlighting the potential for microbiome-targeted interventions in the management of substance use disorders. The future question for this research is: what are the successional changes in the liver and prostate over an extended period of natural death vs. accidental death (drug overdose)?

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Heroine; Postmortem Interval; Cadaver

I43 Investigating Flunitrazepam's Influence on *Calliphora Vicina* (Diptera: Calliphoridae) Growth and Its Impact on Microbiome Diversity

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the effect of flunitrazepam, a Central Nervous System (CNS) depressant drug (benzodiazepines class), on the microbiome diversity and dynamics throughout the development cycle of *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae).

Impact Statement: This presentation will offer the forensic science community novel insights into flunitrazepam's influence on *C. vicina* development and microbiome diversity. The data generated will be instrumental in elucidating the impact of this depressant drug on various stages of *C. vicina* development, particularly in relation to shifts in associated microbiome diversity and dynamics.

Abstract Text: The potential for misuse, impulsivity, and adverse withdrawal effects of benzodiazepines, which are categorized as depressants or psychoactive drugs, may lead to an elevated risk of suicide.[1] The misuse or intentional overdose of these drugs can lead to hypoventilation due to their muscle-relaxing effects, and long-term usage can cause cognitive deficits.[2] Flunitrazepam, a specific type of benzodiazepine, is used to manage severe insomnia and assist in anesthesia.

Examining the impact of flunitrazepam on the blow flies' microbiome from a forensic standpoint has multiple consequences. A variety of factors, including the cause of death and temperature, influence both the decomposition process and insect activity and development. The temperature governs the periods of insect emergence and activity, while the cause of death can affect the insects' development cycle, particularly if specific drugs are present.[3] In this context, the present study explored how flunitrazepam alters the diversity and dynamics of the microbiome throughout the development cycle of *C. vicina*, which has implications for forensic investigations following death.

Two colonies of adult *C. vicina* flies were established. The first colony was fed with cow liver inoculated with flunitrazepam, while the second colony, serving as a control, was fed only with cow liver. The adult flies were reared at a constant temperature of 24°C and a humidity of 45%. Following oviposition, the experiment was conducted in triplicate to rear all *C. vicina* immature specimens under identical experimental conditions. A concentration of 25mg of flunitrazepam was spiked into 200g of minced cow liver for each rearing jar. *C. vicina* specimens were collected in triplicate for each developmental stage. The microbiome was investigated via metabarcoding approach, by analyzing the V3-V4 regions of the 16s rRNA gene via Illumina MiSeq and pre-processed using qiime2 v. 2024.2.[4] The data analysis was performed with R studio using Linear discriminant analysis Effect Size (LEfSe) to identify differentially expressed Amplicon Sequence Variants (ASVs) between control and flunitrazepam fed specimens.[5]

The results showed an increased weight for the specimens treated with flunitrazepam. Considering the microbiome analysis outcomes, no significant differences were found for the alpha diversity between treated and untreated specimens, while the beta diversity showed clear clusters according to treatment and developmental stage. Proteobacteria was predominant in the flunitrazepam samples, and a few bacterial markers that could be used to discriminate insect specimens feeding on decomposed remains with flunitrazepam were identified.

The resulting data is crucial toward understanding the effect of this depressant drug on *C. vicina* development by examining the microbiome diversity and dynamics associated with different life stages, providing more comprehensive information applicable in death investigations.

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Entomotoxicology; Flunitrazepam; *Calliphora vicina*

I44 Microbial Succession of Necrophagous Beetles and Soil During Small Carcass Decomposition: Potential Uses in Understanding Body Translocation Scenarios

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Learning Objectives: After attending this presentation, attendees will have a better understanding of the succession of bacterial communities in the soil and the internal microbiomes of necrophagous beetles throughout the process of decomposition. 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 and internal beetle microbiomes change predictably during decomposition, suggesting they can be used to support body translocation evidence. Microbes are a critical element in the community of organisms associated with decomposing bodies and soils, so understanding the insect and soil microbial ecology during cadaver decomposition could also greatly inform future studies of forensic utility.

Abstract Text: In forensic investigations, insect development data can be used to estimate the minimum amount of time between death and discovery of a body, or the Postmortem Interval (PMImin). Blow flies (Diptera: Calliphoridae) can detect and begin to colonize a carcass within minutes of death.[1] It has been reported that the bacterial community of a carcass will also change in a predictable way, suggesting that microbes can be used in the future to support insect data and calculate PMImin.[2] Beetles are another group of forensically relevant insects that will visit a body during the decomposition process often to feed on the carcass itself or the colonizing blow fly larvae. Several families of beetles are necrophagous and are found to interact with carrion throughout decomposition, though most commonly in the later stages of decomposition.[3,4]. When human or animal remains decompose outdoors, decomposition fluid and organic material is introduced to the soil, changing the microbial communities.[5] Microbes are an important piece of the community of organisms associated with decomposing carrion, so understanding their succession is important to a more general understanding of cadaver decomposition and forensics.[5] Beetles are often understudied and may impact the soil communities at a body because their behaviors are tied to the soil; it is important to investigate the microbial communities of both together.[6]

During a decomposition study completed in the fall, spring, and summer seasons of one calendar year, pitfall traps were used to collect necrophagous beetles visiting the carcass, along with soil samples from underneath stillborn swine carcasses (n=5 (summer), 6 (fall and spring)). Control soil samples consisted of three homogenized subsamples collected one meter from all carcasses at all points. Each beetle was surface decontaminated with two 10% bleach washes. The DNA of beetles and homogenized soil samples was extracted and the bacterial V4 region of the 16S rRNA gene was amplified using 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.

In the fall season, it took 32 days for the carcasses to be fully skeletonized, while the spring and summer seasons progressed more quickly, twelve and nine days, respectively. This was due partially to temperature and weather variation among seasons: fall temperatures ranged from 35.4°C to –3.3°C and spring from 31.6°C - 3.0°C. During each season, the beneath carcass soil samples had communities that were significantly different from the control samples, and each season had communities that were significantly different from each other. The soil communities also followed previously reported trajectories in other decomposition studies where Firmicutes was the most dominant bacterial phyla but was replaced by Proteobacteria as decomposition progressed.[2] During the fall and spring seasons, seven of the top ten bacterial families that increased in abundance in the soil by the end of decomposition were the same, suggesting that these taxa may be able to be used to identify if a body has been moved (e.g., the soil community found under a carcass is inconsistent with the observed stage of decomposition).

Preliminary analyses reveal overlap between beetle microbiomes and soil bacterial communities in both control and carcass soil samples. Overlap of beetle microbiomes and the soil between control and carcass will be evaluated to understand microbial overlap of necrophagous beetles and the soils associated with decomposing bodies. These results indicate that at different time points during decomposition, there are predictable changes in the soil bacterial communities which could allow for future soil evidence to be used to identify translocation of bodies and inform PMImin estimates.

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Microbial; Decomposition; Entomology

I45 Differential Biochemical Decomposition in Single and Mass Grave Deposition

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Learning Objectives: This presentation will inform attendees on the impact of differential decomposition in single and mass grave depositional contexts. The approach presented will cover state-of-the-art mass spectrometry-based biomolecules profiling and multi-omics integration.

Impact Statement: The presentation will demonstrate a new approach to study bone material from commingled depositional contexts, showing that omics techniques represent a valuable alternative to traditional molecular methods for the documentation and analysis of human remains.

Abstract Text: Omics techniques are becoming more frequently used in the analysis of human remains for forensic purposes. Their primary application is in understanding body decomposition dynamics and estimation of the postmortem interval. Furthermore, advances in omics integration techniques allow moving away from the traditional biological data modeling to a more holistic and mechanistic interpretation of the biological phenomena occurring postmortem. We hypothesize that this approach could be extremely beneficial in the investigation of complex decomposition contexts, such as mass graves. As a result, the study will focus on the analysis of both bone materials from donors buried in single and mass graves via metabolomics, lipidomics, and proteomics.

The experimental graves were set up at the Forensic Anthropology Center at Texas State's outdoor taphonomy facility and consisted of one mass grave with six human donors and three individual graves, each containing one donor. Bone samples were collected from the anterior portion of the tibia, both before burial and after 18 months. After this period, the graves were fully excavated, and the bodies were recovered. Peptides were injected in a reverse phase C18 column and subsequently analyzed on an Exploris 480 Quadrupole-Orbitrap Mass Spectrometer. Metabolites and lipids were injected in Hydrophilic Interaction Chromatography (HILIC) and in C18 columns and analyzed respectively on a high-resolution quadrupole Time-Of-Flight (qTOF) mass spectrometer. Both the proteomic and themetabolomic datasets were processed via Progenesis QI for Proteomics and Progenesis QI respectively for Tandem Mass Spectrometry (MS/MS) identification and relative quantification. Categorical statistical analysis showed correct classification of individuals buried in the mass grave compared to those from single graves. Furthermore, we discovered the possibility to distinguish between the individuals buried on the bottom of the mass grave from the ones on top of the grave.

Multi-omics integration allows us to simplify the highly dimensional nature of the datasets highlighting the presence of specific markers to distinguish between single and mass grave. Overall, the minimally invasive nature of the sampling and the vast amount of compound profiled suggest that mass spectrometry omics techniques are suitable for the analysis of complex depositional contexts.

Mass Graves; Omics; Decomposition

I46 A Metabolomic Regression Model for PMI Estimation on Human Pericardial Fluid

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Learning Objectives: During this presentation, attendees will be given information about the potential application of Nuclear Magnetic Resonance (NMR) metabolomics on an unusual biological matrix, namely pericardial fluid, easily collectable in routine forensic casework. This approach allows us to investigate Postmortem Intervals (PMIs) when traditional approaches cannot be applied or can be alternative/additional to other matrices of interest (e.g., vitreous humor).

Impact Statement: This presentation will impact the forensic community displaying results based on a metabolomic investigation conducted on postmortem human pericardial fluid. The results provided follow a proof-of-concept in which the approach was found suitable to investigate PMIs up to 100 hours. Increasing sample numerousness allows us to extend the window of interest up to 140 hours postmortem.

Abstract Text: In this day and age, PMI estimation still represents a conundrum for forensic pathologists. The available tools are scarce, inaccurate, and often not reliable in court. Among novel approaches, -omics sciences, especially metabolomics, are candidates as promising tools for accurate PMI estimation. The significant number of variables (metabolome), taken into account through a metabolomic approach and its evaluation through multivariate statistical analysis, is indeed potentially able to face the inter-individual variability related to in-life and death peculiarities. Pericardial fluid (PF) is a biological matrix of particular interest in the forensic field due to its distinctive physiology. Most of the available literature is focused on postmortem biochemistry and forensic toxicology investigations on PF. Our group has recently published a proof-of-concept study based on ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy on postmortem human PFs demonstrating a robust prediction ability in estimating PMI, particularly for intervals up to 100 hours.[1]

This approach was extended on a larger sample and on a wider time-window in order to corroborate the preliminary results. PF samples were collected in 65 judicial autopsies performed at Legal Medicine Institutes of University of Cagliari (n=42) and of Catholic University of Rome (n=23). Among the samples collected in Cagliari, 24 coincide with the previous dataset.[1] These samples were extracted and analyzed again to have identical experimental conditions and ascertain the intra-laboratory reproducibility.

Samples were collected from individuals displaying an inhomogeneous cause of death, a male-to-female ratio of 2:1 and a PMI ranging from 16 to 199 hours (78 ± 43). The only exclusion criterion was quantitative and/or qualitative alterations of PF macroscopically detected at autopsy. Low molecular weight metabolites were extracted using a liquid-liquid extraction procedure, which previously demonstrated better performance with respect to PMI estimation.[1] Metabolomic analysis was conducted via ^{1}H NMR and the obtained spectral data were submitted to multivariate statistical data analysis by using an orthogonally Constrained Partial Least Square regression model to estimate PMI avoiding the effect on metabolome exerted by age.

Fifty metabolites were exactly quantified. Unsupervised principal component analysis demonstrated that individuals' metabolome presented no difference related to site of collection (Cagliari v.s Rome). Samples were divided into a training set and an independent test set for external model validation. The prediction model performed well, corroborating previous results on a wider time-window (199 vs. 170 hours). Of note, best prediction error of our preliminary results was obtained in a time-window up to 100 hours being the prediction error 13 hours (13%). Due to the larger dataset, the present results allowed us to build up a robust regression model up to about 140 hours with an error in prediction of 23.5 hours (16.7%). Most relevant metabolites in the prediction model were identified.

The present study confirms that PF samples collected from a real forensic scenario represent a biofluid of interest for the estimation of the time since death. Notwithstanding a significant inhomogeneity in age, sex, and cause of death, which may potentially represent a bias, our results confirmed that PMI is the main driving force in metabolomic modifications occurring after death. Moreover, the larger dataset increased significantly the inter-individual variability and, consequently, the complexity of the underlying biological phenomena. Despite that and the increase of almost two days in the PMI window under investigation, the prediction error showed just a slight relative increase. While PF may not represent the biofluid of choice in the first 48 hours, it candidates as the proper biological matrix to be investigated in longer PMIs, at least up to 140 hours after death, when no reliable tools are currently available.

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Postmortem Interval; Metabolite; Death Investigation

I47 Estimation of the Postmortem Interval in Human Vitreous Humor by Infrared Spectroscopy.

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Learning Objectives: This presentation will provide a deep understanding of the challenges forensic pathologist face in case of Postmortem Interval (PMI) evaluation. After attending this presentation, attendees will understand how vibrational spectroscopy techniques like Fourier Transform Infrared (FTIR) and Attenuated Total Reflectance (ATR) can help assess the structural and chemical degradation of macromolecules inside vitreous humor. In fact, it demonstrates the potentiality of spectra to detect PMI.

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.

Abstract Text: **Background and Aim:** Determining the PMI is a challenging task. Traditional methods are effective for early PMI (less than 24 hours), but longer PMI (over 24 hours) require multiple methods to ensure accurate measurement and reduce bias. New approaches, such as protein/nucleic acids (DNA & RNA) degradation evaluation using western blot or mass spectrometry, tissue immunohistochemical analysis to identify a time-stable marker, and microbiome assessment by whole sample DNAs or 16S rRNA gene analyses have been applied for this purpose.[1-6] Our study aims to introduce FTIR spectroscopy as an innovative method for PMI estimation. It can detect how vitreous humor macromolecules progressively degrade after death and can help in reducing bias in long PMI when used in combination with other methods.

Methods: Seventy vitreous humor samples (40 males, 30 females), collected in the 2-187 hours postmortem range, were immediately stored at -80°C until the analysis. The age range was 31-99 years. The VH samples were divided into 6 groups based on the PMI range, each containing several samples. The groups were as follows: 16 samples belonged to Group 1, characterized by PMI in 2-10 hours range; 11 samples to Group 2 with PMI 10,5-15 hours; 20 samples to Group 3 with PMI in the range 16-33 hours; 9 samples to Group 4 with PMI in the range 35-55 hours; 6 samples to Group 5 with PMI in the range 60-72 hours; 8 samples to Group 6 with PMI over 81 hours. A sophisticated Invenio R interferometer with a Platinum ATR accessory equipped with a diamond crystal and a DTGS detector was used to capture the spectra. Spectra were then processed using OPUS 7.5 software for vector normalization and baseline correction. Multivariate and Univariate analyses were used to calculate and statistically analyze specific band area ratios. Principal Component Analysis (PCA) was performed to evaluate the ability of different groups of spectra in discriminating PMIs.

Results: The representative IR spectrum of human vitreous humor was in the range of ,1800-900 cm^-1. The most relevant peaks were correlated to known components of the vitreous humor, such as hyaluronic acid, collagen proteins (mainly type II collagen), and non-collagenous proteins, like glycoproteins and proteoglycans. Several peaks were ascribed to proteins, like those at around 1,657cm^-1 (Amide I), around 1,586cm^-1 (NH and CN bonds), around 1,410cm^-1 (COO- moieties of free amino acids), and around 1,314cm^-1 (Amide III), and some others to carbohydrates, like those at around 1,120cm^-1 (C-O moieties) and around 855 cm^-1 (CO and CC moieties). As time since death passes, the absorbance associated to the spectra of the various macromolecules changed in a statistically significant manner. The absorbance of collagen and non-collagen proteins presented a progressively decrease over time, showing a statistically significant difference between groups 1-2-3 and 4-5-6; free amino acids absorbance tended to increase over time, showing a statistically significant difference between the group characterized by PMI < 55 hours and PMI > 60 hours; phosphate groups absorbance tended to grow significantly in groups 5-6, if compared to 1-2-3-4. PCA showed that FTIR-ATR analysis is effective in discriminating PMI< 33 hours from PMI > 33 hours.

Discussion/Conclusions: For the first time in human samples, this study demonstrated FTIR-ATR's potential role in detecting macromolecular alterations correlated to PMI in a large group of vitreous humor samples. The observed spectral patterns reflected the time-dependent variations of multiple chemical components in the VH. This study showed that FTIR-ATR spectroscopy is a promising technique for PMI evaluation.

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Forensic Science; Postmortem Interval; Spectroscopy

I48 Why "Novel PMI Estimation Methods" Are Rarely Applied in Forensic Routine: An Example on a Decade of Research on Postmortem Protein Degradation

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Learning Objectives: Attendees of this presentation will learn about the essential elements required to establish the foundation for dependable Postmortem Interval (PMI) estimation methods across a diverse range of cases. Factors like institutional pressure to publish, peer competition, and editorial demand for "novelty" will be discussed in the context of the effortful process from the measurement of PMI-dependent alterations of a biomarker to the establishment of a method that can be confidently applied in practice.

Impact Statement: This presentation aims to improve our understanding of the necessary elements to develop reliable PMI estimation methods in the context of the challenging and demanding environment of research in forensic science.

Abstract Text: The estimation of the time since death, or PMI, represents a critical task in forensic routine. A small number of "classical methods," including the analysis of postmortem body cooling, the assessment of postmortem lividity, or rigor mortis, as well as forensic entomology are frequently employed, but often yield unsatisfactory results.[1]

For decades, forensic-intensive research has been conducted with the aim of identifying and establishing new postmortem markers on various targets using a range of different methods. In fact, a multitude of "novel PMI markers," and "new methods for time since death estimation" have been put forth, yet tangible advancement, particularly in terms of routine implementation, remains elusive.

The reason for this is that it is a lengthy and effortful process from the measurement of PMI-dependent alterations of a biomarker to the establishment of a method that can be confidently applied in practice. Additional factors can be identified when considering the researchers' perspectives in designing experiments to optimize the cost–outcome ratio. This encompasses elements such as institutional pressure to publish, peer competition, and editorial demand for "novelty."

This presentation addresses the essential elements required to establish the foundation for dependable PMI estimations across a diverse range of cases. Additionally, it illustrates how valuable insights can be derived from limited background data.[2] We have invested significant effort into researching postmortem protein degradation for PMI estimation ourselves. Over the past ten years, our group has published data from animal models as well as human autopsy cases and body donors from decomposition facilities.[3-7] These studies have investigated the basics of protein degradation, detected changes of biomarkers, described confidence intervals, examined potential individual and environmental influencing factors, and optimized and standardized the methodology for reliable applicability.[8] This background will be used as an illustrative example of what can be achieved after a decade of research on postmortem protein degradation and how the boundaries of what is possible can be continually extended.

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PMI; Method Development; Proteins

I49 Closing the Divide: Evaluating the Discrepancy in Forensic Entomology Research and Casework Needs

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Learning Objectives: Attendees will learn which forensically relevant blow fly species are commonly encountered in human casework across North America. Specifically, they will learn the distributions of these forensically relevant species and what information is available about them and how this information can aid in legal investigations. They will also learn where some of the largest gaps exist within the field and what research and training avenues should be considered to reduce these gaps to strengthen the field.

Impact Statement: This presentation will offer recommendations on which forensically relevant blow fly species should be considered for research to strengthen the use of forensic entomology in casework. Additionally, this presentation will highlight the gaps in entomological evidence collections at a state level to determine where professional trainings should be considered.

Abstract Text: Forensic entomology is the use of insects and other arthropods in legal investigations.[1] Due to the valuable information insects can provide, it is a continually growing field. Even though there has been an increase in its use, there are still very large gaps in the information that is available to access when analyzing entomological evidence. Blow flies (Diptera: Calliphoridae) are the most often-encountered insects in investigations, making them one of the most valuable assets in this field. One way they are utilized in casework is to help determine how long remains have been in an area. This is done by determining the insect's age and calculating a Time Of Colonization (TOC), or when eggs were laid, making developmental data extremely important.[2] However, there is a disconnect between what insects are being encountered in investigations and the forensic entomology research that is occurring.

A survey was sent to active forensic entomologists across North America to investigate which blow fly species have been encountered in each state and Canadian Province. The survey asked the participant to list the species of blow flies that were collected associated with human remains during casework. Additionally, publications that referenced human casework in North America were also included. Responses were received from 14 individuals and of the data collected, three species were found to be predominant in casework across North America, including *Phormia regina* (Meigen) (31 states/provinces), *Lucilia sericata* (29 states/provinces), and *Calliphora vicina* Robineau-Desvoidy (27 states/provinces).

This presentation will discuss all blow fly species collected from human casework across North America and compare that information to the current published research in the field. Specifically, we will focus on blow fly distribution and developmental data available to ascertain where our largest gaps are in the field. Additionally, beetles are the second-largest groups associated with human remains and yet, the amount of research conducted on them in miniscule compared to those of flies.[3] Specifically, skin beetles (Coleoptera: Dermestidae) are common on human remains throughout the country but are prevalent in desert environments. Therefore, skin beetles associated with human remains in desert environments will also be discussed with a cross comparison on available literature. Recommendations on how to reduce these gaps through research and strengthen the field overall will be provided.

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Forensic Entomology; Blow Flies; Distributions

I50 Who Let the Dogs Out? A Comparison of Antemortem and Postmortem Canine Activity

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Learning Objectives: During this presentation, the audience will have reviewed canine activity in a case of dog mauling in the setting of decomposition changes. Afterward, these individuals should be able to identify characteristic findings and patterns at autopsy to differentiate antemortem injury from postmortem predation.

Impact Statement: This presentation will augment the ability of the audience and the forensic community to determine differences in antemortem and postmortem findings at autopsy to facilitate the determination of cause of death and manner of death in cases that involve canine activity.

Abstract Text: In 2023, canines were the number one most popular pet in the United States with around 65.1 million households owning a pet dog.[1] However, interactions with these playful and loving pups are not always friendly. Fatalities from being bitten or struck by a dog during 2011-2021 average at about 43 deaths per year in the United States.[2] Thus, antemortem canine injury at autopsy is an uncommon finding, making recognizing these injuries more challenging. Moreover, determining the role animal activity plays in the cause and manner of death can be difficult, especially in the setting of decomposition changes.[3] However, not all canine activity at autopsy is injury and may be postmortem predation as dogs are well known for scavenging.[4] Understanding the implication of key findings and differences between antemortem and postmortem canine activity in such cases is critical in making determination of cause and manner of death.

The decedent was a 28-year-old male with a history of asthma and tobacco use who was found deceased with decomposition changes in the backyard of another individual's residence. He was last known to be alive approximately five days prior when he called his significant other and described having car troubles near where he was found. Per the investigative report, upon returning home from a trip, the homeowner found the decedent while cleaning his backyard. There were multiple dogs known to live at the residence with free access to a fenced-in section of the backyard and a bus that had been converted into a dog shelter. The decedent's body was located between the bus and the fence, lying prone and partially underneath the bus. Sections of the fence were noted to be broken at the top near where the decedent was found. Additionally, on the scene, there was evidence of illicit substance use in the form of a small blue plastic bag containing an unknown white, powdery substance.

At autopsy, there were numerous puncture wounds, lacerations, and abrasions of the face, neck, torso, and extremities consistent with canine activity. The only significant natural disease process noted was patchy myocardial scarring. Furthermore, the decedent was in a stage of early-to-moderate decomposition. Postmortem toxicology was positive for delta-9 carboxy THC and cocaine in liver tissue. Investigation of the canine activity was determined to be antemortem injury based on comparison to postmortem canine predation, particularly in relation to pattern, extent, associated wounds, and location. Additionally, the position and location of the body and evidence from the scene, including the disturbed fence, supported the conclusion of the injuries being antemortem. Several aspects of the case posed complications in determining the cause and manner of death, such as the positive toxicology, decomposition changes, and lack of blood at the scene, among others.

Canine activity at autopsy creates challenges for forensic investigation, especially when this is further complicated by postmortem changes. Delineating antemortem injury versus postmortem activity is crucial for determining cause and manner of death in some cases. These changes typically present differently; therefore, the investigation of the circumstances of death and scene often play a critical role for these cases. In this case, considering all the evidence available at the time from forensic investigation and autopsy findings allowed for the determination of the cause of death to be due to injuries from dog mauling with a manner of death of accident.

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Forensics; Canine Mauling; Postmortem Predation

I51 Impacts of Vertebrate Scavenging on Blow Fly Oviposition on Human Remains

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Learning Objectives: Decomposition ecology is important to consider when examining assumptions of oviposition.

Impact Statement: The results directly contradict the long-standing assumption that scavenging openings and/or penetrating trauma sites are preferentially and/or primary sites of oviposition.

Abstract Text: Blow flies are primary colonizers of carrion due to their innate ability to detect, locate, and colonize vertebrate remains quickly after death.[1] A long-held belief in forensic entomology states that sites of trauma (ante-, peri-, and postmortem) should act as suitable oviposition (i.e., egg-laying) sites for flies. Though some research exists to support this theory, recent empirical work demonstrated that postmortem trauma does not always result in fly attraction and oviposition.[2,3] Furthermore, no empirical research exists investigating the impacts of postmortem trauma on fly oviposition using human remains.

This project aimed to examine the relationship between vertebrate scavenging events and blow fly oviposition on human remains at the Anthropology Research Facility (ARF) in Knoxville, TN. Over the course of two years, a total of 31 human donors were placed supine on the soil surface and monitored for oviposition and scavenging, with six donors having pre-existing penetrative trauma (gunshot wound, surgical incisions, colostomy bag). No enrollment restrictions (e.g., weight, age, trauma) were placed on these donors. An additional 22 donors were placed along with game cameras to capture more high-resolution scavenger behavior during early decomposition. As the additional 22 donors were part of another study, enrollment in the project was restricted to donors without trauma (including autopsy), weighing less than 170kg, and between the ages of 26-95. Blow fly oviposition and scavenging events were documented for the first three days after placement of each human donor.

Blow fly oviposition occurred on all 53 donors with a mean of 504ADH, with 24 donors experiencing vertebrate scavenging prior to fly oviposition. In the six donors with antemortem penetrative trauma, oviposition occurred exclusively on the head region of the body away from any trauma, supporting recent research in this area.[2,3] Overall, raccoons were the most active scavengers (occurring on 81% of donors) and exhibited proportionally more scavenging activity on the arms and legs of the human donors compared to other body regions. Out of the 24 donors that were scavenged prior to fly oviposition, only four individuals were observed with oviposition in scavenging openings (16%).

The literature states that oviposition should occur preferentially in trauma openings regardless of etiology, with recent documentation of fly oviposition in putative vertebrate scavenging sites on non-human remains.[4-9] These results of our study directly contradict the long-standing assumption that scavenging or traumatic openings are primary sites of blow fly oviposition. As previous work has demonstrated that microbially-produced Volatile Organic Compounds (VOCs) can mediate fly oviposition, we hypothesize that vertebrate scavenging may disrupt interkingdom communication on the remains, specifically quorum sensing and microbial signaling to flies, resulting in the almost total avoidance of flies to scavenged sites. Clearly, there is a need to further explore fly attraction to carrion resources, including the mechanisms mediating fly oviposition decisions in the heterogenous decomposition landscape. We suggest more holistic research examining specific interkingdom cues associated with human remains, including those that may have significant impacts on decomposition trajectory.

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Scavenging; Blow Fly; Oviposition

I52 Morphometric Analysis of Posterior Spiracles for Species Identification of Necrophagous Third Instar Larvae

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Learning Objectives: This presentation will help attendees understand: (1) the necessity of species identification of fly larvae in forensic entomology; (2) the newly proposed posterior spiracle structures (ABS, DBS, and LBS) for species identification of fly larvae; and (3) the newly proposed Error-rate Based Morphometrics (EBM) method for species identification.

Impact Statement: This presentation will demonstrate a new direction and utility for fly larvae identification. As a tool accessible to non-experts, it can be used for rapid species identification in the early stages of scene investigations.

Abstract Text: The identification of fly larvae and pupae collected from cadavers is essential for estimating the minimum Postmortem Interval (mPMI).[1] However, unlike adult flies for which identification keys are well established, distinguishing the species of fly larvae and pupae remains challenging even for experts.[2,3] For species identification, raising larvae or pupae to adulthood or relying on DNA barcoding incurs additional time, effort, and cost. Therefore, a method to easily identify species through the morphology of fly larvae and pupae would be highly useful in forensic entomology practice. The authors have devised a method to easily distinguish species by measuring the posterior spiracle structures of third instar larvae for nine species of the family Calliphoridae, which are commonly encountered in actual death cases in Korea.

Through geometric analysis and measurement of the posterior spiracular structures of third instar larvae from nine species of blowflies in Korea, it was confirmed that three metrics—Angle Between Slits (ABS), Distance Between Slits (DBS), and Length BiSlit (LBS)—are effective for species identification. By analyzing 151 wild type specimens, species identification criteria were established and validated using a statistical method devised by the authors, known as Error-rate Based Morphometrics (EBM). First, the indices (ABS, DBS, LBS) of the unknown species are measured, and the error rates between these measurements and the standard values for each species are calculated. The species with the lowest sum of error rates is then selected for identification.

Using the new method based on EBM, it was confirmed that species identification is possible among the nine species, except for two sister species pairs: the *Lucilia illustris-Lucilia caesar* and the *Chrysomya pinguis-Chrysomya megacephala* pairs.

This study demonstrates that it is possible to distinguish most species of Korean blowflies encountered in forensic cases through morphometric analysis of the relatively simple-to-measure posterior spiracles of larvae. Currently, additional research is being conducted on the identification of species in the family Sarcophagidae and Muscidae, comparison of features such as the arrangement of papillae around the posterior spiracles, and species identification through AI image analysis. It is anticipated that once species identification for major Korean species encountered in forensic cases becomes more streamlined, the identification of fly larvae collected at the scene can be performed more rapidly. This will enable quicker estimation of the mPMI and potentially assist in directing investigations during the initial stages of forensic inquiry

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Forensic Entomology; Scene Investigation; Entomology

I53 *Lucilia Mexicana* and Its Population Structure in Texas

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Learning Objectives: Attendees will learn about the use of a microsatellite panel to assess the population structure of *Lucilia mexicana* in Texas. The panel discussed and the resulting clusters can lead to insights regarding *L. mexicana*, its biology, and its forensic importance.

Impact Statement: The research presented shows that the populations structure of *Lucilia mexicana* is correlated to the abiotic factors present across the Texas ecoregions. The identification of three distinct populations of *L. mexicana* suggests different developmental needs or adaptations that could be of forensic importance. Additionally, the microsatellite panel can be applied to gain insights into other *Lucilia spp*. and *L. mexicana* throughout time to determine if changes have altered the population structure in recent years.

Abstract Text: Forensic entomology involves the use of insects in criminal and civil investigations, necessitating an understanding of the species encountered and their populations for accurate Postmortem Interval (PMI) estimations. PMI estimations rely on the knowledge of insect biology, such as the development of blow flies. Calliphoridae (Order Diptera) are among the first insects to arrive at decomposing carrion and play a crucial role in PMI estimations. *Lucilia mexicana* Macquart 1843 is a blow fly of forensic importance that can be collected in the southwestern United States.

A 2010 survey revealed that *L. mexicana* was the first to arrive at carrion traps in shaded areas across Texas during the summer.[1] Texas, with its diverse ecoregions, provides different habitats that can lead to subpopulations. To determine if *L. mexicana* populations correlate with Texas ecoregions, the population structure of specimens collected during the survey was assessed using an updated eight microsatellite panel. This panel included new and previously published markers and was designed to amplify *Lucilia spp.* DNA.[2,3] The panel was designed to assess the population structure of *L. mexicana* (n=114) collected from the 11 Texas ecoregions. Amplification was performed using Type-it Microsatellite PCR Kit (QIAGEN) and underwent capillary electrophoresis using an ABI 3500 with genotyping Genemapper ID-X v 1.6. All loci were polymorphic with more than five alleles at each locus. Six loci were in Hardy-Weinberg disequilibrium ($p < 0.05$), and no linkage was detected. A moderate to high genetic differentiation was detected (Fst=0.205) suggesting the potential of genetic clusters. A Principal Component Analysis (PCA) indicated the presence of three clusters, supported by STRUCTURE analysis. Additionally, sequencing data revealed that Short Tandem Repeat (STR) motifs are not shared between *Lucillia spp.*, although there is amplification of the markers. The observed clusters could arise from adaptations to the temperature and precipitation gradients in Texas.

Further research is needed to refine the panel as well as determine if climate change has affected the population structure. This study shows the population heterogeneity in *L. mexicana*, highlighting its importance in forensic entomology and the need for continued exploration.

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Entomology; *Lucilia mexicana*; Texas

I54 Visualizing and Clustering the Species in the Mixture of Pollen Flow Cytometry

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Learning Objectives: After attending this presentation, attendees will have a better understanding of how flow cytometry data of pollen mixtures can be visualized on 2D projection by Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) and clustered by Gaussian Mixture Model (GMM).

Impact Statement: This presentation will impact the forensic science community by introducing dimension reduction methods and clustering techniques to analyze pollen mixtures by flow cytometry.

Abstract Text: 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]

Transmission Light Microscopy (TLM) or Scanning Electron Microscopy (SEM) are currently accepted methods to identify pollen grains based on morphological features; however, these techniques require time and effort.[1,4,5] In addition, the limited available information about the technique, a shortage of specialized palynologists, and funding reduction for forensic pollen studies have all contributed to its almost complete absence in United States crime labs.[1] To address 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 is based on the same fundamental principles of traditional pollen morphological analysis, while also providing high-throughput screening capabilities needed by modern crime labs.

One fundamental of flow cytometry analysis is gating that is a technique to identify homogenous groups of individual grain. However, the technique might overlook the high dimensionality of flow cytometry data that may not have fully represented in the histogram and the 2D scatter plots.[6] We implemented unsupervised machine learning techniques that could reduce multi dimensions to two dimensions by UMAP. UMAP is based on manifold theory and topology theory and is being applied into many fields.[7] Furthermore, the result of the mixture pollen was clustered by applying GMM and K-means.

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 contained forward and backward scattering, as well as fluorescence. After applying UMAP to the mixtures of pollen, a scatter plot showed that the data points were separated into three clusters. Additionally, K-means and GMM were used to analyze the result of UMAP. We found that GMM could separate the background from the species and the species were identified on their own groups.

We also considered flow cytometry of each pure species of willow and mustard. The result of the pollen mixtures analyzed by UMAP was projected onto each species. The visualizations of each species showed that data points were spread across the same areas as the clusters in the pollen mixtures analyzed by UMAP. Clustering techniques were then applied to the results, so GMM could identify both the background and each pure species.

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Mixture of Pollen Grains; Flow Cytometry; Dimension Reduction

I55 A Comparison of Drying Characteristics of Insect Stains and Human Bloodstains

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Learning Objectives: Attendees of this presentation will understand the "coffee ring" principle of drying human blood, the unique drying characteristics of stains produced by adult flies following consumption of human blood, and be able to distinguish human bloodstains from insect stains based upon radial crack patterns.

Impact Statement: This presentation will impact the forensic science community by serving as a source of foundational information that can augment traditional means of investigation of trace evidence and insect pattern evidence associated with violent crimes characterized by bloodshed.

Abstract Text: Insect stains produced by necrophagous flies can be considered time stamps of crime scene succession. This concept considers that insect stain formation is dependent on specific events that in turn have time dependent characteristics. For example, deposition of insect stains can only occur if blood shed occurs. Flies rarely have immediate access to wet blood, meaning that a period of time has elapsed between blood release and when a fly interacts with shed blood.[1] Consequently, the time required for blood to dry must be considered under the conditions of the crime scene, as well as an estimate of the length of time before the adult fly likely accessed the scene and then interacted with blood. Of course, numerous factors can influence these events, thereby yielding a range of time estimates for adult fly-bloodstain interactions.

Adult calliphorids demonstrate relatively stereotypic behaviors during interactions with blood, especially when foraging on dried bloodstains. The process includes wetting dried blood through regurgitation, followed by proboscis probing of stains before imbibing liquids, storage in the crop, which may lead to regurgitation or chemical digestion in the midgut, that culminates with fecal elimination to yield defecatory stains.[2-4] Once regurgitate or defecate is deposited onto a surface, the onset of drying ensues. To our knowledge, there are no published reports that detail the characteristics of insect stain drying.

In this investigation, the drying characteristics of insect stains produced by the blow fly *Calliphora vicina* following ingestion of human blood were examined using image analysis of stains deposited or produced on different surface materials (i.e., ceramic tile, glass, polystyrene plastic, cotton shirt) that varied in porosity and topography. The duration of drying and final morphologies of insect stains were influenced by the surface material used for deposition, ambient conditions (predominantly temperature), and volume of fluid. The latter was most apparent with regurgitate stains, which typically required 2-3x longer to dry under ambient conditions than comparable sized bloodstains without anti-coagulant.

As human blood dried on non-porous surfaces, a perimeter ring (coffee ring effect) formed, followed by radial cracks, and eventually lateral cracks between the radial lanes.[5-7] The entire process occurred in >10min at 25°C on ceramic tile when 2µl of blood was deposited directly onto the tile surface. In contrast, regurgitate stains deposited directly by adult flies never formed perimeter rings comparable to human bloodstains and rarely formed radial cracks. When radial cracks did form, they were most commonly associated with regurgitate stains on glass and lacked symmetry like human bloodstains. Defecatory stains never formed coffee ring-like perimeter edges nor were radial or lateral cracks ever observed with dried fecal fluids. In experiments in which regurgitate was forcibly exuded from individual adult flies after consumption of human blood, the drying patterns were more consistent with blood than naturally deposited regurgitate in that most stains formed thickened perimeters and radial and lateral cracks during drying on all non-porous surfaces. The results of this investigation indicate that some insect stains can be distinguished from human bloodstains based on morphological differences of dried stains.

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Forensic Entomology; Bloodstains; Insect Artifacts

I56 Optimizing Efficiency: Evaluating the Role of Limited Examination in Non-Suspicious Deaths Outside the Residence

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Learning Objectives: Participants will learn to identify cases in which proper evaluation of contextual scene findings and medical history can yield efficiencies in limited examination. They will learn the prevalence of natural disease, toxicologic findings, and occult trauma in decedents found outside their residence or near/inside their vehicle with otherwise non-suspicious scene findings.

Impact Statement: This presentation examines atraumatic cases where the decedent is found outside their residence or near/in their vehicle, potentially identifying a population that could be included in limited examination policies, alleviating workload issues and conserving resources. This will improve Medical Examiners' (MEs') ability to identify cases, which could be accurately certified with a limited examination. This would improve office performance and efficiency, without impacting the stakeholder (general population/county) with less accurate medicolegal death investigation.

Abstract Text: The field of forensic pathology is facing a significant challenge due to rising workload and a critical shortage of forensic pathologists.[1] The current workforce is less than half of the estimated national requirement.[2] ME offices have seen a significant increase in cases, especially due to the opioid epidemic and COVID-19 pandemic, which has led some to expand policies allowing for the use of limited examination in cases with documented medical history and benign circumstances.[3] The Harris County Institute of Forensic Sciences (HCIFS) experienced a 20.44% increase in ME cases from 2019 to 2022.[4]

This study examines the utility of full autopsy in cases where the decedent was found outside of their residence or near/in their vehicle with otherwise non-suspicious circumstances. The HCIFS database was queried for all adult deaths from January 1, 2019, to December 31, 2023, where the medicolegal death investigator reported the death as occurring outside without external trauma. The following types of cases were excluded: found in water, witnessed collapse, moderate/advanced decomposition, and cases with a clear class I cause of death visible on external examination. We identified 163 cases meeting these criteria.

The most frequent manner of death was natural (n=103; 63.2%), followed by accident (n=54; 33.1%), with undetermined (n=3; 1.8%), suicide (n=2; 1.2%), and homicide (n=1; 0.6%) comprising the remainder. Most decedents were found near or inside their vehicle (n=119; 73%), with 44 (27%) found outside their residence. There was no significant difference in the manner of death between the two groups ($\chi^2(4) = 8.35$, $p = 0.08$). Cardiovascular disease was the most common cause of natural deaths (n=87, 84.5%). Accidents were overwhelmingly toxicologic (n=52, 96%). Only 16 cases (9.8%) revealed significant internal pathology without prior history or associated external findings, primarily acute myocardial infarction and pulmonary thromboembolism (each n=5, 29.4%).

External examination with toxicology would have accurately classified 97.1% (n=101) of cases with documented medical history (n=104, 63.8%). Only three cases had trauma, only one of which, a subdural hemorrhage, had no external findings. Use of advanced imaging techniques, such as Computed Tomography (CT), would be able to diagnose the trauma cases (one gunshot wound and two subdural hemorrhages) and many of the natural cases with significant internal pathology, including pulmonary thromboembolism and ruptured acute myocardial infarction. Including decedents found outside their residence or near/in their vehicle in ME office policies allowing limited examination for those with significant past medical history and benign circumstances could alleviate workload without compromising the accuracy of the medicolegal death investigation and forensic process.

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Autopsy; Death Investigation; Medical Examiner

I57 An Application of 3D Virtual Animation in Complicated Suicide Scenarios: A Multidisciplinary Approach to Forensic Investigations

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Learning Objectives: From this presentation, the attendees will learn how to manage complex crime scenes through a shared working protocol. Advanced technologies such as 3D computer-aided design and animation will allow for an accurate interpretation of the dynamics of the events.

Impact Statement: Through our presentation, the forensic science community will have the opportunity to learn about the benefits derived from the use of technology and acquire new knowledge aimed at creating a shared working protocol. The new skill acquired will enhance crime scene reconstruction and evidence interpretation in the future. These advancements will lead to more precise and insightful investigative outcomes, positively impacting both professionals and stakeholders involved in forensic cases.

Abstract Text: The study of the crime scene and event dynamics is a continuous challenge in the field of forensic sciences. It represents a fundamental tool in determining the time, cause, and means of death. Professionals often find themselves working in highly complex scenarios characterized by the presence of numerous objects and alterations caused by the sequence of events.

The authors present a case report of a young adult's suicide involving a penetrating traumatic brain injury caused by a telescopic metal rod.[1] The death occurred due to a sophisticated system of weights and levers ultimately resulting in the penetration of the metallic object into the skull through the temporal bone. To determine the time and cause of death, an external examination of the body, a full forensic autopsy, and histological and immunohistochemical examinations were performed, along with a Computed Tomography (CT) scan of the body, which helped in understanding the severity of the brain injury.[2] Substances of abuse were investigated through toxicological examination.

Considering the infrequency of such events, to better understand the victim, the scene, and the dynamics of the suicide, and assess the various hypotheses of the investigators, 3D Computer-Aided Design (CAD) and animation were used.[3] The 1:1 scale virtual environment facilitated visualization and was crucial in understanding the actual movement of the machinery components, which finally led to the fatal impact of the rod. Physical properties such as mass and moment of inertia were accurately reproduced, and the dynamics were calculated via software, ensuring realism and physical consistency. Videos and photos extracted from the animation highlighted specific moments of the dynamics.

This case posed a significant challenge for both forensic pathologists and forensic engineers who collaborated in the investigations. Especially in such complicated cases, technology serves as an essential tool for visualizing the scene and the victim, validating investigative hypotheses, and facilitating exposition and understanding during court proceedings.

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Penetrating Traumatic Brain Injury; Suicide; 3D Reconstruction

I58 Enhancing Medicolegal Death Investigations: The Successful Adoption of Postmortem Computer Tomography (PMCT) Technology at the Institute of Forensic Sciences in Puerto Rico

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Learning Objectives: In this presentation, attendees will learn how the integration of PMCT can enhance the efficiency and effectiveness of medicolegal death investigations. Specifically, they will gain a comprehensive understanding of how PMCT reduces the workload of conventional autopsies, prioritizes cases, and optimizes resource allocation. Furthermore, participants will be able to assess the practical steps involved in implementing PMCT, including investment, staff training, and handling logistical challenges, particularly those faced in the aftermath of Hurricane María. This presentation will demonstrate how PMCT can serve as a valuable routine screening tool in forensic investigations.

Impact Statement: This presentation will significantly impact the forensic science community by demonstrating the practical benefits and successful implementation of PMCT at the Institute of Forensic Sciences of Puerto Rico. Attendees will gain valuable insights into how PMCT can enhance medicolegal death investigations, streamline autopsy processes, and ensure efficient resource allocation. By sharing our experiences and detailed steps taken for the integration of PMCT, we aim to provide a replicable model that other medical examiner offices can adopt. This will ultimately lead to improved accuracy in forensic investigations, adherence to professional standards, and better service to communities, particularly in high-volume and crisis situations.

Abstract Text: After attending this presentation, attendees from medical examiner offices may find it beneficial to consider integrating PMCT as a standard screening tool within their facilities. This approach offers several advantages, including enhanced case prioritization, more efficient resource allocation, and improved guidance for determining the necessity of selective or targeted autopsies. By potentially reducing reliance on conventional autopsies in certain cases, PMCT has demonstrated its ability to optimize forensic processes and expedite investigations.

The aftermath of Hurricane Maria in September 2017 presented profound challenges for Puerto Rico, particularly concerning the management of over 4,000 deaths possibly linked to the natural disaster. The logistical complexities related to cadaver storage, identification, autopsy procedures, and family support overwhelmed existing protocols and risked compromising compliance with the National Association of Medical Examiners (NAME) autopsy standards, risking accreditation. This crisis underscored the critical need for strategic interventions.

Once the purchase of refrigerated wagons was made to increase storage space, the integration of PMCT emerged as a practical approach to reduce the number of conventional autopsies being done at our institution. Implemented in 2021, PMCT notably reduced autopsy numbers by 715 in 2022 and by 565 in 2023, compared to 2020. This highlights its efficacy in optimizing forensic procedures and ensuring prompt investigations for our communities.

While increasing the number of forensic pathologists would be the ideal long-term solution, we recognize that it would take considerable time to train an adequate number of professionals to meet national demand. Therefore, it is crucial to explore immediate and effective approaches, such as the adoption of advanced technologies like PMCT scans. With this tool, these challenges can be overcome, ensuring timely and accurate forensic investigations for the benefit of our communities.

By sharing our jurisdiction's experience on the initial inversion, installation, image storage capacity, and staff training, we aim to provide valuable insights and practical strategies to assist other high-volume jurisdictions facing similar challenges.

CT Scan; Autopsy; Disaster

I59 The Potential Help of Technology in Rare Diseases: The Case of a Child With Gorham-Stout Syndrome

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WITHDRAWN

I60 Bayou Blues: Unraveling Mysteries of the Murky Depths of the Houston Bayou System

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Learning Objectives: Harris County, home to the city of Houston, TX, contains more than 2,500 miles of bayous and creeks. Although the bayou system serves multiple practical functions, such as natural water filtration, drainage and flood reduction, and recreation, it poses a unique forensic risk. By the end of this talk, participants will be able to recognize the unique forensic hazards associated with the Houston bayou system, enhancing their ability to effectively investigate and interpret cases involving bodies found in these environments.

Impact Statement: This presentation will discuss deaths occurring in or near bayous in Houston while highlighting the specific challenges and considerations necessary while working on these cases. This knowledge will improve participants' ability to accurately assess postmortem changes, identify potential causes of death, and gather relevant forensic evidence from this unique environment.

Abstract Text: The Houston bayou system, comprising a network of more than 2,500 miles of waterways interconnecting the city and its surrounding areas, presents unique challenges and considerations for forensic pathologists which are not commonly encountered in other geographical locations.[1] The goal of this retrospective study is to investigate the deaths occurring near or within a bayou, summarize the major findings commonly seen in these cases, and review the challenges faced by forensic pathologists when working on these cases.

The database at the Harris County Institute of Forensic Sciences (HCIFS) was queried for all deaths occurring at or near a bayou from January 2003 to June 2024. Four hundred sisty-six deceased individuals were found in or near the bayou system during this timeframe. The bayou with the most deaths was Buffalo Bayou followed by Braes Bayou. The most common manner of death was accident (45%, 209/466), followed by undetermined (27%, 126/466). Drowning was the most common cause of death (44%, 204/466), followed by undetermined (20%, 95/466). Homicides comprised approximately 11% (53/466) of the bayou deaths, with gunshot injuries being the most common cause of death (43%, 23/53).

As evidenced by the alarmingly high undetermined rate of approximately 20%, the bayou system poses challenges in the determination for an accurate cause of death. The single most important factor in this is the decomposition changes encountered in these cases. Not only does the bayou water environment accelerate decomposition changes, but the fluctuating water levels can also conceal or carry bodies large distances, delaying time in recovery and resulting in further decomposition. Another obscuring feature occasionally encountered is the presence of aquatic animal activity, such as alligator predation, which can mask critical findings precluding an accurate cause of death.

Although bayou deaths comprise a relative minority of the overall cases encountered at the HCIFS, one of the major functions of the medical examiner is to provide vital statistics regarding the community. Motor vehicle collisions and drownings comprised approximately 46% (216/466) of the total bayou-related deaths. One of the major reasons for these deaths is the open access nature of the water system. In many areas, roads and walkways traverse adjacent to the waterways and lack physical barriers. This design enables people to freely interact with the bayous, posing a serious hazard and providing a convenient dumping ground for bodies.

While the Houston bayou system enriches the city with its ecological benefits and recreational opportunities, it is a hidden reservoir of death posing challenges for medical examiners. Understanding these complexities is crucial for improving safety measures and enhancing forensic investigation capabilities.

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Water Deaths; Medical Examiner; Retrospective Study

I61 Non-Traumatic Companion Cases: A Ten-Year Review and Examination of Investigative and Autopsy Findings

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Learning Objectives: The goal of this presentation is to highlight principles of death investigation and pathology examination in cases where more than one decedent is present at the same location.

Impact Statement: This presentation will impact the forensic science community by providing increased awareness regarding investigative strategies and considerations for pathology in companion cases; and the importance of regular welfare checks for individuals in need of assistance even when caretakers are present.

Abstract Text: Companion death cases can be defined to include two or more deaths that occur at the same location or were transported to a hospital from the same location.[1] The investigation into these deaths illicit additional responsibilities and inquires. Companion cases present considerations for environmental concerns, such as carbon monoxide, hypothermia, hyperthermia, and chemical contaminants; drug or alcohol abuse; infectious disease; and concerns for foul play. To determine the cause and manner of death for non-traumatic companion cases, a more in-depth investigation is usually required.

For carbon monoxide toxicity, identifying the source includes a thorough search for generators and active gas appliances. In suspected hyperthermia or hypothermia, investigators record the ambient and body temperature, check for operational electricity, and determine how the decedents attempted to regulate their temperature. Observations like diaphoresis or paradoxical undressing can also indicate temperature-related deaths. With chemical contaminants, the type of chemical, source, and decedent activities at the time of exposure are recorded. When infectious disease is a concern, investigators inquire about recent complaints and look for signs of illness, such as medications, used tissues, and an abundance of electrolyte or water beverages. With drug and alcohol involvement, investigators ask about any history of use, search for signs of illicit substances, paraphernalia, alcohol containers, and signs of drug use on the body, such as puncture wounds or foam near the nose or mouth. Companion cases raise concerns for foul play prompting searches for injuries, potential weapons, blood, and signs of disarray, missing valuables, or forced entry. In cases where decedents exhibit varying stages of decomposition, investigators explore the relationship between decedents. Often, this scenario involves a caregiver and a dependent.

The electronic database of the Harris County Institute of Forensic Sciences was searched for all companion fatalities between 2013 and 2023, excluding traumatic causes of death such as gunshot wounds or motor vehicle accidents. This study presents 96 deaths occurring at 44 locations.

The number of decedents at each scene were two (n=40), three (n=1), four (n=2), or five (n=1). The manner of death was accident (n=60), natural (n=32), or undetermined (n=4). The cause of death was due to drug or alcohol toxicity (n=34); natural causes (n=22), including cardiovascular disease and diabetes mellitus; carbon monoxide toxicity (n=15),three of which also had the presence of illicit drugs; infectious disease (n=12), including acute bronchopneumonia, H1N1 influenza viral infection, and coronavirus disease 2019; chemical exposure at work (n=6); undetermined (n=4); hyperthermia (n=2); and choking (n=1).

Twenty-three decedents had decompositional changes. In several cases, variable states of decomposition were noted, suggestive of one decedent's death preceding the other. Many of these cases involved a caretaker who was discovered to be in a more advanced stage of decomposition than the decedent they cared for.

Different manners of death from the same scene occurred in three incidents. The first was the death of a caretaker that included blunt trauma to the lower extremities and whose dependent spouse died from dehydration and malnutrition complicating dementia. The second was in a skilled nursing facility, where on the same day one person choked on a food bolus and the other died from dehydration and cardiovascular disease. The third was the natural death of a son from cardiovascular disease, who was the carer for his elderly mother. She subsequently died due to unintentional neglect, and the manner of death was accident.

In conclusion, reviewing these cases enhances our understanding of the complex analysis involved in companion cases, provides greater awareness of the importance of welfare checks, especially in caregiver-dependent situations, and highlights the critical role of a thorough medicolegal death investigation.

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Companion Cases; Scene Investigation; Drug Toxicity

I62 A Meeting With Death: How Medical Examiner/Coroner Offices Can Help Support the Surveillance of Respiratory Illnesses

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Learning Objectives: Attendees will learn ways that point-of-care tests can be utilized in the morgue, specifically in times of epidemics/pandemics; discover how the medical examiner's office can benefit from having an epidemiologist on-site; and understand the value of medical examiner/coroner cases being utilized to supplement respiratory viral surveillance performed by public health.

Impact Statement: Our project shows how an on-site epidemiologist facilitates timely reporting of health events, grants assistance with data requests and annual/quarterly reports, and can establish a more secure thread of communication between public health and the death investigation community. Tests performed in-house can decrease turnaround time in negative cases for respiratory illnesses and diagnostic results from the public health lab allow medical examiners to make fully informed decisions when determining cause of death.

Abstract Text: Respiratory viral surveillance in Georgia is typically acquired through hospitals and clinics volunteering to report specified health events. In 2021, the Council of State and Territorial Epidemiologists (CSTE) offered a grant to examine "Unexplained Respiratory Deaths" with the intent to expand respiratory viral testing in the postmortem environment and encourage collaboration between public health and medical examiner/coroner offices. Cobb & Douglas Public Health (CDPH) and the Cobb County Medical Examiner's Office (CCMEO) partnered for this grant opportunity.

All accepted medical examiner cases were tested for 18 respiratory viruses by a CDPH epidemiologist stationed onsite at CCMEO. Two point-of-care platforms were utilized to decrease test turnaround time. The Abbott ID NOW platform was utilized to test for COVID-19 from January 2021 through December 2023. The Biomerieux BIOFIRE SPOTFIRE respiratory/sore throat panel was utilized to test for an additional 15 respiratory viral targets from April 2023 through December 2023. Additional swabs were sent to the Georgia Public Health Laboratory (GPHL) for confirmatory testing. Tests include the Centers for Disease Control and Prevention (CDC) Influenza SARS-CoV-2 (Flu SC2) Multiplex test for COVID-19 and Influenza A/B, the Illumina Respiratory Virus Oligos Panel V2 (RVP) for 15 common respiratory viruses from January 2021 through July 2022, and the Truemark Respiratory Panel 2.0 Taqman Array Cards System from July 2022 through December 2023. The epidemiologist from CDPH stationed at CCMEO was to assist in sample collection, testing, results/disease reporting, and anything else needed.

By implementing point-of-care testing at CCMEO, autopsy delays due to testing backlogs during the COVID-19 pandemic were eliminated. While the sensitivity of the Abbott ID NOW and Biomerieux BIOFIRE SPOTFIRE have been highly variable, the specificities of each platform have remained high at 94.5% and 99.2%, respectively. Over the course of three years, more than 2,200 decedents have been tested with 373 cases positive for COVID-19, 16 cases positive for Influenza, and 303 cases positive for a respiratory virus other than COVID-19 and flu. Approximately 215 of the cases tested were reported to public health due to the decedent's death being caused by a reportable disease. It was also found that 126 cases were infected with more than one respiratory virus, with a maximum of five viruses infecting a single person.

Utilizing CCMEO cases for surveillance can be effective in establishing a more accurate incidence rate for pathogens circulating in the general population by testing a population that is less likely to have sought medical intervention. The epidemiologist onsite has encouraged timely reporting of health events while also assisting in compiling data requests, analyzing data for annual reports, and establishing a more secure thread of communication between public health and the CCMEO. Point-of-care testing performed by the epidemiologist can be utilized to determine which cases will most likely need additional respiratory testing performed and which cases are truly negative. Additionally, diagnostic results from GPHL allows medical examiners to make fully informed decisions when determining cause of death, specifically in cases with respiratory illnesses.

Postmortem; Surveillance; Forensic Epidemiologist

I63 When Lightning Strikes! A 22-Year Review of Fatalities in Houston, Texas, and New York City

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Learning Objectives: After attending this presentation, attendees will understand the characteristics of fatalities caused by lightning strikes, including scene investigation and autopsy examination, as well as the mechanism of injury and death.

Impact Statement: This presentation will impact the forensic science community by reviewing fatalities due to lightning strikes, most of which are preventable deaths, enhancing their ability to analyze a death scene, understand the mechanism of injury and death, perform an autopsy examination, and consider public education and safety regulations to reduce these deaths.

Abstract Text: Lightning is a transient, high-voltage electric discharge consisting of direct current of up to 50,000 amperes. It represents a significant natural hazard, striking the earth approximately eight million times per day.[1,2] Every year in the United States, approximately 23 people are killed due to a lightning strikes, although this number may be underreported due to factors such as misdiagnosis.[3] Over the past ten years, Florida had the highest number of deaths, Texas was second, and New York was equal in the eighth position.[4] There are multiple mechanisms of injury, including direct or indirect strikes such as electrification of the ground. Lightning can result in injury to the body through four means: light, heat, barotrauma, and electricity, which can cause cardiac arrythmias and sudden death due to depolarization of the entire myocardium.[5]

The electronic database of the Harris County Institute of Forensic Sciences and the Office of Chief Medical Examiner of the City of New York was searched for all fatalities due to lightning strike between 2002 and 2023. Twenty-two cases were identified.

Of the 22 fatalities, 19 were male and 3 were female. The average age was 35 years (range: 13 to 65 years). There was no survival interval in 11 cases; and a survival interval ranging between 1 day and 34 years in 11 cases, with the longest survival interval related to epilepsy following a remote lightning strike. Eight cases underwent external examination only where there was a survival interval, and 14 cases underwent an autopsy examination. In 21 cases, the cause of death was due to a lightning strike; and in one case, the lightning strike caused a residential fire, resulting in death due to smoke inhalation and thermal injuries. The manner of death in all cases was accident.

All cases of lightning strike occurred outdoors (except the residential fire) and the circumstances surrounding the lightning strike were: a soccer game (n=5) – either sheltering under a tree (n=3) or on the soccer field (n=2); at work (n=5) – landscaper (n=2), utility line worker (n=1), installing drain lines (n=1), working on fence (n=1); on a business property (n=2); walking on a street (n=2); riding a bicycle (n=1); electrification of the field (n=1); residential yard work (n=1); golf course (n=1); residential park (n=1); on a beach (n=1); and on the roof of a building (n=1). Cases mostly occurred during the summer: August (n=6), July (n=3), and June (n=2); and on a Sunday (n=6) or Thursday (n=5). Five scenes were associated with other people being struck by lightning at the same scene who survived. One scene (sheltering under a tree) was associated with two fatalities.

Autopsy findings included electrical burns (n=21), singed hair (n=7), tympanic membrane perforation (n=5), Lichtenberg figures (n=3), thalamic hemorrhage (n=1), myocardial hemorrhage (n=1), and soot deposition (n=1). The body regions where electrical burns were observed were the torso (n=21), lower extremities (n=14), neck (n=6), head (n=6), and upper extremities (n=5). Clothing examination revealed defects and burns. Extensive thermal injuries with charring and soot deposition in the airway were noted in the residential fire fatality.

Fatalities associated with lightning strikes are rare but are certainly at risk of being unrecorded due to inadequate investigation and examination. The approach to the case begins at the scene to document any environmental evidence such as burn marks, tree damage, or soil disruption; witness statements; surveillance cameras; and review of the recent weather pattern, including lightning activity. Most fatalities were found in young men engaged in outdoor activities or who were at work, and there is a clear role for public education and safety regulations to reduce these deaths.

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Lightning; Scene Investigation; Autopsy

I64 Foreign Matter and Surgery: A Forensic Approach to Identification and Source Determination

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WITHDRAWN

I65 Did George Floyd Die of Cardioinhibition From Pressure on His Neck?

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Learning Objectives: The attendee will be enabled to: (1) recall the physiologic responses to pressure on the neck; (2) become acquainted with the clinical and forensic literature on instantaneous deaths; and (3) describe what to look for in cases of neck trauma and of instantaneous deaths.

Impact Statement: The forensic pathology community will understand the possibility of instantaneous deaths and of allegations of such in court.

Abstract Text: We were motivated to study whether the carotid sinus might have been involved in the death of George Floyd from the knee on his neck by Derek Chauvin.

We define Instantaneous Neurogenic Cardiac Arrest (INCA) as an immediate collapse or death mediated through a vagal neural reflex. The subject of neural reflex deaths has been controversial and not adequately studied or discussed.[1-6] We have performed a comprehensive review of the clinical and forensic literature to understand the subject matter, although there are significant gaps in the literature. Specifically, we reviewed the carotid sinus anatomy, baroreceptor physiology, and the known mechanisms of neurally mediated syncope.[7-9] We have also reviewed carotid sinus massage and carotid hypersensitivity, along with any known case reports involving deaths thought to be due to carotid sinus stimulation from the literature.

The carotid sinus is not fully developed until adolescence.[10] It is the primary baroreceptor of the body and the principal method for blood pressure control.[7] Reflex syncope occurs from vagal cardioinhibition and vasodepression. When the carotid sinus is stimulated, the heart can come to a complete standstill. However, it almost always recovers.

We found 8 cases in the clinical literature of INCA death from carotid sinus massage; all are old case reports, not all of which are well documented.[11-17] These deaths appear to be due to the development of ventricular fibrillation, not asystole.

We have found 12 cases of INCA without trauma to the neck reported in the forensic literature and 13 cases of INCA with trauma reported in the forensic literature.[18-31] The documentation of these cases varies. Authors have pointed to an absence of hemorrhage and other vital signs in some cases and the presence of hemorrhage in "reflex zones" in some cases involving trauma.[25,27]

INCA appears to occur, but rarely, from injury or pressure on the neck at the carotid bifurcation without compromise of the vascular or airway. Still more rarely, INCA can occur from other neural triggers, such as injury to the eyes, celiac plexus, or pleura. The mechanism of death in these cases is from ventricular fibrillation, not asystole. Neck trauma is not a necessary finding. Underlying cardiovascular disease or carotid bifurcation traumatic hemorrhage need not be present in these cases. These deaths have occurred in the young and old but have not been reported in children. We conclude that George Floyd did not die from INCA. His cardiac arrest was not instantaneous or near instantaneous but prolonged. Floyd died of pulseless electrical activity, not asystole or ventricular fibrillation.

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Sudden Death; Neck Injury; Cardiac Death

I66 Drug-Related Deaths in the Elderly: A Five-Year Overview of Western Michigan

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Learning Objectives: After attending this presentation, attendees will recognize that elderly Americans experience drug-related fatalities, though they are not typically the population that comes to mind when discussing drug overdoses. Attendees will gain a better understanding of the substances and medications implicated in these deaths as well as the distribution of manners of death among these individuals.

Impact Statement: This presentation will impact the forensic science community by highlighting that elderly Americans, a population in which the leading causes of death are natural diseases, including cardiac disease, cancer, COVID-19, and cerebrovascular disease, are not immune to drug-related fatalities. The development of unique strategies for overdose prevention may be necessary for these individuals.

Abstract Text: In a Census Bureau brief from 2023, it was reported that older adults aged 65 years and over accounted for 55.8 million (16.8%) of the total United States population in 2020.[1] This age group tripled in size from 2010-2020 in comparison to the growth seen from 2000 to 2010.[1] From 2000 to 2020, the rate of drug-related deaths among adults aged 65 years and over also tripled from an age-adjusted rate of 2.4 to 8.8 deaths per 100,000.[2] In 2023, 2,646 deaths of older adults aged 65 years and over were reported to the Medical Examiner's Office (MEO) at Western Michigan University Homer Stryker M.D. School of Medicine (WMed). This number represents 58.9% of the deaths reported to the MEO and about 20% of the total deaths in the counties served, which includes 13 counties throughout western Michigan. More than half of Medicare enrollees in this older population take four or more prescription drugs daily, increasing the risk of intentional and unintentional drug-related deaths.[3] The authors sought to examine the impact of the opioid crisis on the aging population within this region.

An advanced search was performed within the web-based case management system that houses deaths reported to the MEO within WMed. This search included all deaths of adults aged 65 years and older reported in one of 13 western Michigan counties between January 2019 and December 2023 in which a drug, substance, or poison was listed as contributory to death. The minimum age was then extended to 60 years and older to capture a larger-than-expected group of drug-related deaths in older adults and allow for the comparison of decade age groups.

A total of 301 cases were initially identified, with 255 cases of adults aged 60 years and over confirmed to be drug related. Forty-six cases were excluded due to non-natural contributors, such as drowning or asphyxia. The decedents ranged in age from 60 to 91 years with the average age at death set at 65 years. Over 82% of these deaths were of individuals in their sixties. Of the confirmed 255 cases, 116 (45.5%) were between the ages of 60 and 65 years.

Accidental deaths accounted for 84.7% of these cases while deaths by suicide accounted for 13.3%. There were also four indeterminate deaths and one homicide. Of the 255 total cases, 163 (64%) involved one or more opioids and just over 47% of deaths by suicide included an opioid. Methamphetamine proved noteworthy within this population, contributing to 25.5% of these deaths singularly and in combination with other substances. From 2019 to 2023, this age group represented 15.6% of all (1,629) drug-related deaths reported to the MEO at WMed.

While forensic pathologists pay special attention to elderly deaths for concerns of abuse or neglect, the authors demonstrated the importance of a thorough medicolegal death investigation, including pill count(s) and searching for other signs of illicit or prescription drug use. Without this, forensic pathologists run the risk of missing intentional or unintentional drug-related deaths that require full autopsies and comprehensive postmortem toxicological examinations. Thorough death investigations and examinations can inform health care and community partners in the design and implementation of unique overdose prevention strategies for this vulnerable population.

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Overdose; Elderly; Substance Abuse

I67 Fatal Intoxications vs. Congenital Cardiac Disorders in Sudden Death: Where to Draw the Line?

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Learning Objectives: The presentation will cover a frontier in forensic research and an actual challenge in forensic practice: the differentiation of fatal acute intoxication vs. arrhythmogenic deaths in young (< 35 years) cases. The focus will be put on the indication to a combined approach based on rigorous traditional autopsy examination and the correct toxicological and genetic methodology

Impact Statement: The presentation is aimed to impact on different forensic experts, in particular on forensic (cardio)pathologists, toxicologists and geneticists, stressing (through a combination of real cases and general considerations) the criteria for indication of specific examinations, their correct application, and the relevance in terms of legal and public health goals.

Abstract Text: Most cases of sudden death in the young (< 35 years) are related to fatal acute intoxications or to cardiac conditions.[1-3] Since these latter disorders mainly are congenital (i.e., congenital cardiomyopathies and channelopathies), with autosomal dominant inheritance, incomplete penetrance, and variable expressivity, when they are found at postmortem investigation, the entire first-degree family must be screened.[4-6] Arrhythmic deaths cause by congenital cardiac disorders and fatal intoxications not only share the main age group but also the lack of frequent/explicit autopsy signs in the young victims. Indeed, microscopic signs like focal myocarditis can be the only signs of both scenarios, with the differential diagnosis being particularly difficult especially when the correct toxicological methodology is not applied (or the toxicological values are not high) or the postmortem genetic testing is not indicated after the autopsy.[6-8] That being said, rigorous differential diagnosis is pivotal, because the diagnosis (traditionally per exclusionism) of congenital cardiac disorder has serious implications of the relatives of the victim, while identifying the intoxication as the cause of the death entails legal consequences for the drug dealer.

The presentation will start with a general description of the recurring macroscopic and microscopic features in deaths in young subjects caused by congenital cardiac disorders, fatal intoxications or by a combination of them, also showing and describing specific cases investigated by our group for each of these categories. We have already performed an unpublished review of the issue and we have collected three cases, with iconography, to be used as examples during the presentation. Then, a proposition of multidisciplinary workflow based on the combination of rigorous toxicological, pathological, and genetic testing will be presented. Finally, it will be discussed if and when specific drugs or combinations of different drugs are actually able to elicit electrical responses that mimic congenital disorders (e.g., QT prolongation caused by fentanyl) and what information the pathologist should consider in order to infer whether a trigger role of illicit substances on congenital substrate is possible.[9] In conclusion, as we will discuss, due to the many confounders in these cases and the lack of specific findings, it is fundamental to share a combined diagnostic routine based on multiple technologies and know-hows, and in particular a rigorous toxicological testing, (cardio)pathology examination, and postmortem genetic testing.

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Sudden Cardiac Death; Cardiomyopathy and Channelopathy; Forensic Toxicology

I68 Illicit Drug Deaths in Decedents Over 60 Years of Age in the City and County of San Francisco From 2020 to 2024

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Learning Objectives: At the end of this presentation, attendees will understand that a significant number of older people use and die from illicit substance use and will order toxicology testing appropriately to capture this data. Additionally, attendees will know that many of the symptoms of illicit drug use can be confused with symptoms of natural disease, the presence of natural disease does not rule out drug use, and that the presence of natural disease may not reflect an accurate cause of death. Illicit drug use in older people is not a new problem, but attendees will learn that this issue is not well known and has continued after the COVID-19 lockdown.

Impact Statement: Deaths in older people are often certified as natural deaths after review of medical records and without toxicology testing. Without an increased level of awareness, deaths can be misclassified as natural when they are actually due to illicit drug use. Older persons do use and die from illicit drugs, and this information needs to be captured for public health purposes and to improve statistics on drug use in all populations and keep older people alive and healthier through better funding of drug rehabilitation programs specifically for elderly people.

Abstract Text: The United States Census Bureau projects that the number of older adults will increase from 40.3 million and 72.1 million between 2010 to 2030, due to the aging of the "Baby Boomers" (those born between 1946 to 1964).[1] As people age, they acquire medical conditions, but don't necessarily discontinue the bad habits from their youth, such as illicit drug use. The reasons for this include physical addiction, chronic pain, heightened anxiety about their health and about the aging process or circumstances around loss and living conditions, finances, depression, or grief. Additionally, the type of drugs used between older and younger people seem to differ.

Between 2003 to 2012, illicit drug use in those between the 60 to 64 years of age has increased from 1.1 percent to 3.6 percent.[1] This percentage is similar to what was found in older decedents by our office between the years 2005-2016.[2] More recently from 2020-2024, the age group from 55-64 years routinely had the highest percentage of drug overdoses.[3] When presenting to their physician, elderly people (many of whom have existing natural disease diagnoses) may have symptoms that may be indistinguishable from those of drug use such as altered mental status or memory loss, and their clinicians may attribute these symptoms to natural disease such as dementia or stroke rather than screen for drug use. Drug treatment programs specifically designed for older people are not widely promoted or available, although older adults do respond well to treatment.[1]

In our office, approximately 23 percent of the older people (those between the ages of 60-86) died of illicit drug use in the years 2020-2023, remaining relatively stable before and after the COVID -19 pandemic.[3] In contrast to younger people (those under the age of 60), where fentanyl was found in approximately 78% of overdose deaths, only 63% of older overdose deaths had fentanyl detected. Younger people also tended to have more methamphetamine (56%) than older people (42%), and older people tended to have more cocaine (55%) than younger people (35%). The vast majority of the elder deaths due to drug overdose had paraphernalia at the scene and most were found in their own residence (that is, they were housed at the time of death). Most of these deaths are found in more economically challenged and socially isolated seniors, not only from the homeless and COVID-19 pandemic as might be expected in younger people.

Substance use has been declared a public health emergency.[4] This presentation will illustrate that older people, often with natural disease, may use illicit substances as well. Screening for illicit drug use after death gives insight and information to those treating live people and may prompt interactive treatment to prolong the life of elders using illicit substances and increase their quality of life.

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Drug Abuse; Elderly; Death

I69 Sodium Azide/Nitrite Suicides in the Netherlands Between 2015 and 2022

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Learning Objectives: The attendees will learn about the prevalence of sodium azide and nitrite suicides. They will learn how to investigate these suicides. The attendees will learn about the characteristics of the individuals using these methods compared to what is known in literature.

Impact Statement: Case reports about the use of sodium azide and nitrite have been presented at AAFS before, but no research on population basis has been performed until now. After this presentation, pathologists, toxicologists, and forensic physicians have the tools to recognize and analyze these suicides in their own state. By showing characteristics and trends, we give starting points for suicide prevention.

Abstract Text: In 2017, a "right-to-die" organization publicly announced the legally available and existing chemical sodium azide as a humane method of suicide. Sodium nitrite was found as an alternative by members of the organization. Sodium nitrite ($NaNO_2$), a less expensive alternative that was easier to purchase, also became more well-known.[1] Sodium azide is a toxic chemical known for its use in laboratories as a fungicide, and its former use in airbags because of its explosive nature. Sodium nitrite is also a toxic chemical that is used as a preservative for foods such as meat and as a coloring agent.[2] Both chemicals are possible to detect using postmortem analysis after external examination or during autopsy.[3]

The announcement in 2017 caused commotion in the Dutch society. Internationally, case reports have been published about these methods, and suicide kits with this substance were offered online.[4,5] In this study, we investigated how many sodium azide/nitrite suicides were reported before and after the announcement, as well as characteristics of the deceased.

Based on the registration of forensic physicians working at the Public Health Services and data from Statistics Netherlands, an observational study was conducted into the number of sodium azide/nitrite deaths in the period from 2015 to 2022 in the Netherlands. The number of sodium azide/nitrite suicides in that period was compared with the total number of suicides and suicides by auto-intoxications in that same period.

Between 2015 and 2022, 172 sodium azide/nitrite suicides were identified on the basis of external examination reports drawn up by forensic physicians. The first case was reported in 2017. The total group contained approximately an equal number of men (53%) and women; 38% of all suicides were 70 years or older at the time of death with an average age of 59 years. The majority of the people using sodium azide/nitrite died at home. In 22% of the cases (n= 38), other people, such as family and/or friends, were present during the suicide. In 10% of the cases (n=18), the decedent had been taken to the hospital prior to their death after being found intoxicated; 19% of the individuals had asked their doctor for euthanasia and more than half were denied.

Compared to all suicides in the Netherlands, people who opted for sodium azide/nitrite were more often female ($p < 0.001$) and on average older ($p < 0.001$); 70% (n= 120) had a history of psychiatric illness. The use of sodium azide/nitrite for suicide increased between 2017 and 2022, with a provisional peak (n= 50) in 2021. The rate sodium azide/nitrite suicides in relation to the total number of suicides and intoxications per year increases gradually in the period 2017–2021.

This study is the first to our knowledge that investigates the numbers of sodium azide/nitrite suicides and the associated characteristics of the individuals. Since 2017, a rise in these suicides was observed. More research to investigate characteristics of the individuals should be done in order to create accurate suicide prevention methods in the future and pay attention to the trends regarding the use of these suicide powders.

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Suicide; Forensic Medicine; Sodium Azide

I70 Fatal Drug Intoxication in the Pediatric Population of El Paso, Texas

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Learning Objectives: After presenting this abstract, attendees will be able to: (1) identify the demographic patterns and risk factors associated with pediatric drug intoxication in El Paso, TX; (2) understand the high incidence of polysubstance use and the common substances involved; (3) recognize the significance of accidental deaths versus suicides and the role of psychiatric history; (4) highlight the importance of targeted prevention efforts and education for the most affected demographic, including the need for parental awareness and safe medication storage practices; and (5) compare the demographic data from El Paso, TX, with data from the National Fatality Review-Case Reporting System (NFR-CRS).

Impact Statement: This presentation provides critical insights into the unique pediatric population of El Paso, TX, a region not extensively covered in previous forensic studies. By detailing demographic patterns, risk factors, and substance use trends, it fills a significant gap in the forensic literature. The findings underscore the need for region-specific prevention and intervention strategies, enhancing the forensic community's understanding of local pediatric drug intoxication issues. This data can inform tailored approaches to forensic investigation and public health initiatives, potentially influencing policy and practice to better address the needs of similar populations.

Abstract Text: **Background:** Pediatric fatal drug intoxication is a devastating and often preventable event. Understanding the demographic patterns, risk factors, and trends associated with drug-related incidents among pediatric patients in El Paso, TX, is crucial for developing effective prevention and intervention strategies.

Methods: This study retrospectively reviewed all pediatric cases (ages 0–18 years) documented by the El Paso County Office of the Medical Examiner between years 2009 to 2024. Data on cause of death, manner of death, medical history, and social background were analyzed to elucidate demographic characteristics and identify risk factors associated with acute drug intoxication.

Findings: A total of 24 cases were analyzed, with ages distributed as follows: 14 years (2 cases), 15 years (2 cases), 16 years (8 cases), 17 years (7 cases), and 18 years (5 cases), with a notable male predominance (70.83%). The demographic composition revealed that all decedents were White, and 75% of the cases were of Hispanic ethnicity. The primary manner of death was accidental (75%), while 25% were certified as suicide. Polysubstance death accounted for 50% of the cases, with fentanyl (11), alprazolam/benzodiazepines (8), and cocaine (5) being the most common substances. Sixteen cases (66.67%) had a history of illegal drug use, and nine cases (37.5%) had a documented history of psychiatric illness. Additionally, 16% had experienced recent personal loss. Notably, 62% of incidents occurred at home. One suicide case involved a patient with extensive medical history and chronic pain who was denied access to a pain specialist until adulthood.

Conclusions: Understanding the demographic patterns and risk factors associated with fatal drug intoxication among pediatric patients in El Paso, TX, has significant implications for prevention strategies and clinical management. The majority of cases involved Hispanic males aged 14 to 18 years, indicating a higher risk for this demographic. The predominant manner of death was accidental (75%), with a notable portion certified as suicide (25%). Many decedents had a history of psychiatric illness (37.5%) and/or recent personal loss (16%), highlighting the need for accessible mental health services and support systems. Half of the cases involved polysubstance use, emphasizing the complexity of substance abuse issues and the importance of comprehensive prevention programs. The most abused substances included fentanyl, benzodiazepines, and cocaine, suggesting these should be primary targets for drug education and intervention. The high percentage of incidents occurring at home (62%) underscores the need to educate parents and guardians on recognizing signs of drug abuse and the importance of safe medication storage.

These findings correlate with data reported by the NFR-CRS in the United States from 2004 to 2024, particularly regarding the notable prevalence of opioid intoxication (63%), the significant occurrence of deaths at home (63%), the age distribution of decedents (66% aged 15-17), the sex distribution (59% male), and the racial demographics (78% White).[1] Fentanyl and cocaine were also among the top substances involved in illegal drug-related deaths.[1] Finally, the case of a suicide patient denied access to a pain specialist until adulthood highlights gaps in the health care system that need addressing to prevent similar tragedies.

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Pediatric; Drug; Intoxication

I71 A Multicentric Study on Forensic Investigation of Bodies Exposed to Fire: The Importance of an Interdisciplinary Approach

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Learning Objectives: After this presentation, participants will gain information on the main circumstantial, radiological, autoptic, histopathological, and laboratory findings in cases of corpses exposed to fire and their differences according to the degree of the heat-related injuries.

Impact Statement: This presentation will have an impact on the forensic scientific community as it highlights the importance of a multidisciplinary approach when assessing the cause and manner of death in bodies exposed to fire. It also proposes an operational protocol to guide these assessments.

Abstract Text: Fire may produce deep and destructive changes in tissues and organs, depending on the anatomical areas affected, temperatures, and duration of exposure. Injuries can range from localized burns to extensive charred areas or complete carbonization. This study aims to investigate the presence of different patterns between bodies with second-to-third-degree burns and charred corpses potentially useful for forensic pathologists in establishing the cause and manner of death of subjects exposed to fire.

A retrospective analysis was conducted using autopsy data involving burnt or charred bodies referred to various Italian institutes of legal medicine between 2017 and 2023. The cases (51) included were categorized according to the degree of fire-related injuries: bodies with second-to-third-degree burns (24) and charred corpses (27).

Epidemiological (victim's gender and age), clinical (e.g., history of psychiatric disorders), circumstantial (e.g., site of the recovery, fire source, presence of weapons near the corpse), and forensic data from radiological examination, external inspection, autopsy, histological, toxicological, genetic, and odontological analysis (e.g., presence of foreign bodies, traumatic fractures, organ injuries, heat-related injuries as well as injuries unrelated to heat, carboxyhemoglobin blood concentration) were recorded for each group.

Key results indicate a high percentage of male subjects in both groups (75% in bodies with second-to-third-degree burns and 68% in charred corpses). Burned bodies were most commonly recovered from homes (33.3%) and workplaces (25%), while charred corpses were frequently found inside vehicles (48.1%). Heat-related injuries such as fractures, amputations, loss of substance, and skin splits were present in 50% of burned bodies, compared to 96.3% of charred corpses. Blunt and sharp force injuries were reported in 8.3% of burned bodies and 22.2% of charred corpses. Among burned bodies, only one case had fire-related injuries classified as postmortem, whereas 30% of charred bodies had postmortem injuries caused by heat. In both groups, exposure to flames was the most frequent cause of death (62.5% in burned bodies, 70.4% in charred corpses). Accidental deaths were most common among burned bodies (66.7%), whereas charred corpses did not show a predominant manner of death (37% homicide, 33.3% accident, 29.7% suicide).

The results of our study, obtained through a multidisciplinary approach that includes death scene investigations and the collection of radiological, autopsy, toxicological, genetic, and odontological data, enabled us to identify characteristic patterns in burned and charred bodies. These patterns are related to the dynamics of death and may be useful to forensic pathologists.

Charred Body; Burn; Multidisciplinary

I72 Fire and Blood: Homicide With Combined Sharp Force Injury and Carbonization

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Learning Objectives: Our presentation is particularly interesting because it describes a unique case of a combined homicide that took place in a brutal manner. The key message of this presentation is the importance of integrated crime scene analysis in association with investigations in order to identify the burned body and to find the killers.

Impact Statement: This presentation will impact the forensic science community by describing how to analyze the circumstantial data and the results on-site inspection. In addition, an in-depth description of the particular lesions found will also help in future cases of combined homicide.

Abstract Text: Damage to the tissues arising from the application of heat is commonly encountered in forensic pathology and sometimes provides a challenging problem in the distinction between antemortem and postmortem burning. This may lead inexperienced observers to suspect that antemortem wounds have been inflicted, with the fire being used to cover up a criminal offence.[1]

Carbon monoxide in the blood is a valuable indicator that the victim was alive after the fire began. Where a dead body is disposed of by arson, no absorption can take place as the gas can gain entry only through the pulmonary interface. Thus, a Carboxyhemoglobin (HbCO) blood concentration of more than 5% suggests that breathing occurred after the fire began. However, the fact that a body in a fire does NOT have HbCO in the blood does NOT mean that they must have been dead before the fire began.[2]

The purpose of this work is to present a case of combined homicide characterized by carbonization that occurred on a living victim, motivated by the aggressors' will to hide the body. As expected, in homicides, the discovery of more than one potentially lethal instrument can make the reconstruction of the dynamics and the ultimate cause of death by the medical examiner difficult. This can be particularly challenging when carbonization is involved, such as in the present case. In these circumstances, it is therefore essential to carry out a meticulous forensic inspection and analyze the crime scene first to assess the position of the victim's body and to isolate any weapons or harmful means. In addition, it is essential to analyze macroscopically and microscopically the skin flaps and soft tissues adjacent to the lesions in order to assess their viability. Moreover, carbon monoxide value in the blood is mandatory. This is necessary first to understand if the victim at the time of fire was still alive or had already died.

This study presents the case of a young unknown boy found dead in a deserted ground. Forensic examination, external examination, complete autopsy, routine microscopic histological examination, and toxicological investigation were performed. At the time of the discovery, the body presented the "pugilistic attitude." It was characterized by carbonization of the trunk, the left upper limb and the lower limbs as well as two weapon wounds on the neck.

At the autoptic examination, subgaleal hemorrhagic infiltration in the right frontotemporal area, cerebral edema, fracture of hyoid bone; glottis' edema, subpericardial and subpleural hemorrhagic petechiae, pulmonary edema, reddish-bluish blood as well as parenchymal section surfaces of a bright red color in the context of multi-visceral congestion were found. Microscopically, hematoxylin and eosin staining were performed, confirming the macroscopic findings and showing vitality of all the weapon wound. Finally, carbon monoxide in the blood was 9-11% and no drugs were found.

Despite the carbonization, we were able to identify the victim by IV e V right hand fingerprint, thanks to hard synergically work of the coroner and police. After that, it was then possible to identify the killers and manner of death.

The postmortem investigations showed the cause of death of the young boy to be murder by a combination of two different modes, that is, attributable to the combined action of a cutting injury and carbonization.

Finally, based on our experience, it is essential to search, even without macroscopic and microscopic signs suggestive of carbonization, such as soot in the tracheal mucus. for HbCO in blood to determine whether and when the carbonization has acted in the determinism of death.

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Homicide; Identification; Forensic Biometrics

I73 Models of Autopsy Techniques Compared in the Era of Minimally Invasive Techniques

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Learning Objectives: After attending this presentation, attendees will understand the importance and feasibility of new minimally invasive autopsy techniques in forensic practice

Impact Statement: This presentation will impact the forensic science community by demonstrating the innovation and usefulness of new minimally invasive autopsy techniques, which can replace classic autopsy techniques in selected cases.

Abstract Text: **Introduction:** Various advances in medicine have not replaced the diagnostic value of autopsy; on the contrary, they have increased the potential information that can be obtained from it. In recent years, ethical, legal, and socioeconomic issues have contributed to declining autopsy rates. The traditional autopsy techniques, dating back to the 19th century, proposed by Virchow, Ghon, Letulle, and Rokitansky, are currently a fundamental point of reference in the forensic field. Minimally invasive autopsy has recently been introduced as an alternative to the traditional open approach. The aim of this work is to compare the minimally invasive autopsy techniques with the standard autopsy procedure, evaluating the contribution that these new methods can offer in forensic practice.

Materials and methods: A review of the scientific literature was carried out through the analysis of the search engines Pubmed NCBI, SCOPUS, and Google Scholar. The following keywords were used "new autopsy techniques," "modern tools in autopsy practice," and "minimally invasive autopsy." Papers published in the past 15 years were analyzed. The selection criteria included only papers that analyzed the utility of new autopsy methods. Papers that only dealt with standard autopsy results in the absence of the evaluation of a new method or technology in autopsy were excluded. After removing duplicates, a preliminary selection was made based on title and abstract and then on the full text. A total of 30 papers were selected. The results were examined and compared, especially regarding the advantages and diagnostic sensitivity and specificity of new methods with respect to traditional autopsy.

Results: Our literature review demonstrates that there are emerging and significant minimally invasive autopsy techniques. Conventional and endoscopic autopsies have been described in literature. The results of the two procedures showed a high correlation (100%) for detecting intraperitoneal, thoracic hemorrhages, and hepatic, splenic, and diaphragmatic injuries. The results showed a slightly lower correlation (60-80%) for mesenteric and retroperitoneal injuries.[1,2]. Brain lesions were identified (95%) with a reduction in the risk of inhalation of bone dust. These minimal approaches were used mainly in cases of deaths due to infectious diseases SARS-CoV-2 or neonatal deaths.[3,4]. Collection of body fluids and tissue samples is equally possible by laparoscopy. In addition, endoscopic autopsy using the fiberscope allows the localization and extraction of bullets and shrapnel, also making use of the support of imaging techniques (ultrasound, MRI). The technique proved to be accurate, faster than conventional autopsy, and left the body intact. Our review also analyzed thoracoscopic, laparoscopic, endoluminal, and endovascular approaches. A cadaveric thoracoscopic examination has been described inserting the trocars on the seventh, fifth, and third intercostal spaces to evaluate the mediastinum and pericardium.[5,6] The review of the literature showed that cadaveric laparoscopy can be performed by making a subumbilical-based incision and other small incisions to insert four trocars. The data found that in comparison with traditional complete autopsies, the accuracy of diagnosis by minimally autopsy was 94.4%, sensitivity was 90%, specificity was 100%.

Discussion and conclusion: The minimally invasive autopsy technique has various potentials and advantages: it provides information on the cause of death while also minimizing the disfigurement of the body; it is more tolerated by the relatives of the deceased; it helps in determining the cause of death even in the most disadvantaged populations with a high mortality rate; it reduces the risk of contagion for health care personnel; it reduces the cost and time of a postmortem study. This is a modern approach, proving to be an alternative in situations where conventional autopsy is not possible. However, extensive research in larger study groups is needed to determine whether the minimally invasive autopsy can totally replace conventional autopsy.

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Forensic Science; Autopsy Techniques; Minimally Invasive Autopsy

I74 The Role of Forensic Anthropology and Radiography in Supporting Identifications in a Medical Examiner's Office

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Learning Objectives: After attending this presentation, attendees will better understand the role of forensic anthropologists in supporting or refuting decedent identifications using radiographs. Forensic anthropologists are able to effect identifications by comparing evidence of antemortem skeletal trauma, surgical interventions, and other pathologic conditions as seen on radiographs to those specified in medical records or provided by next of kin.

Impact Statement: This presentation will impact the forensic science community by showing that the expertise of forensic anthropologists can be utilized in a less traditional manner to support identifications of decedents.

Abstract Text: Forensic anthropologists contribute to the identification of individuals in a variety of ways. Practitioners may contribute to identifications by limiting potential matches to the individual through the development of a biological profile and the documentation of skeletal anomalies and pathologic conditions. This is traditionally done when there is no indication or suspicion of who the individual may be. If an individual does have a presumptive identification based on circumstantial evidence, forensic anthropologists may assist in establishing a positive identification if antemortem and postmortem records match sufficiently. For example, forensic anthropologists may compare an antemortem radiograph of the suspected individual with a postmortem radiograph of the decedent, searching for consistencies or inconsistencies in bone morphology, antemortem trauma, or pathologic conditions in order to include or exclude that individual.[1,2] However, if antemortem radiographs for the suspected individual are not available and direct antemortem/postmortem comparisons cannot be made, forensic anthropologists may still use postmortem radiographs to either confirm a positive identification or exclude that individual. Less traditional methods of using radiographs to support identification include comparing evidence of antemortem skeletal trauma, surgical interventions, and other pathologic conditions or anomalies as seen on radiographs to those that are specified in the suspected individual's medical records or provided by their next of kin.[3,4]

In the Maricopa County Office of the Medical Examiner (MCOME) in Phoenix, AZ, forensic anthropologists are frequently relied upon to perform such radiographic analyses with the purpose of confirming or refuting identification. The aim of this study was to perform a retrospective analysis of the cases for which the forensic anthropologists at the MCOME and Arizona State University Forensic Anthropology and Bioarcheology Laboratory (FAABL) were asked to review radiographs to aid in identifications of decedents with presumptive or suspected identifications. Cases from January 2020 to July 2024 were included in this study and retrospectively analyzed. During this timeframe, 3,054 individuals with presumptive identifications were received at the MCOME and FAABL, and forensic anthropologists were asked to perform radiograph reviews with the purpose of confirming identification in 240 (7.9%) of these cases. During the radiographic reviews of these cases, 441 points of concordance were used, broadly grouped under: antemortem skeletal trauma without fixation (22.4%); antemortem skeletal trauma with internal or external fixation (16.1%); evidence of surgical intervention such as sternotomy wires, joint replacements, stents, meshes, surgical clips, etc. (29.0%); pathologic conditions such as scoliosis, sclerotic lesions in bone, vertebral fusion, severe osteoarthritis, vascular calcifications, soft tissue lesions, etc. (31.1%); and direct comparisons of antemortem and postmortem radiographs (1.4%). In 319 cases (72.3%), these features were confirmed by medical records or information provided by next of kin, and identification could be supported. In the remaining 122 cases (27.7%), medical records were either not available for the suspected individual or there was no next of kin to provide antemortem information for comparison.

These results show that the expertise of forensic anthropologists is extremely valuable in supporting identifications of suspected individuals. Results also indicate that forensic anthropologists are frequently relied upon to perform radiographic reviews for this purpose, despite many forensic anthropologists not having sufficient radiological training or experience.[5] Radiological training should therefore be included in undergraduate, graduate and professional development courses for forensic anthropology students and practitioners.

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Anthropology; Radiology; Identification

I75 The Suicide: An Experimental Study of the Expression of the Neurotrophic Factors BDNF and GDNF in the Brain of Subjects Who Died by a Suicidal Act

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Learning Objectives: The attendees will be able to notice the critical role of the neurotrophic system, which is still slightly known, and how this can influence the phenomenon of suicide. The study highlights the importance of the immunohistochemical method for obtaining precious data directly on the brains of suicidal subjects.

Impact Statement: This study will impact the forensic community by showing the relevant role of the neurotrophic system in the biology of suicide. Since there are no further similar studies in the literature carried out on suicidal deaths using immunohistochemical methods, Brain-Derived Neurotrophic Factor (BDNF)and Glial cells-Derived Neurotrophic Factor (GDNF)could be proposed as promising and reliable biomarkers of suicidal risk.

Abstract Text: Suicide represents a critical public health problem whose neurobiology is not yet fully understood. The suicidal risk factors are related to both individual and social factors. Furthermore, there is a strong connection with psychiatric illnesses, particularly with major depressive disorder.

According to the literature, the suicidal phenomenon could be considered a psychopathological entity independent of psychiatric comorbidity, which follows the "diathesis-stress" model. This model establishes that a behavioral disorder derives from a genetic predisposition that makes the individual more vulnerable to stress.[1]

An emerging pathophysiological hypothesis underlines the role of neurotrophic factors, the proteins involved in neurogenesis, synaptic plasticity, response to stress, and numerous additional mechanisms.[2] Among the neurotrophins, the BDNF and the GDNF are particularly interesting.[3-5]

This study aims to analyze the correlation between suicide and the expression of BDNF and GDNF on autopsy samples in many specific brain areas to evaluate their possible role as a neurobiological marker predictive of suicidal risk, with any relevant repercussions in the clinical-psychiatric and medicolegal fields.

The analyzed sample consists of 20 cases of subjects who died by suicide, compared to 10 control cases (who died by traumatic causes); decomposed bodies, drug addicts, subjects on antidepressants and neuroleptic therapy, and subjects affected by neurodegenerative diseases have been excluded. Six cases had no psychiatric diagnosis, while of the remaining 14 cases, 5 cases were diagnosed with major depressive disorder and one with bipolar disorder.

An autopsy was carried out on each case, with brain sampling fixed in a formol solution. After 21 days, the authors proceeded to sample the prefrontal cortex, cingulate gyrus, basal nuclei, and hippocampus, subsequently treated with immunohistochemical stains with a panel of antibodies anti-BDNF and anti-GDNF. A statistical analysis was conducted; a P value < 0.05 was considered statistically significant.

The results show that BDNF was underexpressed in the brain parenchyma of subjects who died by suicide compared to controls in all brain areas examined. Regarding GDNF, there was overexpression in the cerebral parenchyma of suicide victims, except in the basal nuclei, where a slight increase was recorded compared to controls, which, however, was not statistically significant. The hippocampus was the area with major BDNF underexpression and GDNF overexpression.

The sample was further divided, examining exclusively the 14 psychiatric subjects: the expression of BDNF and GDNF in subjects suffering from mood disorders was compared to the other psychiatric patients, but no statistical differences were found between the two groups.

The results obtained support the hypothesis that lower levels of BDNF and higher levels of GDNF are related to an increased suicidal risk. Therefore, such neurotrophins could be used as markers of suicidal risk, which can be useful in some fragments of populations with specific risk factors, thus implementing effective prevention policies.

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BDNF; GDNF; Suicide

I76 A Postmortem Diagnosis of Bloodborne Infectious Diseases Using Point-of-Care Test Kits

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Learning Objectives: This study demonstrates the positivity rates of blood-borne infectious diseases that may be exposed during autopsies and evaluates the effectiveness of rapid diagnostic kits in forensic settings. Attendees will gain insights into the use of point-of-care test kits for detecting blood-borne infections during autopsies. The use of these kits can provide quick information about the decedent's medical history, assist in diagnosing the cause and circumstances of death, reduce the risk of infection exposure for autopsy personnel, and shorten response times to potential infection exposure incidents.

Impact Statement: The findings of this research are expected to impact the forensic science community by enhancing competence in infectious disease screening during autopsies and improving risk assessment and management performance. Furthermore, it can contribute to more comprehensive postmortem examinations, benefiting both practitioners and stakeholders in the medicolegal system. However, further research is needed to validate the diagnostic accuracy of these kits and expand their applicability in the field of forensic science.

Abstract Text: Unlike clinical patients who undergo blood tests before procedures or surgeries, autopsies are often performed without accurate knowledge of the decedent's medical history, including infectious diseases. As a result, forensic professionals are always exposed to the risk of blood-borne infectious diseases during autopsies, yet testing for infections typically takes over a day.[1-3] This delay complicates immediate responses when exposure to pathogens through tools like scalpels or needles is strongly suspected.[4] However, research on infectious diseases related to autopsies is very scarce worldwide, including in the Republic of Korea, and the prevalence of infectious diseases in decedents referred for autopsy is not well understood.[5] Therefore, this study aimed to use newly released rapid diagnostic kits for blood-borne infections to evaluate simultaneously: (1) the prevalence of infectious diseases in decedents referred for autopsy, and (2) the feasibility of point-of-care test kits for selected blood-borne infectious diseases in autopsy settings.

From July 2019 to July 2024, autopsies were performed on 221 cases at the Seoul National University Medical Examiner's Office. Five types of point-of-care test kits were used for identifying syphilis, Human Immunodeficiency Virus (HIV) infection, Hepatitis B Virus (HBV) infection, and Hepatitis C Virus (HCV) infection. One kit detected antibodies for HIV-1 and HIV-2 simultaneously. Two kits were used for HBV, detecting HBsAg and Anti-HBs, respectively. The others each detected HCV and syphilis. Among the 221 cases, 27 (12.2%) were positive for at least one of the kits: syphilis in 10 cases (4.5%), HIV in 2 cases (0.9%), HBsAg in 4 cases (1.8%), anti-HBs in 6 cases (2.7%), and HCV in 5 cases (2.3%). In a few cases with medical histories, the test results were consistent with their underlying infectious conditions. Their protocol recommended reading the results within 20 minutes after applying the sample, which implies that they are useful in the rapid and easy detection of infectious diseases. This advantage is urgent in autopsy procedures. The authors are planning and conducting a comparison of these results with other tests, such as serologic tests or Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) for each microorganism, which are used as standard or confirmatory methods, to assess sensitivity, specificity, and overall diagnostic accuracy.

Using point-of-care test kits for infectious diseases during autopsies would provide valuable information about the decedent's medical history, assist in diagnosing the cause and circumstances of death, and protect autopsy personnel by promoting infection prevention. Moreover, should an infection incident occur, these kits would protect autopsy personnel by facilitating rapid and accurate responses. Further research is needed to validate the diagnostic accuracy and expand the applicability of these kits.

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Blood-Borne Infection; Point-of-Care Test; Autopsy

I77 It's a ME...MA! When Genetics Is the Actual Cause of Death

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Learning Objectives: Attendees will learn about other causes of Acute Aortic Dissection (AAD), such as Mucoid Extracellular Matrix Accumulation (MEMA), which is quite unknown, given its microscopic identification and scarce literature. Detailed macroscopic and microscopic analyses are shown to suggest when and how to investigate this particular cause of AAD at the postmortem examination.

Impact Statement: This presentation will enhance the diagnosis of AAD during autopsies by improving the competence and ability of forensic professionals, especially regarding histological analysis in individuals with no significant medical history of cardiovascular diseases. Attendees will be able to apply their enhanced knowledge in real-world forensic scenarios, improving their ability to differentiate between AAD by MEMA and by other causes of sudden death, by the systematic execution of such assessments that can define the role of connective tissue diseases and alterations of the extracellular matrix in the development of AAD.

Abstract Text: AAD is a life-threatening condition where a tear occurs in the aorta's inner layer, thus forming a false lumen. This can lead to compromised organ blood flow, organ failure, and shock. It is a critical cause of sudden or unexpected death due to its rapid onset and severe complications. Key-risk factors include hypertension and genetic disorders.[1-3] Usually, unexpected deaths by AAD are filed as medical malpractice. Postmortem examination and histological studies play a crucial role in confirming the precise cause of death, especially in AAD, where symptoms can mimic other conditions. Autopsy findings are characterized by striking macroscopic alterations of the aortic wall. Histological studies should be performed routinely as they have a central role in confirming the diagnosis, elucidating the underlying causes and providing insights into potential genetic factors, contributing to better prevention and management strategies. MEMA is a possible finding in histopathological examination in cases of AAD.[4,5] It refers to the buildup of a gelatinous substance in the extracellular matrix, which retains water, leading to a swollen, jelly-like consistency. Mucoid accumulation can impair the normal function and flexibility of tissues and lead to structural changes compromising organ efficiency. MEMA has been linked to various medical conditions. Further studies are necessary to clarify the pathologic and prognostic significance of this nosological entity. In this context, the Azan-Mallory stain should be used to distinguish cells from the extracellular matrix and to highlight the collagen fibers. Three cases of fatal aortic dissection are presented.

The first case (a 41-year-old male) was discovered deceased in his apartment; toxicological examinations resulted positive for cocaine. The autopsy revealed hemopericardium associated with a laceration in the ascending aorta; delaminations were also observed in other aortic segments. Microscopic analysis disclosed MEMA within the media layer, arranged both translamellarly and intralamellarly, along with fragmentation of elastic fibers, focal loss of smooth muscle fibers and microhemorrhages. The cause of death was ruled to cardiac tamponade from aortic dissection associated with MEMA, in the presence of acute cocaine intoxication.

The second case (a 44-year-old male) died a few hours after presenting to the emergency department with complaints of fatigue, palpitations, and diffuse abdominal. Medical malpractice was filed. At autopsy, hemopericardium was found, along with a dissection extending from the media to the adventitia of the ascending aorta. Delaminations of the aortic media were also observed in the thoracic and abdominal segments. Microscopically, MEMAs were identified in both translamellar and intralamellar distributions, with disruption of elastic fibers, focal loss of smooth muscle fibers, and associated microhemorrhages. MEMA was ruled as the cause of the aortic dissection.

The third case (12-year-old male) presented with sudden chest pain after dinner and was discharged from the emergency department with negative initial tests. Medical malpractice was filed. He was later found unresponsive by his parents. At autopsy, external examination revealed a slender physique, long limbs, and tapered fingers, suggestive of a marfanoid habitus. Internal examination showed hemothorax and aortic dissection 7cm below the aortic arch with the presence of a full-thickness tear. Marfan syndrome, caused by FBN1 gene mutations, leads to defective connective tissue and can cause severe cardiovascular issues like aortic dissection, even in young individuals, and was ruled as the cause of death. Postmortem and histological analyses are crucial for understanding AAD, particularly in unexpected deaths. The three cases described demonstrate that detailed autopsies and histological examinations, including the identification of MEMA, provide key insights into the pathology of AAD. These findings improve diagnostic accuracy and help guide future research for better prevention and management of AAD, even in medical malpractice suits.

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Autopsy; Unexpected Death; Malpractice

I78 The Application of Intracerebral Aquaporin-4 (AQP4) Expression in Differential Diagnosis of Drowning: An Immunohistochemical Study Related to the Timing of the Autopsy

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Learning Objectives: After attending this presentation, attendees will deepen their understanding of the intracerebral AQP4 expression in cases of freshwater and saltwater drowning. This study also puts particular emphasis on autopsy-performing timing.

Impact Statement: This presentation will impact the forensic community by highlighting the importance of histopathological and immunohistochemistry analyses in differential diagnosis of drowning, leading to a better understanding of the pathological process of drowning. Moreover, this study proves that an early autopsy conducted within 72 hours of death provides better microscopic results and a reliable level of accuracy for immunohistochemical markers. Additionally, the evolution of immunohistochemical pattern identified in the study could be useful even to evaluate the postmortem interval.

Abstract Text: The role of forensic pathologists is pivotal in definitively diagnosing drowning cases. Currently, a combination of autopsy, histopathological examination, diatom tests, and toxicological analyses is routinely employed to diagnose drowning. Further differentiation becomes essential for distinguishing between Freshwater Drowning (FWD) and Saltwater Drowning (SWD). Aquaporins (AQPs) are a group of proteins specialized in transporting water and small molecules across the plasma membrane of cells in specific tissues such as the kidneys, lungs, and brain. These proteins are situated in the middle of the membrane lipid bilayer. To date, 13 classes of AQPs (AQP0–AQP12) have been identified, each differing in transport capacity, tissue allocation, and function.[1,2] AQP4 is the most common AQP found in the mammalian brain, located in astrocytes near capillaries and pia, as well as in ependymal cells.[3] AQP4 plays a crucial role in water homeostasis and neural signal transduction, with its expression rapidly induced by stimuli such as osmolarity and mechanical or chemical stress.[4,5] Our study aims to examine the expression of AQP4 in the brain as a potential marker for differentiating between FWD and SWD relating to autopsy-performing timing.

A total of 23 cases were classified into three groups: eight FWDs, nine SWDs, and six cCntrols (CTRs). All samples were classified upon autopsy-performing timing into two subgroups: within and after 72 hours of death. The samples were then subjected to histological and immunohistochemical investigations. The immunohistochemical investigations used specific antibodies to detect the presence of AQP4 in the brain tissue. Afterward, the data was analyzed using Jasp software. The analysis involved conducting an Analysis of Variance (ANOVA) test for the examination of variance and employing the post hoc Scheffé's F test for multiple comparisons.

For autopsies performed within 72 hours of death, we found a significantly higher value of AQP4-positive astrocytes in cases of FWD compared to SWD and control groups. This suggests that the expression of AQP4 in the brain could be a valuable marker for identifying cases of FWD. We also found a significantly lower AQP4 expression in SWD cases compared to the control group. This could indicate that the regulation of cerebral volume is different in cases of SWD. For autopsies conducted after 72 hours, the immunohistochemical staining does not reveal the peripheral terminations of astrocytes, which appear blurred and only recognizable as halos. The other localizations of aquaporins (perivascular and submeningeal) are also less pronounced than the control group. This suggests that the expression of AQP4 changes over time, even after death, making it less reliable as a marker for drowning in these cases.

In summary, our findings indicate that AQP4 can be a promising marker for distinguishing between FWD and SWD. The implications of this study extend beyond just differentiating between FWD and SWD to the potential for a direct diagnosis of FWD-related death. Therefore, conducting a comprehensive analysis of AQP expression across multiple organs in conjunction with other established markers is essential to achieve a more precise differentiation between FWD and SWD. However, it is crucial to highlight that the best results were obtained in autopsies conducted within 72 hours. Thus, it is critical to emphasize that a reliable level of accuracy for the differentiation between FWD and SWD is only maintained if autopsies are performed within 72 hours of death. Further investigations are necessary to better understand the postmortem modifications identified by the immunohistochemical study, and potentially correlate these results with the postmortem interval.

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Drowning; Histological; Aquaporin

I79 The Essential Role of Genetic Analysis in Sudden Cardiac Death: Advancing Molecular Autopsies and the Potential for Preventive Screening

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Learning Objectives: Several studies suggest the presence of a significant heritable component of arrhythmogenic diseases in Sudden Unexpected Death (SUD). This work illustrates the case of an SUD in which genetic analysis had a key role in identifying the cause of death. This paper aims to highlight the importance of adopting a multidisciplinary approach to resolve unexplained cases through genetics and to improve the understanding of certain nosological entities causing death that would otherwise remain unknown without an in-depth study of such peculiar cases.

Impact Statement: This presentation will impact the forensic science community by showing the growing significance of molecular autopsies as a fundamental tool in the diagnosis of SUD and Sudden Cardiac Death (SCD). Moreover, it underscores the crucial role of whole exome genetic analysis in building a widespread database supporting the creation of a screening model, thereby finding individuals at risk of SCD and preventing sudden death.

Abstract Text: Forensic medicine defines Sudden Death (SD) as an unexpected death either within 1 hour of symptom onset or within 24 hours of having been observed alive and symptom-free. SD is a major health issue, accounting for 15–20% of all deaths and over 300,000 fatalities annually in the United States.[1-3] Most SDs are of cardiac origin and are termed SCD. However, in up to 40% of SD cases, no definite cause of death is identified by autopsy, so they are referred to as SUDs.[4,5]. Since SUDs are often due to an underlying inherited arrhythmogenic cardiac disease, postmortem diagnosis of SUDs involves genetic testing to identify potential hereditary conditions such as cardiomyopathies or channelopathies, which lead to the reclassification of the death.[6,7] Moreover, in most of cases of genetic predisposition to SCD, the first symptom is sudden death itself.

This case involves the death of a 40-year-old male who collapsed at work. An autopsy was performed and, given the negative histological and toxicological analyses, a genetic investigation was carried out using Next-Generation Sequencing (NGS). The analysis of 70 genes associated with cardiomyopathies or channelopathies identified a mutation in the SLC4A3 gene (NM_201574.3). It appeared as a missense mutation of a nucleotide, resulting in a different amino acid (c.1318G >T, p.Asp440Tyr).

This variant has not been previously described in the scientific literature, and its pathogenic significance remains unknown. According to the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines, it has been classified as a "variant of uncertain significance" (VUS), meaning that no pathogenic variants of recognized relevance were found compatible with the clinical suspicion. Specifically, the evidence for its pathogenic role has been classified as moderate in category PM2 (absent from controls or at extremely low frequency if recessive in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium) and supportive in category PP3 (multiple lines of computational evidence support a deleterious effect on the gene).[8] However, the SLC4A3 gene (OMIM:106195) encodes a plasma membrane protein that acts as an anion exchanger, and mutations in this gene have been associated with short QT syndrome.[9]

It should therefore be considered that unexplained SCDs are often linked to a genetic predisposition. Additionally, the diagnosis is made postmortem and results from a negative autopsy with positive genetic mutations. Therefore, it is crucial to encourage genetic analysis and research to identify other gene mutations, in order to expand our knowledge of potential additional genes beyond those known to be pathognomonic.[2] This approach is critical for building a comprehensive database capable of eventually facilitating the development of a screening model. Such a model would enable early diagnosis of individuals at risk of SCD and aid in the implementation of preventive strategies to avert sudden death.

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Sudden Cardiac Death; Genetics; SLC4A3

I80 Extra-Articular Gout Deposits Can Be Clinically Significant and Contribute to Death

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Learning Objectives: Attendees will learn that gout, while thought of as a manageable disease, can have unusual and significant complications of crystal deposition from the disease process that may not be considered. These complications can rarely cause or contribute to death and should be reflected on the death certificate for more accurate public health information.

Impact Statement: Common diseases can have unusual manifestations that may not be widely known and can have significant morbidity, affecting death certification, and should be listed on the death certificate. Accuracy in death certification is how forensic pathologists can influence how diseases are monitored and treated and highlight unusual complications of common diseases that can have unappreciated morbidity.

Abstract Text: The decedent is a 51-year-old man who was found dead in his home on a well-being check. He had a history of hypertension, chronic ethanolism, and poorly controlled gout with tophi on all the extremities. He had felt unwell for several days prior to death. At autopsy, he had alcoholic cirrhosis and erosive gastritis with a gastro-intestinal hemorrhage. He also had an acute hemorrhage in the left psoas muscle, with the hemorrhage expanding the psoas muscle and accompanying retroperitoneal hemorrhage. Medications at the scene (including indomethacin) were severely underutilized and old. There was no trauma, infection (including tuberculosis), or mass that could account for the muscle hemorrhage. Other differentials of the hematoma could include bleeding diathesis from gout treatment (i.e., Non-Steroidal Anti-Inflammatory Drug [NSAID] use) or cirrhosis, spontaneous hemorrhage, hypertensive bleed, or prior abscess with pseudoaneurysm, or focal ischemia.

On microscopy, the hemorrhagic psoas muscle and retroperitoneum had refractile crystals consistent with uric acid crystals.

Gout is a common and treatable disease caused by the deposition of monosodium urate crystals in articular and non-articular structures. Chronic inflammation and urate crystal deposition in joints with gout is well known. Monosodium urate deposits, either from overproduction or underexcretion, in other extra-articular sites are thought to be rare and of little clinical significance. Recent diagnostic testing, including dual energy computed tomography, has led to the recognition that urate deposition occurs in any organ system, including the vasculature, and that this happens more commonly than previously thought.[1,2] Crystalline monosodium urate is a damage-associated molecule and can stimulate immune pathways in a two-step process.[3] Reviews of the mechanisms in gout have postulated gout monosodium urate crystals from phagocytosis by macrophages along with signaling by TLR2 and TLR4 activates the NALP2 inflammasome with release of interleukin-1 beta and plays a major role in the initiation of the gout "flare" with resulting tissue damage and tophus formation.[3,4] This generation of proinflammatory cytokines can also lead to atherosclerotic cascade activation and progression (1,2), infiltrative granulation tissue, degeneration of the elastic lamina which may cause pseudoaneurysm formation and ultimate vascular rupture .[1,2,5] A case report states this is what caused a pulmonary pseudoaneurysm with resulting pleural hemothorax when all other etiologies had been ruled out.[5]

This decedent had hemorrhage in his psoas muscle and retroperitoneum from a non-articular gouty tophus, along with alcoholic cirrhosis and erosive gastritis with upper gastrointestinal hemorrhage. The psoas muscle hemorrhage and gout were noted in the other conditions contributing to death on the death certificate.

This case demonstrates an unusual significant complication from a common manageable condition (gout), which contributed to his death.

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Gout; Complication; Morbidity

I81 Digital Pathology in Education: Shaping Career Choices and Recruitment Strategies in Forensic Pathology

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Learning Objectives: Attendees of this presentation will learn how digital pathology and online educational platforms are reshaping medical education and influencing career choices.

Impact Statement: By showcasing how digital pathology education influences career choices and enhances training, this presentation will provide actionable insights for optimizing educational strategies and recruitment efforts.

Abstract Text: In the past decade, digital pathology has experienced a remarkable rise in popularity, emerging as a versatile and transformative tool that enhances integration, data analysis, and decision-making processes within the medical field. This digital revolution has fundamentally reshaped the educational landscape, leading to significant changes in curriculum development and professional practices. Notable digital platforms such as Uworld, Pathoma, Path Dojo, and Online MedEd have redefined medical education by offering interactive, real-time content that challenges the traditional reliance on textbooks like Robbins and Cotran's *Pathologic Basis of Disease*. These platforms provide a dynamic and engaging learning experience, making complex pathology concepts more accessible and easier to grasp.

A recent study underscores the pivotal role of rotation schedules and early clinical exposure in shaping medical students' residency choices and specialty preferences.[1] To explore this further, a survey was conducted at the University of Toledo Medical Center, where medical students and residents shared their insights on the educational resources they rely on for board exam preparation and training. The survey revealed a pronounced preference for digital media over traditional textbooks and highlighted the significant influence of early exposure to various medical disciplines on career decisions.

In response to these findings, an educational video library is being developed to address the specific needs of the institution. This library will focus on critical aspects of pathology, including grossing techniques, evisceration methods, and common autopsy findings. By tailoring the content to the institution's unique specimen requirements, the library aims to enhance the practical learning experience for students and residents, providing them with valuable, hands-on knowledge.

The study hypothesizes that medical students and residents prefer digital educational resources as their primary learning tools and that early exposure to digital pathology education positively influences their career trajectories. The overarching goal of this research is to identify and cultivate resources that not only support pathology recruitment efforts but also underscore the critical role of forensic pathologists in the medical field. By leveraging digital tools and early exposure, the study aims to improve educational outcomes and bolster the profession's future.

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Education; Career; Forensic Professionals

I82 A Fatal Citrobacter-Induced Meningitis Complicated With Pneumocephalus in an Infant: A Case Report

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Learning Objectives: Attendees will learn about the serious risks associated with *Citrobacter koseri* infections in late preterm infants, including the potential severity of these infections. The presentation will also discuss the importance of understanding these risks from both a forensic and clinical perspective.

Impact Statement: This presentation highlights the significant threat posed by *C. koseri* in late preterm infants and underscores the potential severity of infections caused by this organism. The knowledge of this case can be useful not only from a forensic point of view but also in the area of preventing the risk of hospital infections, especially in the neonatal field.

Abstract Text: Neonatal infections caused by *Citrobacter koseri* present a significant clinical challenge due to their potential for severe outcomes. *C. koseri*, a facultative anaerobic gram-negative bacillus belonging to the family Enterobacteriaceae, is commonly found in the gastrointestinal tracts of humans and animals, as well as in environmental sources.[1] The mode of transmission of *C. koseri* to neonates remains unclear, with possibilities including vertical transmission or acquisition from environmental sources.[2] Neonates under two months of age and preterm infants are especially susceptible to infections such as meningitis, encephalitis, and brain abscesses, often associated with high mortality.[3] One rare complication associated with this gas-producing organism is pneumocephalus, the presence of an intracranial air or gas collection.[2]

This report details the case of a female infant born at 30 weeks' gestation via cesarean section, with a birth weight of 1,870 grams, following a pregnancy complicated by Premature Rupture Of Membranes (PROM) a month earlier. Immediately after birth, she was admitted to the neonatal intensive care unit for respiratory distress syndrome. Two weeks later, she developed hyperpyrexia and sepsis, with blood cultures identifying *C. koseri* as the causative agent. Her clinical condition rapidly worsened over the following days, with the onset of seizures and cerebral distress observed via Electroencephalograph (EEG). Concurrently, cranial Computed Tomography (CT) revealed the presence of intracranial gas embolism, with localization observed within the cerebral tissue, the ventricular system, and the subdural space. She succumbed to her illness 17 days after birth, and an autopsy was performed to ascertain the cause of death. The external examination of the corpse revealed cyanosis of the extremities, while the macroscopic autopsy findings included the release of abundant gas upon opening the cranium, inflammatory aspects of the meninges, a vacuolated appearance of the brain on section, parenchymal hemorrhages, and ventricular dilation. Tissue samples were taken, with particular attention to the meninges and brain. Subsequent histological examination revealed severe purulent meningitis, encephalitis with foci of acute inflammation, pneumocephalus, intraparenchymal cerebral hemorrhage, and acute cerebral edema. The cause of death was identified as sepsis due to *Citrobacter koseri*, which resulted in meningoencephalitis with diffuse pneumocephalus and cerebral hemorrhage.

To our knowledge, this is the first documented case of fatal meningoencephalitis complicated by pneumocephalus in a preterm infant in Italy. This represents an exceptionally rare complication, as it is only the seventh case reported in the literature. Furthermore, it highlights the significant threat posed by *C. koseri* in late preterm infants and underscores the potential severity of infections caused by this organism. The knowledge of this case can be useful not only from a forensic point of view but also in the area of preventing the risk of hospital infections, especially in the neonatal field.

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Infant Death; Microbiology; Pediatric

I83 Death After Spontaneous Splenic Rupture in a Newborn Child With Severe Hemophilia A Caused by *De Novo* Mutation: A Case Report With Literature Review

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Learning Objectives: After this presentation, attendees will be aware of the clinical features and possible fatal consequences of rare conditions such as spontaneous splenic rupture in a newborn affected by Hemophilia A. Furthermore, the crucial role of individual genetic assessment setting to detect *de novo* mutations and their possible pathogenetic contribution to death will be emphasized.

Impact Statement: This presentation will impact the forensic science community by providing a peculiar case report, which presented a challenge from both a diagnostic and forensic point of view.

Abstract Text: Spontaneous splenic rupture in newborns is an extremely rare emergency, even among hemophiliac patients. Indeed, in over 50 years of medical literature only a handful of such cases have been reported, in which all of the newborn survived.[1-5]

The present study reports the case of a 5-day-old neonate who suffered spontaneous splenic rupture, which rapidly resulted in death on Day eight Of Life (DOL8). In the afternoon of DOL4, after a heel-stick test (performed in the morning), the baby showed persistent bleeding along with an elevated Thromboplastin Time (PTT) of 62.3 seconds (internal standards 32 – 34.5), with no other alterations in laboratory tests related to coagulation or hemoglobin: the patient was then discharged after the bleeding had ceased. The day after (DOL5), the newborn was admitted to the same hospital because of sudden onset of intense pallor in the lower half of the body. The first medical assessment pointed out a really critical scenario, characterized by hypotonia, bradycardia, extensive pallor, cold extremities, and oxygen saturation of 85% on room air. Laboratory tests revealed severe metabolic acidosis (pH level < 6.80, K+ 6.18, lactate >20), hematocrit level of 10%, hemoglobin values of 2.9g/dl, and prothrombin time of 44% with an International Normalized Ratio (INR) of 1.87. The patient was immediately referred to the Neonatal Intensive Care Unit of a nearby hospital, where he underwent chest and abdomen radiography in order to identify the source of his severe anemia. After radiologic investigations showed no significant findings, the infant underwent exploratory laparotomy, which revealed massive hemoperitoneum with numerous large blood clots, as well as multiple actively bleeding lacerations of the splenic parenchyma. Therefore, an emergency splenectomy was performed. The spleen, with a weight of 8.8g and volume of 4.5 x 2.7 x 1cm, was found normal at the histopathologic examination. Postoperative laboratory tests showed severe deficiency of all coagulation factors. Moreover, the clinical condition of the infant, characterized by persistent metabolic acidosis and renal failure due to acute hemorrhagic shock, remained extremely severe. On DOL 7, cerebral ultrasounds revealed critical ischemic hypoxia and, on DOL8, the infant died.

Forensic autopsy identified hemorrhagic shock due to non-traumatic spleen rupture as the primary cause of death, whereas subsequent genetic investigations found out a de novo inversion of intron 22 on X chromosome, which codes for coagulation Factor VIII, thus leading to a diagnosis of Hemophilia A.

In the present case, several circumstances contributed to determine the fatal outcome, such as no family history of hemophilia, vague clinical manifestations, inconclusive laboratory tests (elevated PTT vs. normal range PT and INR), as well as the sudden onset of hemorrhagic shock and the following fast evolution to irreversible metabolic acidosis, renal failure, and cerebral ischemia, despite the proper performing of splenectomy and intensive care treatment.

This case highlights the importance of performing a thorough autopsy, followed by histopathologic examination and postmortem genetic testing. In fact, the diagnosis was made only postmortem, after all these investigations. In order to prevent further tragic deaths of hemophiliac newborns, the suggestion is to strictly follow up cases of persistent bleeding, performing appropriate clinical management and including genetic investigations, if needed, to promptly detect any possible coagulation disorder.

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Spontaneous Splenic Rupture; Hemophilia A; *De Novo* Mutation

I84 Fatal Neonatal Herpes Simplex Virus Infection

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Learning Objectives: After attending this presentation, one will be more familiar with the lethal effects of disseminated neonatal herpes.

Impact Statement: This presentation will impact the forensic science community by highlighting the significant lethality associated with neonatal viral infections.

Abstract Text: **Background:** Herpes Simplex Virus (HSV), is an enveloped double-stranded DNA virus belonging to the Herpesviridae family.[1] The virus, which can be classified into HSV-1 and HSV-2, is most known for being ubiquitous within adult populations; HSV-1 has a predilection for oral lesions while HSV-2 is known for genital infections. It is important to note that either subtype of the virus can inhabit either location. Most infections can be asymptomatic or go unrecognized compared to the more classic painful blisters and ulcers associated with herpes. The lack of visible lesions and unknown carrier status lends itself to being such a pervasive presence. Herpes is especially problematic in neonates, as along with other Toxoplasmosis, Others (syphilis, hepatitis B), Rubella, Cytomegalovirus, Herpes simplex (TORCHes) infections, it can be transmitted vertically from mother to child at the time of birth.[2,3] While rare, neonatal herpes still affects 10 out of 100,000 births worldwide, with the greatest risk coming from mothers contracting HSV late in pregnancy.[4] Disease can either manifest with Skin-Eye Mucous Membranes (SEM), the Central Nervous System (CNS), or disseminated disease, the deadliest type.[2]

Case: An 8-day old White male was taken to urgent care by his mother after he coughed up yellow mucus, then quickly became lethargic and pale. Emergency services transported the decedent to the local medical center where he was hypothermic with low oxygen levels. He was intubated and stabilized before being transferred to a neonatal intensive care unit in critical condition. His condition continued to decline, and he was pronounced dead within a few hours after admission. Medical history was significant for term spontaneous vaginal delivery eight days prior. He had been diagnosed with birth-related blood collection of the scalp tissues and was treated with phototherapy for elevated bilirubin levels. Autopsy revealed disseminated HSV infection with severe disease involving the liver, exhibiting diffuse speckled areas of congestion with yellow discoloration of the parenchyma. Herpetic changes were also detected in the adrenals and lungs with noted viral cytopathic changes, including intranuclear inclusions, glassy nuclei, peripheral margination of chromatin, molding, and multinucleation evident on microscopy. Immunohistochemical staining further confirmed the presence of both HSV viruses with nuclear and cytoplasmic positivity within the liver and lungs.

Conclusion: Neonatal HSV infection can range in severity from mild symptoms to fatal disseminated disease. Vertical transmission from viral shedding at the time of vaginal delivery is the most common mode of infection. Shedding can take place asymptomatically, as in this scenario, as the mother was unaware of her positive carrier status for both HSV-1 and 2. It is this exposure to viral shedding during the birthing process that can result in non-specific symptoms, including fever, lethargy, seizures, hepatosplenomegaly, and hypotension. Symptoms typically appear 10 to 21 days after infection, with the disseminated disease type usually presenting at seven days after birth, or eight as in this case. SEM type is notable for rash and vesicular appearance while the CNS variety is more prone to neurological issues such as lethargies and seizure.[2] The United States sees an incidence of 1,500 neonatal herpes cases annually, with more current studies suggesting an increasing trend.[1] The case-fatality rate is thought to be 60% for untreated cases and significantly high even when treatment is administered in a timely manner.[5] Our case had a hospital course that transpired over the course of hours, and specific antiviral treatment was not delivered as the mother was unaware of her status. While relatively rare, an estimated 1,271 deaths have been attributed to neonatal herpes within the United States from the years 1995 to 2017 and is important to keep in mind when assessing infant fatality.[6]

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Neonatal; Herpes; Virus

I85 Sudden Pediatric Death: The Lethal Intersection of Influenza and Hypoplastic Coronary Artery Disease

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Learning Objectives: Attendees will learn about the pathological and forensic significance of Hypoplastic Coronary Artery Disease (HCAD) and its association with sudden deaths, especially in pediatric cases. The presentation will cover the interplay between HCAD and viral infections such as Influenza B, as demonstrated in a real case of a two-year-old male. Key learning outcomes will include understanding the role of congenital coronary anomalies in forensic pathology and recent advancements in studying the underlying mechanisms of HCAD, including genetic mutations.

Impact Statement: This presentation will enhance competence in identifying and diagnosing HCAD in forensic settings, particularly in cases involving sudden pediatric deaths. By integrating review on the molecular mechanisms of HCAD and its interaction with viral infections, attendees will be better equipped to assess similar cases, ultimately improving both diagnostic accuracy and stakeholder confidence in forensic evaluations.

Abstract Text: The underdevelopment of one or more coronary arteries with a markedly diminished lumina is a rare but well-documented entity since 1970, known as HCAD.[1] Also, backed by a vast amount of epidemiological data, technology has proved a significant association between laboratory-confirmed influenza infection and cardiovascular death.[2] In this occasion we present the tragic incident of a two-year-old male toddler found unresponsive, face down with signs of emesis, while napping. The toddler had been given cough syrup and acetaminophen by his parents minutes earlier for a presumed episode of fever. Once found, the toddler was taken to the hospital and pronounced dead the same day. On autopsy, significant findings included a positive viral nasopharyngeal swab for Influenza B and epicardial coronary arteries with less than 1 millimeter in diameter, particularly the circumflex artery, which focally measured less than 0.5 millimeter in diameter. Additionally, there was no identifiable posterior descending artery. Histologic sections of the lung showed congestion, edema, and patchy atelectasis. Neuropathologic forensic examinations demonstrated acute hypoxic-ischemic changes in the bilateral hippocampi. The finalized pathology determined the cause of death as Influenza B infection in the setting of HCAD, and the manner of death as natural.

The interplay brought by HCAD and Influenza virus raised our interest to review the more recent literature on the mechanisms and advances in the study of HCAD. Recent advances have allowed the study of the crucial role of nitric oxide synthase-3 in down-regulating the expression of Gata4, Wilms tumor-1, vascular endothelial growth factor, basic fibroblast growth factor, and erythropoietin, inhibiting the migration of epicardial cells in the pathogenesis of HCAD in mice models.[3] Additionally, the use of nuclear medicine procedures such as Thallium-201 perfusion scintigraphy has documented evidence of persistent myocardial ischemia with barely any clinical symptoms in patients with HCAD.[4] Myocardial injury seems to be an important factor in recent cases of HCAD, where ischemia-induced or scar-related reentrant ventricular arrhythmias induced by HCAD were identified as possible causes of sudden death.[5] Worth mentioning is the groundbreaking genomic sequencing of a new mutation in NOTCH1, the first pathogenic-like mutation reported among HCAD cases.[6]

Due to the multifactorial components of the surrounding events leading to the death of the present case, there may be a discussion about the role of Influenza B in the final sequence of events leading to this child's death, raising questions about the primary culprit. There is no doubt that HCAD was a predisposing factor for a cardiovascular event and that Influenza B could have exacerbated this effect. However, the mechanisms behind these interactions remain unclear. In the end, it might have been a febrile seizure that was the ultimate cause. Considering this case, it becomes evident that congenital coronary anomalies like HCAD should be critically considered in forensic pathology, especially when investigating sudden unexplained deaths in children. The intricate interplay between HCAD and viral infections such as Influenza B underscores the complexity of determining the precise cause of death. This case not only highlights the need for heightened awareness but also sparks further inquiry into the mechanisms at play, potentially leading to more accurate diagnostics and preventative measures in the future.

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Sudden Death; Influenza; Hypoplastic Coronary Artery Disease

I86 The Occasional and Rare Finding of Choroid Plexus Papilloma in the Cerebellopontine Angle in an Infant: A Case Report

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Learning Objectives: Attendees of the American Academy of Forensic Sciences conference will learn how to recognize the diagnostic challenges posed by rare neoplasms like Choroid Plexus Papilloma (CPP) in neonates with complex medical conditions. They will gain insights into the importance of comprehensive neuroimaging protocols, the role of autopsy in uncovering undiagnosed conditions, and the need for vigilant surveillance of hydrocephalus in infants with brain tumors. The presentation will provide measurable outcomes by highlighting specific diagnostic gaps and offering strategies for early detection and management in similar cases, with a focus on interdisciplinary approaches in neonatal intensive care.

Impact Statement: The presentation will enhance the forensic science community's understanding of pediatric brain tumors, emphasize the importance of thorough autopsies and interdisciplinary collaboration, and inspire advancements in diagnostic protocols and research.

Abstract Text: This abstract pertains to a rare case of Choroid Plexus Papilloma (CPP) diagnosed postmortem in a premature infant who passed away due to respiratory complications related to prematurity. The case highlights the diagnostic and therapeutic challenges posed by such rare neoplasms, emphasizing the need for surveillance and innovative diagnostic approaches in neonates with complex medical backgrounds.

The clinical case involves a male infant born prematurely at 29 weeks via emergency cesarean section. The infant, suffering from microdeletion syndrome, bronchopulmonary dysplasia, ocular coloboma, and notable dysmorphic features (microcephaly and retro-micrognathia), required immediate neonatal intensive care, including mechanical ventilation. His complicated clinical course involved two months of initial hospitalization and multiple subsequent admissions, with numerous instrumental examinations and close clinical monitoring, yet CPP was never diagnosed. Choroid Plexus Papillomas are rare, accounting for about 1-4% of pediatric brain tumors.[1,2] They are typically located in the ventricular system and can cause hydrocephalus by obstructing Cerebrospinal Fluid (CSF) flow or by overproducing CSF.[3,4] In neonates, especially those with conditions like microdeletion syndromes, diagnosing and managing CPP presents significant challenges due to overlapping symptoms and the severe potential consequences of associated hydrocephalus.[5,6]

CPP was identified through autopsy and ancillary histological examinations. A gelatinous, reddish area was found in the fourth ventricle, which was sampled for histological examination by hematoxylin eosin staining. The histopathological analysis revealed papillomatous proliferation of the choroid plexus. The autopsy revealed a CPP in the ventricular system, a finding not previously detected despite rigorous monitoring. Histological examination showed typical features of CPP, such as papillary growth patterns and cellular characteristics resembling normal choroid plexus tissue. This discovery highlighted a critical gap in current pediatric neuroimaging protocols, which failed to detect the tumor during the infant's life. The case also illustrated the severe implications of such a tumor in an infant with significant pre-existing health issues, as the infant ultimately died from prematurity-related complications before CPP could be clinically addressed.

This case illustrates the complexities in managing neonates with genetic syndromes and brain tumors, highlighting the diagnostic importance of autopsy examinations. The overlapping symptoms of microdeletion syndrome and CPP complicated the clinical picture, making timely diagnosis difficult. The presence of CPP significantly increased the risk of developing hydrocephalus, a potentially fatal condition if not promptly identified and managed. The findings underscore the need for vigilant hydrocephalus surveillance in all neonates diagnosed with brain tumors, regardless of their benign nature. The occurrence of CPP in this premature infant with microdeletion syndrome underscores the need for comprehensive diagnostic protocols and an interdisciplinary approach in neonatal intensive care. Enhancing early detection strategies and considering the complex interplay between genetic and neoplastic factors is crucial for improving outcomes in similar cases. Future research should focus on developing targeted surveillance and diagnostic techniques that account for the unique challenges posed by congenital anomalies and neoplastic conditions in the neonatal population. Finally, this study reiterates the importance of performing autopsies for clinical purposes in all neonatal deaths.

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Autopsy; Infant; Tumor

I87 Undiagnosed T-Lymphoblastic Lymphoma Mimicking Asthma and Leading to Sudden Death in a Pediatric Patient

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Learning Objectives: The objective of this presentation is to educate attendees about the difficult diagnosis of T-lymphoblastic lymphoma in pediatric patients and the preceding concern for asthmatic symptoms masking this patient's diagnosis.

Impact Statement: This presentation will impact the forensic science community by representing an unusual case in which T-lymphoblastic lymphoma, accounting for up to 70–80% of cases of Non-Hodgkin Lymphoma (NHL) in children, adolescents, and young adults,[1] presented with asthmatic symptoms in a pediatric patient and resulted in sudden death.[1] Historically, up to 90% of cases of lymphoblastic lymphoma present as bulky mediastinal disease in young males, often with shortness of breath, chest pain, or superior vena cava syndrome.[2]

Abstract Text: In this case, a 9-year-old male with a history of asthma at age 2 (but no symptoms or medical management since) without a significant family medical history developed a progressively worsening cough with difficulty breathing, shortly after moving residence. Other than a tonsillectomy and adenoidectomy, he had no surgical or other medical issues.

For two weeks, the patient had a persistently worsening cough that was intermittently productive and worsened with both activity and at nighttime. Due to the persistent nature of the cough and a new onset of difficulty breathing, when the family initially sought care, they were discharged home with albuterol and concerns for recurrent asthma. After three days of medication use, his symptoms did not improve, and he developed an intermittent fever prompting an Emergency Room (ER) presentation. A significant amount of anterior cervical adenopathy was noted on physical exam, along with diffuse expiratory wheezes in all lung fields. The patient was afebrile. A chest X-ray was obtained with initial interpretation by the emergency physician as showing a large thymus, but no definite infiltrates. The patient received two albuterol nebulizer treatments with slight improvement in wheezing and saturations remaining around 93%. Following continuous levalbuterol and prednisolone, though, the patient greatly improved and was discharged in stable condition with albuterol per nebulizer and prednisolone for a diagnosis of acute dyspnea, acute bronchitis, acute asthmatic exacerbation, and acute cervical lymphadenopathy. Over the course of the next few days, the patient developed lethargy, nausea, vomiting, diarrhea, and diaphoresis. He was then found dead in bed in the morning by his father.

At autopsy, the lungs were somewhat displaced laterally and the heart was displaced downward and to the left due to a mediastinal mass. Within the mediastinal soft tissues, there was an 870gram, 17 x 10 x 8cm, firm, infiltrative white-tan mass that had a somewhat soft to focally firm nodular surface. This mass was found to be T-lymphoblastic lymphoma, staining positive for CD1A, CD2, CD3, CD4, CD7, CD99, TdT, and MIB-1. There was involvement in the mediastinum, lymph nodes, lungs, epicardium, pericardium, pleura, trachea, kidney, spleen, liver, and bone marrow. Additionally, 100cc of serosanguineous fluid was collected within both pleural cavities and within the pericardial cavity. On histology, there was no significant evidence of asthma.

The present case both highlights the difficulty of diagnosing T-lymphoblastic leukemia/lymphoma and raises questions as to whether the decedent's "asthma" symptoms were entirely related to the developing lymphoma. Of note, the thymus is rarely seen on X-ray after 8 years of age.[3] This case stresses the difficulty of diagnosis, and the need for physicians to continually consider this rare but serious diagnosis on their differentials for pediatric patients presenting with shortness of breath and/or persistent cough.

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T-Lymphoblastic Lymphoma; Asthma; Pediatric

I88 A Comparison of Histomorphological Changes of Atherosclerosis and Lipid Profile in Non-Cardiac Deaths Among Children and Young Adults at Autopsy

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Learning Objectives: After attending this presentation, attendees will gain an understanding in initiation and progression of atherosclerotic disease in children and young adults. The presentation highlights histomorphological changes in the coronary arteries in non-cardiac deaths at autopsy and their association with hypercholesterolemia in children and young adults.

Impact Statement: This will impact the forensic science community to elucidate the genetic influence in initiation of Atherosclerosis (AS), a disease often attributed to lifestyle choices. This may further help in understanding the atherosclerotic disease process with or without lifestyle modification. Further, the genetic predisposition may be utilized to prevent morbidity and mortality in the young population.

Abstract Text: AS, rooted in Greek for "gruel" and hardening, traces back to 3300 BCE.[1] Sudden death in young adults without medical history often raises suspicions. Sudden deaths due to myocardial infarction or strokes (CVA) remain globally concerning, yet hardly any data is available, especially from India in this age group.[2] This study aims to illuminate these gaps and understand atherosclerosis patterns in children and young adults.

The primary objectives of this study were to evaluate the pattern of atherosclerotic changes in children and young adults aged 1 to 30 years through histopathological examination of coronary arteries. In addition, we determined pattern of hypercholesterolemia using postmortem serum lipid profiles and correlated with histomorphological atherosclerotic changes. Further, the grade of atherosclerosis and lipid profile were compared with demographic details.

In this study, a total of 71 autopsy cases were analyzed to determine the degree of coronary involvement due to atherosclerotic changes. Both macroscopic and microscopic examinations of the coronary arteries were performed. Furthermore, postmortem biochemical analyses of lipid profiles were conducted on 61 cases. The data were analyzed to determine the extent of AS, hypercholesterolemia, and their associations with various demographic and lifestyle factors.

The results of the study revealed that 21 out of 71 cases (29.58%) exhibited atherosclerotic changes upon gross examination. None of the cases had more than 50% luminal narrowing on gross examination. Microscopic examination showed that 69 cases (97.18%) had some form of atherosclerosis, predominantly affecting the Left Anterior Descending (LAD) and Left Circumflex (LCX) arteries. On examining the coronaries, triple vessel involvement was most common, followed by double vessel, and none of the cases showed only one vessel being involved. It was observed that the proportion of double- and triple-vessel disease increased with increasing age. On assessing the postmortem serum lipid profile, we found that only two (3.28%) cases had hypercholesterolemia, the majority being in the normal range. These cases were males, though the results were statistically insignificant ($p=0.580$). The cases showing hypercholesterolemia belonged to age >10 years and had BMI >18.5 Kg/m2. The two cases with hypercholesterolemia showed a direct correlation with atherosclerotic changes though statistically insignificant. We also observed that smoking and alcohol intake was significantly related to hypercholesterolemia. ($p=0.006$). We observed that males had higher hypercholesterolemia, increasing with age and mean BMI though statistically insignificant ($p >0.05$). The findings indicate a high prevalence of atherosclerotic changes starting from as early as one year of age, with progression observed as age increases. The significant role of genetic influences on the development of AS, beyond lifestyle factors, is highlighted, emphasizing the necessity for early intervention and a deeper understanding of genetic predispositions.

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Histological; Autopsy; Pediatric

I89 A Multivessel Sudden Coronary Artery Dissection During the Peripartum Period

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Learning Objectives: The goals of this presentation are to: (1) define sudden coronary artery dissection, (2) recognize which populations are at risk of SCAD, (3) explain how SCAD goes misdiagnosed, (4) describe the gross findings of SCAD, single vessel and multivessel, and (5) discuss the possibility of familial inheritance

Impact Statement: Because sudden coronary artery dissection typically occurs in those without cardiovascular risk factors, it may be misdiagnosed before death and can present as sudden unexplained death at autopsy. This, coupled with its poorly understood etiology and subtle gross findings, makes it easy to miss during postmortem examination. This presentation will impact the forensic science community by bringing awareness to its prevalence, high-risk populations, gross findings, and its possible familial inheritance patterns.

Abstract Text: A 29-year-old woman in the early postpartum period (18 days since cesarean section) was found unresponsive in her bed. Family and responding emergency personnel attempted resuscitative measures that were ultimately unsuccessful; she was pronounced dead at the scene. She had recently complained of migraine, burning eyes, blurry vision, anxiety, back pain, and her arms "feeling like Jello." Her medical history included gestational hypertension, marijuana use, migraines, an unspecified congenital heart abnormality, anemia, anxiety, and multiple miscarriages.

At autopsy, she was found to have a healing Pfannenstiel incision and a geographic burn of her upper back consistent with the use of a heating pad (no other injuries). Internal examination identified mild cardiomegaly (361g with an expected weight of 242-323g) with prominent-appearing left anterior descending and right coronary arteries. The myocardium of the left ventricle was slightly mottled; no congenital anomaly was identified. Sectioning of the coronary arteries revealed dissecting hemorrhages creating near-complete compression of the lumina of two coronary arteries, involving mid- to proximal segments (~6.1cm for Left Anterior Descending [LAD] and ~6.5cm for Right Coronary Artery [RCA]). The circumflex and remaining coronary branches demonstrated patent lumina. Histology confirmed multi-vessel involvement in Spontaneous Coronary Artery Dissection (SCAD).

SCAD is an intimal tear of an epicardial coronary artery, causing a disruption between the tunica intima and adventitial layers of the artery. It is characterized by intramural hematoma and false lumen formation leading to the obstruction of blood flow and myocardial ischemia; 85-90% of the published cases of SCAD occur in young to middle-aged women; current or recent pregnancy is a known association.[1] SCAD usually presents with signs and symptoms of ST-elevation myocardial infarction, including chest/shoulder pain, syncope, dyspnea, diaphoresis, and nausea; ventricular arrhythmias or shock are rare. Although SCAD is an emerging cause of Acute Coronary Syndrome (ACS) in women under 50 years without cardiovascular risk factors, it is still a relatively rare cause that accounts for < 1% of myocardial infarctions.[1]. The multifactorial etiology of SCAD is poorly understood; its overwhelmingly higher incidence in women suggests the hypothesis that female sex hormones play a role in its occurrence. Other possible contributory factors are underlying arteriopathies such as fibromuscular dysplasia, and emotional and physical stress. Because SCAD typically occurs in those without cardiovascular risk factors, it may be misdiagnosed; it can present as sudden unexplained death.

Multivessel SCAD, less well documented in the medical literature, makes up 9-23% of SCAD cases.[2] Pregnancy-Associated Spontaneous Coronary Artery Dissection (PASCAD) accounts for 5% of SCAD cases; sufferers average a decade younger than those with non-pregnancy related SCAD. PASCAD has a predilection for left main, multivessel, and proximal segment involvement, creating a markedly higher risk of major adverse cardiovascular events, including sudden death. This decedent suffered pregnancy-associated multivessel SCAD, which led to her death in sleep, with recognition of the disease only at autopsy.

Recent genome-wide association meta-analysis has identified 16 risk loci for SCAD; although the exact inheritance is not known, the autopsy diagnosis in this case both answered family questions and may be important for prevention of cases in her kindred.[3]

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Sudden Death; Pregnancy; Cardiac Death

I90 Pregnancy-Associated Manners of Death in Western Michigan, 2016–2024

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Learning Objectives: After attending this presentation, attendees will learn how the manners of deaths of individuals who are peripartum/postpartum, a vulnerable population, may show a different distribution compared to those who were not pregnant within one year of death in Western Michigan. Attendees will become familiar with the importance of accurate and complete death certification for the purposes of public health and vital statistics as well as potentially for death prevention.

Impact Statement: This presentation will impact the forensic science community by demonstrating possible differences that exist between the circumstances surrounding deaths in the peripartum/postpartum population and individuals who were not pregnant within one year of death. It will highlight the importance of accurate and complete death certification and the need for collaboration among interdisciplinary teams to improve maternal health and potentially prevent deaths.

Abstract Text: In 2003, the Centers for Disease Control and Prevention (CDC) National Center for Health Statistics revised the standard death certificate to include the decedent's pregnancy status within the past year to more effectively track maternal mortality.[1] Although the addition of this checkbox was meant to allow for better data collection, up to one-third of the positive pregnancy checks are false positives.[2] Checkboxes on death certificates are effective in identifying pregnancy-associated deaths resulting from maternal causes. However, they are far less effective in identifying deaths resulting from non-maternal causes, such as homicide, accidental death, and substance abuse.[3] When investigating maternal deaths, terms such as "pregnancy-associated" (death during and within one year of pregnancy) and "pregnancy-related" (death due to direct sequela of the pregnancy) must be understood to be used correctly.[4] To further examine deaths and potential trends in this population, this study evaluated the distribution of manners of deaths among individuals who were pregnant within the year prior to death compared to a similar-aged group of decedents who were not recently pregnant.

The electronic death investigation database was queried for all cases reported to the Medical Examiner's Office between January 2016 and July 2024 in which "Pregnant at the time of death," "Pregnant within 42 days of death," or "Pregnant 43 days to 1 year before death" was selected on the Medical Certificate of Death. The age range of these decedents was noted and an additional query for decedents categorized as "Not pregnant within the last year" was performed for comparison.

A review of deaths reported to the Medical Examiner's Office revealed 62 individuals who were pregnant within the last year, 21 of whom were noted to be pregnant at the time of death (33.9%). The 62 decedents ranged in age from 19 to 43 years. Of these cases, there were 21 certified as natural (33.9%), 30 as accident (48.4%), 3 as suicide (4.8%), 6 as homicide (9.7%), and 2 as indeterminate (3.2%). In the same time frame, there were 632 deaths of individuals with ages that ranged from 18 to 45 years who were categorized as not pregnant at the time of death or within the year prior to death. Of these cases, there were 270 certified as natural (42.7%), 248 as accident (39.2%), 73 as suicide (11.6%), 23 as homicide (3.6%), and 16 as indeterminate (2.5%). Two cases (0.3%) remained pending further information at the time of this writing. A z-test used to compare percentages between two groups revealed that there were significantly more homicides in the individuals who were either pregnant at the time of death or within the year prior to death than those who were not pregnant within one year of death ($p < 0.05$). While suicides appeared to be a more common manner of death among those who were not pregnant or recently pregnant, this was not statistically significant ($p = 0.1$).

Deaths of individuals who are pregnant or recently pregnant often fall under the jurisdiction of the medical examiner/coroner, as this is generally a young, healthy, and potentially vulnerable population. For public health and vital statistics purposes, death certification should always be accurate and complete. Review of these data sets demonstrates that homicides occur more often in the "Pregnant within one year of death" cohort than those who were not pregnant in the year prior to death. These pregnancy-associated deaths are preventable, and the advocacy of the Medical Examiner's Office in collaboration with Maternal Mortality Review Committees, Public Health Departments, and the CDC can offer additional avenues to improve maternal health in Western Michigan.

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Maternal Mortality; Pregnancy; Manner of Death

I91 An Approach to Placental Examination and Placental Pathology for the Forensic Pathologist

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Learning Objectives: Attendees will learn a systematic approach to placental examination and pathology, identifying key gross and microscopic findings. They will understand the implications of these findings in perinatal and neonatal deaths.

Impact Statement: This presentation will strengthen the skills of forensic pathologists in placental examination, improving their ability to identify and interpret key findings. This knowledge will improve the accuracy and reliability of their examinations as well as cause of death certification in perinatal and neonatal deaths.

Abstract Text: The placenta is a crucial diagnostic organ in forensic pathology in the context of perinatal and neonatal death. This presentation outlines a systematic approach to placental examination and pathology, guided by the Amsterdam Placental Workshop Group Consensus Statement.[1]

A comprehensive placental examination begins with an external gross assessment, noting key descriptors such as weight, dimensions, umbilical cord characteristics, and membrane condition. Significant gross findings, including cord hyper/hypocoiling, abnormal membrane or cord insertion, and atypical placental size for gestational age, can be easily overlooked but may be essential for determining the cause of death. Histologic sampling should include extraplacental membranes, cross-sections of the umbilical cord, and normal-appearing parenchyma from the central two-thirds of the disc.

Key diagnostic entities include Maternal Vascular Malperfusion (MVM) and Fetal Vascular Malperfusion (FVM). MVM, characterized by altered blood flow through the maternal vessels of the placenta, is typically associated with maternal conditions such as pre-eclampsia, diabetes mellitus, and hypertension. Manifestations include placental hypoplasia, parenchymal infarcts, and decidual arteriopathy. FVM is characterized by obstructed blood flow in the fetal vessels, with histologic manifestations including vascular thrombosis, mural fibrin deposition, and avascular villi.

Evaluating ascending intrauterine infections and their inflammatory responses is also critical, with current Amsterdam guidelines recommending documentation of inflammation stages and grades. Maternal Inflammatory Response (MIR) encompasses the spectrum of subchorionitis and chorioamnionitis, while Fetal Inflammatory Response (FIR) includes inflammation in the chorionic plate and umbilical cord vessels. Chronic villitis (of unknown etiology) and other inflammatory lesions such as chronic histiocytic intervillositis and massive perivillous fibrin deposition have also been implicated in fetal demise and carry a significant recurrence risk in subsequent pregnancies.

Placental pathology has profound implications for determining the cause of perinatal and neonatal deaths. Findings of MVM indicate altered maternal vascular flow to the placenta and are associated with poor fetal outcomes, including intrauterine growth restriction, placental abruption, and fetal demise. FVM findings indicate acute or chronic fetal blood flow obstruction, leading to fetal hypoxia, with etiologies such as umbilical cord knot or nuchal cord. MIR and FIR suggest infectious or inflammatory conditions affecting the mother or fetus.

A thorough and systematic approach to placental examination is indispensable in forensic pathology. Pathologic findings in the placenta can guide directed examination of other organ systems, correlate with autopsy and neuropathology findings, and provide a clear etiology regarding the cause of death or intrauterine fetal demise.

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Forensic Pathology; Placental and Perinatal Pathology; Autopsy

I92 Examining the Lung Float Test: Ending the Use of a Broken Tool for Pregnancy Criminalization and Wrongful Convictions

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Learning Objectives: Attendees will learn about the Hydrostatic Float test, also known as the Lung Float Test (what is it and when is it used). Attendees will learn about the test's inability to consistently and accurately fulfill its sole purpose: determining whether a neonate was born alive. They will further learn about how the test is used to justify pregnancy criminalization and why/how the test should be challenged in court to end the wrongful convictions of mothers who have suffered pregnancy loss.

Impact Statement: This presentation will inform and educate attendees about this little-known or discussed test and how, despite its widely acknowledged scientific weaknesses, it continues to have massive negative impacts on defendants convicted of felony charges based on this insufficient evidence at criminal trials. The presentation will explain why medical examiners should cease use of the Lung Float Test, and/or think critically about how they write about their findings and discuss the test with prosecutors, police, and juries. The presentation will also explain why prosecutors and police should not rely on lung test float evidence or introduce Lung Float Test evidence at trial, and why defense lawyers should challenge the admission of Lung Float Test results in court. The ultimate goal of this talk is to eradicate the use of the Lung Float Test as evidence in criminal prosecutions and prevent the possibility of future wrongful convictions.

Abstract Text: The Lung Float Test is a 17th-century practice whose basic methodologies have not advanced in the hundreds of years since its invention.[1] The test is used by medical examiners to determine whether a neonate was born alive or stillborn. Over the centuries, scientists and doctors have spoken out against the test's many flaws, including its high error rate, and determined it to be unreliable.[2-4] Despite loud scientific voices railing against its use, the test is still performed in medical examiner offices nationwide and relied on as definitive proof in criminal homicide prosecutions to this day.[5-7]

Pregnancy Justice has identified multiple cases in which women were charged and/or convicted, at least one wrongfully for the deaths of their neonates, with the prosecution relying on the Lung Float Test as the sole evidence used to argue the neonate was born alive.[8] In the vast majority of these cases, the mother reported to police that the neonate was stillborn.

Recently, the Lung Float Test has been used in cold case investigations in which parents are identified, sometimes decades after the neonate's death, through the use of genetic genealogy, thus dangerously pairing hyper-advanced crime solving technologies with the least modern forensic assessment in a medical examiner's tool belt.

This panel will: (1) present the flaws in the science underpinning the Lung Float Test; (2) discuss the patterns found in cases that utilize the Lung Float Test; (3) outline some of the more egregious cases where the test was used to obtain a conviction; and (4) provide actionable steps that can be taken by stakeholders across the legal system to prevent this test from causing unjustified convictions, including fully ending its use as a medical examiner forensic tool, ending reliance on Lung Float Test results in court, and using *Frye* and *Daubert* motions to contest the Lung Float Test's accuracy and utility.

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Pediatric; Wrongful Conviction; Lung Float

I93 Genetic Insights Into Pediatric Sudden Cardiac Death: A Case Report of Anomalous Left Coronary Artery Originating From the Right Sinus of Valsalva

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Learning Objectives: After this presentation, the audience will understand anomalies where the left coronary artery originates from the right sinus of Valsalva, including the epidemiology and underlying pathophysiology. We will also explore the genetic mutations involved in cardiovascular development. At the end, attendees will gain insights into these complex conditions and the genetic factors that contribute to them.

Impact Statement: This presentation will impact the forensic science and clinical community by identifying anomalies in morphogenesis and proposing future research directions aimed at enhancing genetic databases, refining variant interpretations, and advancing personalized medicine approaches.

Abstract Text: **Introduction:** Sudden Cardiac Death (SCD) among pediatric populations is a rare yet critical event, often linked to congenital and acquired cardiac abnormalities. These include structural heart diseases, cardiomyopathies, coronary artery anomalies, arrhythmias, primary pulmonary hypertension, and commotio cordis. Notably, coronary artery anomalies account for about 10% of all cases of SCD in the young, with most data gathered postmortem. Anomalous origin of coronary arteries, including the left coronary artery originating from the right sinus of Valsalva, is a significant risk factor for SCD, particularly during exertion. This study integrates findings from a case report and broader discussions on the epidemiology and genetic implications of SCD in young individuals.[1]

Case Presentation: An autopsy was conducted on a 15-year-old Hispanic male who suffered SCD during exertion. The patient had no prior medical history, family history, or substance use. Autopsy findings revealed an anomalous left coronary artery originating from the right sinus of Valsalva. Next-generation sequencing was performed, focusing on a comprehensive arrhythmia and cardiomyopathy panel, identifying mutations in the SCN1A, deemed as "Likely Pathogenic;" LAMA4, and TRPM4 genes, considered as "Variants of Uncertain Significance (VUS)" based on the classification by the American College of Medical Genetics and Genomics.

Discussion: SCD among young patients is a tragic occurrence often attributed to cardiovascular abnormalities detected postmortem. Symptoms such as angina and syncope, particularly during exercise, may precede SCD, although sudden death can sometimes be the initial presentation. Mechanisms leading to SCD include ischemia exacerbated by mechanical obstruction of coronary arteries, potentially culminating in fatal arrhythmias during exertion.[2] Various genetic mutations have been implicated in critical stages of cardiac development, including those affecting cardiac morphogenesis, atrioventricular valve and endocardial cushion development, cardiomyocyte differentiation, and local microvasculature and angiogenesis. Genetic testing and screening of relatives using imaging techniques could significantly enhance understanding in this field. Awareness of such anomalies emphasizes the necessity for comprehensive cardiac evaluations in young patients to reduce the risk of SCD.[3]

Conclusion: A comprehensive examination of cardiac structures and genetic testing is vital for determining the precise cause of death, particularly in complex cases where genetics could potentially contribute to anomalies found during the autopsy. Continuous genetic testing and meticulous documentation of anomalies are critical to refining genomic databases and elucidating VUS, thereby supporting improved patient care and genetic counseling. Understanding rare coronary anomalies not only aids in averting adverse events in young individuals but also drives forward cardiovascular genetics research, enhancing risk prediction and management, mainly through genetic counseling for affected families.

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Autopsy Medicine; Coronary Artery Anomalies; Cardiac Genetics

I94 Retinal Fold Apical Attachment of the Internal Limiting Membrane Is Not Specific for Vitreoretinal Traction During Alleged Cycles of Ocular Acceleration-Deceleration Based on Lange's Folds Histology

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Learning Objectives: After attending this presentation, attendees will recognize that attachment of the internal limiting membrane to retinal fold apices is not specific for Vitreoretinal Traction (VRT) from alleged repetitive ocular acceleration-deceleration forces (shaking).

Impact Statement: This presentation will impact the forensic science community by showing that the attachment of the internal limiting membrane at the apices of artifactual Lange's folds is histopathologically identical to its attachment on reported perimacular and pathologic retinal folds.

Abstract Text: VRT is often suggested as the cause of retinoschisis, Perimacular Retinal Folds (PRFs), Retinal Hemorrhages (RHs), and "pathologic" retinal folds during cycles of ocular acceleration-deceleration (shaking). Support for the VRT hypothesis includes the attachment of the posterior hyaloid and Internal Limiting Membrane (ILM) to the apex of the retinal fold. In 1986, Greenwald et al. reported on five infants with severe RHs and retinoschisis, hypothesizing that the retinoschisis resulted from the direct mechanical forces of violent shaking.[1] Two years later, Gaynon et al. concluded that extensive RHs and bilateral, ring-shaped, symmetrical PRFs were associated with VRT based on observations of two presumed child abuse victims.[2] In 1991, Massicote et al. claimed that their case series of three infants confirmed the role of VRT in forming PRFs, as evidenced by partial vitreous detachment except at the apices of the retinal folds.[3]

Breazzano et al. examined ocular findings in 60 eyes of young children (ages 1-30 months) diagnosed with Abusive Head Trauma (AHT) and 46 eyes from controls or cases with alternative causes. They claimed that their findings, including the perimacular ridge, illustrated the physical mechanism of violent forces transmitted by VRT, which they associated with abusive head trauma based on age-related anatomical vulnerability.[4] Abed Alnabi et al. named VRT as the likely mechanism behind PRFs in AHT, based on histopathological findings in five children (ages 2-13 months) diagnosed with AHT who had PRFs. They documented condensed vitreous, and ILM attached to the apices of the retinal folds, with the detached ILM forming the inner surfaces of the schisis cavity.[5]

Lange's folds are well-recognized postmortem fixation artifacts in retinas of neonates, infants, and young children.[6,7] The current study examined the histopathological features of the ILM on Lange's folds and its reported apical attachment on perimacular and pathologic retinal folds.

Our study involved 15 pediatric autopsy cases with RHs found by postmortem monocular indirect ophthalmoscopy between the years of 2016 and 2024. Clinically, child abuse was not suspected and none had a documented antemortem fundal examination. The cohort included eight males and seven females, aged from 1 day to 55 months, with a median age of 6 months. The manners of death were six natural, five accidental, one homicide, and three undetermined; none involved non-accidental head trauma.

All of the eyes showed Lange's folds. Microscopic sections, cut at 6 microns, were stained with Hematoxylin and Eosin (H&E), picrosirius red, fast green, alcian blue (RGB) trichrome, and Periodic acid-Schiff (PAS). In all 15 cases, the ILM was attached to the apices of Lange's folds and detached from the adjacent retina, identical to its appearance on reported perimacular and pathologic retinal folds.

Our findings suggest that ILM attachment to the apices of retinal folds is not specific for VRT resulting from alleged ocular acceleration-deceleration (shaking). Misinterpreting these retinal microscopic findings can lead to wrongful accusations of child abuse against caregivers or families. We urge caution in interpreting the microscopic finding of ILM apical retinal fold attachment out of context due to the potential for causing irreversible harm.

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Vitreoretinal Traction; Lange's Folds; Internal Limiting Membrane

I95 Frequency and Distribution Patterns of Rib Fractures in Deceased Neonates, Infants, and Young Children

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Learning Objectives: By attending this presentation, attendees will gain an insight into the incidence and locations of pediatric rib fractures from distinct mechanisms of injury. The frequency and distribution of rib fractures caused by Cardiopulmonary Resuscitation (CPR) will be described and compared to those produced from accidental and non-accidental trauma. The detection of rib fractures using various imaging modalities will be discussed.

Impact Statement: This presentation will provide medicolegal professionals with the frequency and distribution of fractures for various mechanisms of injury in a large pediatric autopsy cohort. This data is crucial to assist with accurate diagnosis by determining the difference between fractures caused by CPR and by accidental/non-accidental injury.

Abstract Text: Rib fractures found in neonates, infants, and young children in the absence of a congenital bone disorder or a traumatic history are considered highly suspicious for Abusive Head Trauma (AHT).[1-3] However, rib fractures have also been reported in deceased infants with no additional injuries suggestive of non-accidental or major trauma, which have been attributed to CPR.[4] Posterior rib fractures are believed to be the most prevalent in the case of AHT, whereas CPR-induced fractures are most commonly located anteriorly.[1,3,5,6] Typically, medical and scientific literature reports indicate that the incidence of rib fractures in young children resulting from CPR are very rare (approximately 0-2%).[7-10] However, in our postmortem experience, rib fractures occur in approximately 10% of neonates, babies, and young children that have received CPR.

This retrospective study will examine the frequency and locations/patterns of rib fractures attributed to CPR from the autopsy and imaging reports of over 350 children under 3 years of age that required either a forensic (over 100 cases) or coronial postmortem (over 250 cases), undertaken as part of one pediatric pathologist's case load over a 12-year period (November 2010–December 2022). Comparisons will also be made to rib fractures caused by suspected abusive injury and accidental injury. Furthermore, the study will compare the detection of rib fractures reported during the postmortem examination and on hospital and postmortem skeletal surveys and Computed Tomography (CT) scans.

The aim of the study is to aid health, law enforcement, and legal professionals involved in child death investigations by creating a more robust evidence base for accurate diagnoses in cases involving rib fractures. Comparing rib fracture locations reported on full-body radiographs (i.e., skeletal survey), CT scans, and autopsy data will also determine the sensitivity and specificity of each imaging modality within a significantly large case series in the detection of rib fractures attributed to various mechanisms of injury, including abusive handling, CPR, and accidental trauma in children.

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CPR; Rib Fractures; Radiology

I96 Pediatric Autopsies in Luxembourg: A Retrospective Analysis of a Five-Year Period (2018–2022)

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Learning Objectives: After attending this presentation, attendees will have an insight into autopsy rates as well as cause and manner of deaths in infants, children, and adolescents in Luxembourg.

Impact Statement: This presentation will affect the forensic science community by sensitizing investigators and forensic pathologists to the importance of pediatric forensic autopsies in order to discover non-natural/violent deaths as well as natural deaths with relevance to public health.

Abstract Text: As literature on deaths and autopsy frequency in childhood and adolescence is scarce, it is to be feared from a forensic medical point of view that some cases requiring clarification will not be investigated further, and that non-natural and natural deaths will remain undetected.[1]

Luxembourg is a Western European country with approximately 670,000 inhabitants (as of January 2024). All forensic autopsies for the country's two judicial districts are currently carried out at the Institute of Legal Medicine (DML), which is part of the national health institute (Laboratoire National de Santé [LNS]) in the south of Luxembourg. Clinical autopsies are done by the National Center of Pathology (NCP) located at the same facility. The fact that all autopsies are carried out at the same location and that national cause of death statistics are available opens up the unique possibility of fully evaluating the data with regard to type of death, autopsy rate, and age groups.

For the present study, all forensic autopsies carried out at the DML and clinical autopsies done at the NCP from 2018 to 2022 on individuals younger than 20 years, divided into five groups (< 1 year, 1-4 years, 5-9 years, 10-14 years, 15-19 years), were evaluated with regard to the cause and manner of death. In addition, the cause of death statistics for the corresponding years were analyzed and then aligned with the autopsied cases.

In the five-year period analyzed, 150 individuals under the age of 20 (95 [63.3 %] male; 55 [36.7 %] female) died. This corresponds to 0.7% of all deaths in Luxembourg. Most of these deaths involved children under one year of age (n=79), and in particular the neonatal period (n=68). In 104 cases (69.3 %), the manner of death was ruled natural, with the percentage of natural deaths in the different age groups ranging from 29.3% (group 15 to 19 years) to 97.5% (group < 1 year); 46 deaths (30.7 %) were ruled non-natural, with three quarters of those deaths (76.1 %) affecting male individuals.

During the observation period, 35 of the 150 deaths of persons under the age of 20 were subjected to a medicolegal autopsy; this corresponds to an overall forensic autopsy rate of 23.3% of all cases in that age group. 63.0 % (n=29) of all non-natural deaths in individuals below the age of 20 years received a forensic autopsy with the autopsy rates varying significantly in the different age groups. Clinical-pathological autopsies, with n=12, predominantly concerned the age group under one year (n=10), and here mostly neonatal deaths (n=6).

Among the non-natural causes of death, accidents dominated with n=20, followed by suicides (n=7). Homicides were rare (n=2) and exclusively concerned small children—one case of non-accidental head trauma in a seven-month-old infant and one traumatic death of a 2-year-old child caused by a car deliberately hitting him. In one case, no cause of death could be determined.

All autopsy cases received a complete toxicological analysis, with 48.6 % of the analyses rendering a negative result. Most of the positive cases were due to the administration of medication as part of emergency or intensive care treatment.

The evaluation of all deaths and autopsies in infants, children and adolescents presented here shows that, from a forensic point of view, there are clear deficits in the frequency of autopsies in both natural and violent deaths in the age groups examined. This carries the risk that cases of abuse, but also internal diseases with relevance to public health, are overlooked. In our view, a higher, ideally complete, frequency of autopsies should be the aim.

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Autopsy; Pediatric; Cause of Death

I97 Suicides in Children and Young Teens: A Nine-Year Retrospective Review From the Cook County Medical Examiner's Office

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Learning Objectives: After attending this presentation, attendees will better understand the incidence, circumstances, and features of suicides among subjects aged between 8 and 14 years in a large metropolitan area.

Impact Statement: This presentation will impact the forensic science community by addressing pediatric suicide deaths in pre-adolescents, highlighting circumstances, methods, risk factors, and precipitants.

Abstract Text: According to the Centers for Disease Control and Prevention (CDC), suicide deaths in the youngest generations have experienced an upward trend over the past decades.[1] The most recent data show a three-times increase among people aged 10-14 from 2007 through 2018, 57% increase among people aged 15-19 from 2009 through 2017, then persistently high rates in both age groups through 2021.[2]

Suicide in the pediatric population is a topic rarely discussed in the current forensic literature. Since the circumstances that lead a child or young teen to commit suicide can be particularly difficult to evaluate, the determination of the manner of death may be challenging for forensic pathologists.

A retrospective review of cases from the digital database of the Cook County Medical Examiner's Office in Chicago, IL, was performed from January 2015 to December 2023 to search for suicides in the pediatric population. Because of the large number of suicides due to gunshot wounds in the age group between 15 and 18 years, only subjects aged 14 or younger were included in the study. Since no cases of suicide were identified in subjects younger than 8 years, the lower limit was set at that age.

Following the age classification proposed by the CDC, subjects were subcategorized into two age groups: middle childhood (8-11 years) and young teens (12-14 years). Cases were reviewed for age, sex, race, cause of death, suicide method, circumstances of death, month and time of death, location of the event, autopsy or external examination findings, and toxicology findings. When available, information regarding risk factors, previous suicide attempts or ideation, and precipitants was obtained from the investigative and police reports.

Fifty-three cases (29 males and 24 females) were identified. The average age was 11 years. Twelve cases were in the middle childhood age group, while 41 cases were young teens. Most subjects were White (32 cases), followed by Black (18 cases), and Asian (3 cases). The leading suicide method was hanging (36 cases), followed by gunshot wounds (10 cases), self-poisoning (4 cases), train striking pedestrian (2 cases), and jump from a height (1 case). An external examination was performed in 38 cases, a full autopsy in 14 cases, and a head autopsy in 1 case.

This study will examine distinct features of suicide deaths in the pediatric population, addressing an important public health problem through the experience of a large medical examiner's office. The results of this study will be discussed with attendees.

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Forensic Science; Pediatric; Suicide

I98 Tragic Homecoming—Fatal Dengue-Induced Myocarditis in a Maldives Vacationer: A Case Report

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WITHDRAWN

I99 A Nearly Missed Homicide: Pre-Cremation and Delayed Detection of Gunshot Wounds in a Mistaken Love Triangle

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Learning Objectives: After attending this presentation, attendees will: (1) review unusual case features that may obscure detection of gunshot wound homicides; (2) consider the value of skilled pre-cremation body examination; and (3) learn strategies of law enforcement and medical examiner cooperation to detect difficult homicides.

Impact Statement: This presentation would impact the forensic science committee by drawing attention to features of unusual gunshot wound homicides that may lead to overlooking significant injuries, with implications for the judicial process.

Abstract Text: A 53-year-old man, an employee at a liquor store whose significant medical history included obesity, was found dead in bed with his CPAP mask pushed to one side. His personal physician agreed to sign the death certificate; cremation was requested.

The medical examiner who was sent to the funeral home to inspect the body prior to cremation noticed that the decomposition-stained sheets brought from the scene suggested overlapping blood stains. Her examination indicated the possibility of a gunshot wound under the double chin; an overlooked suicide was considered.

Examination of the body at the regional office two days later documented more advanced decomposition partially obscuring a small, atypical gunshot wound defect on the undersurface of the chin, associated with unexplained marks not characteristic of hard contact; suicide was still in consideration at this point. Plain radiographs demonstrated two bullets in the head. Repeat examination then identified a highly atypical entrance gunshot wound without evidence of close-range fire, entering through the right nostril where it overlapped the columella of the nose. Autopsy showed that each bullet penetrated the brain, findings inconsistent with suicide.

Along with a history of obesity and obstructive sleep apnea, autopsy confirmed long-standing medical conditions, including a hypertrophied heart from hypertensive cardiovascular disease, coronary and aortic atherosclerosis, hepatosplenomegaly, prostatic hypertrophy, and a renal stone. Toxicology showed only a low level of ethanol. Death was due to the gunshot wounds.

The scene at the house had been released by law enforcement prior to cremation view and autopsy; the funeral wake permitted dozens of relatives to pass through the scene. Suspicion initially focused on a sister who wished to claim a parental set of china, as the decedent had been heard to say that she would take that china over his dead body. Later, law enforcement identified a different assailant, a man who mistook text messages from the liquor store clerk to his wife as evidence of an affair. The messages, sent over a period of months, were actually communicating with her regarding sales of her preferred liquor.

Further investigation identified a hidden entrance to a basement room in the suspect's house containing large sums of cash, roughly 100 guns, and a journal detailing his obsession with the presumed affair. The suspect later wrote out an extensive confession for his wife in his jail cell, prior to a suicide attempt, which did not end in death. About 15 months after the homicide, he pled guilty without a jury trial.

The literature contains few reports of delayed identification of homicides; case reports often involve autopsy after exhumation.[1,2] Gunshot wound homicides are rarely missed; a single large study of 155 cases of post-exhumation forensic autopsy identified seven missed homicides, three by poisoning, three by blunt force trauma, and one "craniocerebral gunshot."[3] A combination of decomposition artifact, unusual gunshot wound locations, decedent obesity, and an initially unsuspected assailant were factors that caused this gunshot wound homicide to be nearly missed. This case report provides a cautionary tale for those tasked with recognizing postmortem injury.

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Murder; Handguns; Cremations

I100 An Update on Forensic and Medicolegal Aircraft Accident Investigation: New Challenges Meet Novel Technologies

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Learning Objectives: The attendees will learn about the individual disciplines involved in aircraft accident investigations, which comprise police, forensic science, genetics, and pathology, together with specialists from aviation itself including pilots, air traffic controllers, and aircraft technicians. The audience will learn about the need for successful collaboration between those disciplines in order to uncover all factors involved in the chain of events. Attendees will gain insight into new methods in pathology and genetics and their expanding possibilities in medicolegal practice in aircraft accident investigation and death victim identification.

Impact Statement: The German Air Force Centre of Aerospace Medicine provides a highly specialized Legal Medicine Task Force particularly trained for the investigation of aviation mishaps. With a case archive going back to the year 1964, recommendations based on the experience of decades of aircraft accident investigation can be provided. The presentation of casework from both military and civilian mishaps will allow forensic scientists to prepare for a broad spectrum of scenarios, which can become a challenging part of their future work.

Abstract Text: Essential requests on forensic scientists and medicolegal death investigators in military as well as in civilian aviation mishaps include the disclosure of the chain of events resulting in the fatal crash together with the unequivocal identification of the victims. Successful investigations depend on data about the aircraft's technical condition, the flight path and the final flight maneuvers, the pilot's aeromedical history, and the availability of reliable postmortem results.

However, technical information on the aircraft can be classified, notably in military aviation, and air traffic control data about the flight period prior to the crash may be unavailable, particularly in military conflicts. Recent aeromedical challenges, for instance, about the physiological resilience to long-term effects of SARS-nCoV-2 infection or modern, mRNA-based vaccines, need to be covered when evaluating military as well as civilian aviation accidents.

Currently, novel technologies in molecular pathology and forensic genetics increasingly eradicate the limitations from standard medicolegal practice in which the investigative procedures are restricted to basic methods like autopsy and histology. Findings in cells and tissues destroyed by blunt force impact, fire, or putrefaction, which were not retrieved from autopsy or histology, now can be recovered using RNA-based studies on, for instance, hypoxic changes, cellular stress, or inflammatory reactions. New procedures improve DNA-associated findings on disease burden, for instance, single-nucleotide polymorphisms associated with ion channel abnormalities leading to myocardial arrhythmia. DNA-based identification methods recover genetic profiles for identification with rising sensitivity, including data for age estimation and phenotyping. Forensic investigative genealogy opens the door for family-tree-based identification of previously unknown victims, such as on evacuation flights from conflict areas.

This presentation will summarize the requirements and the available methods to conduct up-to-date aircraft accident investigations. Casework examples, practical guidelines, and findings from recent studies will be provided. The attendees will be invited to verify and widen their investigative strategies in aviation accident assessment.

Accident; Autopsy; Victim Identification

I101 Familial Dyadic Deaths: A Sample Study From Southern Italy

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Learning Objectives: After attending this presentation, attendees will gain an understanding about the phenomenon of dyadic deaths by analyzing the forensic characteristics of these crimes.

Impact Statement: This presentation is set to make a significant contribution to the forensic community by sharing the data provided by the analysis of five cases involving dyadic death that occurred in Southern Italy in the Campania region.

Abstract Text: Dyadic deaths, also known as Homicide-Suicide (HS) or murder-suicide, refer to an event where a homicide is followed by the perpetrator's suicide. Most dyadic deaths happen in an intrafamilial context, involving a male perpetrator with psychiatric disorders and a female victim who usually is his intimate partner. Intrafamilial HS can also concern filicide or matricide/patricide. The motivational aspects of familial HS can vary from jealousy or possessiveness toward the current or former partner, to familial and financial issues. A different type of familial HS is the so called "mercy killing," usually happening in older couples, both suffering from significant diseases and/or social isolation. There is no current definition about the time interval between the homicide (H) and the suicide (S). According to some authors, the time elapsed between the H and S should be no longer than 24 hours, while others adopt as inclusion criterion a several days timeframe, up to a week, and still others do not use a timeline at all.

Italy has a population of 59 million individuals approximately and, according to the Italian Institute of Statistics (ISTAT), it shows one of the lowest homicide and suicidal rates among the European countries with less than 400 homicides and 4,000 suicides per year, respectively.[1,2] The lack of a national registry of HS makes it difficult to investigate both the epidemiological and motivational aspects of these crimes. A rate of 0.38 per 100,000 with suicide following the 3.8% of homicides has been reported in the United States.[3] Epidemiological data on HS from European countries show an incidence rate ranging from 0.02 to 1.33 per 100,000 inhabitants considering the national variations.[4,5] A recent Italian survey highlighted an increasing trend of HS over the decade 2009-2018 compared to the 1985-2008 period, also predicting a steady increase in the coming years.[4]

The aim of this presentation is to provide an insight into the forensic characteristics of HSs. Five cases of familial HS that occurred in Southern Italy (Campania region) have been revised. In all cases, the perpetrators were males aged between 48 to 72 years and had a familial relationship (husband and father) with the victims. Firearms were the most used weapons in three out of five cases (two shotguns, one handgun). In one case, the victim was stabbed by her husband, who later committed suicide by jumping out of a window. Asphyxia was the cause of death in another case where the victim was strangled by her husband, who then hanged himself. Motivations varied from unaccepted separation to disputes over money and depression. Previous abusive behavior toward the victim was reported in two cases. The prevalence of male gender among the offenders and the use of firearms as the most common weapon are consistent with data reported in literature. Interestingly, in three out of five cases, more victims were involved: in one case, two mass shootings in public areas were committed by the perpetrator before and after killing his wife and trying to kill his daughter. In another case, a woman was killed by her husband along with her sister and parents. In all cases, suicide was committed by the perpetrator right after the killings.

Despite being rare, crimes involving dyadic deaths represent indeed an alarming phenomenon with significant individual and social implications. In contrast with the suicidal and homicidal decreasing incidence rates through the years, an increase of HS has been estimated in the near future. These data show how crucial it is to provide a definition of the etiology and motivations that lead to committing these kinds of crimes.

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Dyadic Deaths; Intrafamilial Crimes; Victim-Offender Relationship

I102 Par for the Course: Exploring Unexpected Golf-Related Fatalities in Northwest Florida

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Learning Objectives: This presentation will delve into the causes and manners of death associated with golf and golf-related activities in the Florida Panhandle (Florida's First Judicial District). Attendees will gain insights into the various fatal outcomes related to this seemingly innocuous and popular sport.

Impact Statement: This presentation will enhance the forensic science community's understanding by providing a comprehensive analysis of golf-related fatalities. It aims to improve competence in identifying and categorizing such deaths, translating this knowledge into better medicolegal death investigative practices, and potentially influencing public health measures.

Abstract Text: Golf, often celebrated for its physical and mental health benefits, can also pose significant risks, leading to fatal health outcomes.[1]The Florida Panhandle, specifically District One encompassing the counties of Escambia, Okaloosa, Santa Rosa, and Walton, and renowned for its beaches and numerous golf courses, presents a unique setting to study these risks. While previous research by the United States Bureau of Labor Statistics focused on fatalities among golf facility workers, our study is pioneering in addressing the causes and manners of death among golf participants in this region.[2]

Our study sourced data from the district database, covering the years 2020 to 2024. During this period, 20 golf-related fatalities were recorded. The findings reveal a predominant occurrence of accidental deaths, accounting for 65 percent (13 cases), with causes including multi drug toxicity, drowning, pulmonary thromboembolism, and multiple blunt traumatic injuries. Natural causes ranked second, comprising 20 percent (4 cases), with incidents like ruptured aortic aneurysm and hypertensive cardiovascular disease. Additionally, there were 2 cases (10 percent) of homicide due to multiple blunt force injuries and mechanical asphyxia, and a single case (5 percent) of asphyxia due to suicide by hanging.

These findings underscore the importance of comprehensive safety measures and awareness among golf participants and organizers. By understanding the diverse fatal risks associated with golf, stakeholders can implement targeted strategies to mitigate these hazards.

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Golf; Florida Panhandle; Mortality

I103 Military Defendants Exonerated by Independent Autopsy

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Learning Objectives: After attending this presentation, attendees will recognize the necessity of always examining the heart muscle in its entirety to avoid a flawed and negligent autopsy and wrongful prosecutions.

Impact Statement: The forensic science community will be impacted through improved standards of autopsy practice and prosecution of military personnel.

Abstract Text: This case concerns a 19-year Caucasian male who was participating in the "Crucible" to complete his Marine Corps basic training. PFC Beals had no known significant past medical history nor ever had a screening **Electrocardiogram** (EKG) (including none performed as part of his military enrollment physical). On the last day, he was 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 local coroner had jurisdiction 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: 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.[1,2] 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. Etiology was undetermined. Myocardial scarring and interstitial fibrosis distinguished this death from the assumed hyperthermia. 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.[3]

The Marine Corp proceeded against Smiley, Ryan, and Judy based upon it's false presumption of hyperthermia.

Drill instructor SSgt. Smiley was prosecuted for negligent homicide.[4] Thereafter, Capt. Sean Ryan and then Capt. Heather Judy were prosecuted for Beals' death.[5,6]

Naval Criminal Investigative Service (NCIS) conducted an intensive investigation to apparently vindicate Beal's death.[7] The Command Investigation concluded Beals' death was from hyperthermia and likely preventable. However, NCIS had notice of the second autopsy and did not investigate it for Judy's hearing, even though it was performed nine days after Beals' death.

Judy was initially charged and culpable for a substandard performance related to Beals' death and alleged failure to supervise SSgt. Smiley and separated from the Marine Corp. Thereafter, Judy requested a second Board of Inquiry for rehearing due to the undisclosed belated existence of the critical second autopsy's results.[8] A rehearing was granted.

During Judy's rehearing, findings from the second autopsy were again presented that Beals' death was due to a serious and previously unknown heart condition, not hyperthermia.[9] The prosecution focused on alleged training violations (recruit surveys) by Smiley that Judy reported to Ryan instead of the trustworthy autopsy. The prosecution indirectly conceded Judy was not responsible for Beals' death.

The Marine's unavoidable death resulted from the severe preexisting cardiac disease. Without the indispensable contradictory second autopsy, military judicial injustice would have occurred. Smiley, Ryan, and Judy were acquitted of the charges.[10-12] Proper autopsy practice must include complete dissection of the heart and expanded histologic analysis to ensure rare conditions are explored.

Military command and law enforcement investigate through their perspective of correctness, without excluding other possible causation theories for a conclusion. Unfortunately, Smiley, Ryan, and Judy had to prove their innocence due to a negligent autopsy, three successive wrongful prosecutions, and retributive substitute justice.

Disclaimer: The views expressed in this abstract are those of the authors and are not an endorsement by the Marine Corps, Department of the Navy, the Department of Defense, or the United States Government.

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5. Inquiry in Board of the case of Capt. Sean Ryan, USMC, Charges filed May 2022 pursuant to SECNAVINST 1920.6D, enclosure 6, paragraphs 1.a.(1) and 1.a.(3), (Failure to demonstrate qualities of officer's leadership and discharge officer's duties.) with a penalty of separation from the Marine Corp. Capt. Ryan was the company commander for Capt. Judy.
6. Inquiry in Board of the case of Capt. Heather R. Judy, USMC, Charges filed July 15, 2024, pursuant to SECNAVINST 1920.6D, enclosure 6, paragraphs 1.a.(1) and 1.a.(3),(Failure to demonstrate qualities of officer's leadership and discharge officer's duties.) with a penalty of separation from the Marine Corp. Judy was the Series Commander for PFC Beals' platoon and officer in charge of SSgt. Smiley. First BOI in this case was appointed on August 8, 2022. BOI was conducted on November 2, 2022. The Second BOI was appointed on 3 July 2024 and conducted on July 15, 2024.
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11. Capt. Ryan: Board of Inquiry determined, based upon a preponderance of the evidence, in May 2022 the charges were "unsubstantiated", therefore a "no basis" finding.
12. Capt. Judy: Upon rehearing the Board of Inquiry determined, based upon a preponderance of the evidence, on July 15 2024 the charges were "unsubstantiated", therefore a "no basis" finding.

Autopsy; Myocardial Interstitial Fibrosis; Hyperthermia

I104 Promising Autophagic Markers in Traumatic Brain Injury: An Immunohistochemical Study

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Learning Objectives: Attendees will understand the potential of autophagy markers as diagnostic tools for Traumatic Brain Injury (TBI) in forensic pathology, addressing the challenges of postmortem TBI diagnosis. In particular, they will gain an understanding of the potential use of autophagy markers, such as LC3-II, beclin 1, p62, and LAMP2A, as diagnostic tools.

Impact Statement: This presentation will equip forensic scientists with the knowledge to potentially utilize autophagy markers as novel diagnostic tools for TBI, enhancing their ability to accurately identify and assess TBI in postmortem examinations. This improved diagnostic capability will help to improve the forensic investigations and potentially contribute to a better understanding of the pathophysiological processes involved in TBI.

Abstract Text: Autophagy is a cellular response to stress that allows controlled degradation of individual cellular organelles or the entire cell.[1,2] TBI consists of brain damage caused by a traumatic event and is one of the leading causes of death and disability worldwide. However, postmortem diagnosis and interpretation of TBI can be particularly challenging. Since symptomatology is largely clinical, there is often little or no macroscopic or histological evidence of damage in the event of the victim's death, especially without a complete description of the circumstances of death or in cases of mild trauma.[3-6] Literature shows that the autophagy process is active in cerebral cells after a TBI as a defense mechanism against neuronal damage. Therefore, proteins involved in the three pathways of autophagy could be used as diagnostic markers of TBI in forensic pathology and in clinical practice for prognostic evaluations.[7-12]

To the best of our knowledge, only 21 studies described markers of autophagy in TBI models, identifying a total of 24 markers.[13] Among these, LC3-II, beclin 1, p62, and LAMP2A were the most promising, showing increased expression in the brain of murine models of TBI. The aim of the authors is to search for qualitative and quantitative differences in the expression of these four markers using immunohistochemistry, with the final goal to find valuable diagnostic markers for TBI in forensic pathology.

Subjects dead after traumatic brain events (cases) were selected from the Institute's database. In parallel, subjects who died suddenly without cranial trauma or other significant pathologies and with a negative toxicology exam were selected as controls. For each case and each control, immunohistochemical staining for LC3-II, beclin 1, p62, and LAMP2A was performed on a frontal lobe sample. Multiple fields were randomly acquired at the level of the white and gray matter of the cerebral cortex and were evaluated to determine the presence of differences in protein expression in neuronal and glial cells in cases and controls.

The presented data suggest a differential expression of autophagy markers in TBI samples compared to controls in both glial and neuronal cells. Moreover, a preliminary qualitative examination of the slides revealed that, in anti-LC3-II antibody-stained slides, while the neurons in the control samples predominantly showed a diffuse localization pattern of the protein in the cytoplasm, the case samples exhibited numerous neurons with a vesicular cytoplasmic pattern and perinuclear reinforcement indicative of active autophagic activity. Subsequent statistical comparison using Welch's t-test demonstrated an increase of positive neurons in the cases compared to controls.

Given the promising results obtained from the present studies, further experiments should be conducted in support of the presented data, for example, by applying semiquantitative methods such as immunofluorescence and western blotting techniques. Particular attention should be paid to the choice of cases, because it cannot be excluded that concomitant pathological processes, such as neurological disorders, the cause of the death, and the influence of extreme age ranges, could impact autophagic processes. Finally, after setting the appropriate working conditions, the clinical application of these markers should be evaluated for their prognostic significance.

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Autophagy; Traumatic Brain Injury; LC3-II

I105 Gross, Microscopic, and Immunohistochemical Features of Antemortem Thrombi, Agonal Thrombi, and Postmortem Clots: A Pilot Study

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Learning Objectives: Attendees of this presentation will gain information on: (1) common macroscopic presentation of antemortem thrombus, postmortem clot, and agonal thrombus; (2) histology and immunohistochemical features of each category; and (3) the way of integrating macroscopic and microscopic data to gain correct interpretation of intravascular formations detected during autopsy.

Impact Statement: This presentation will impact the forensic science community by providing a comprehensive background on the problematic characterization of clots and thrombi during autopsy and presenting a methodological approach for interpreting intravascular formations detected during autopsy.

Abstract Text: Autopsy pathologists are tasked with differentiating Postmortem Clot (PMC) from Antemortem Thrombo-embolus (AMT), as this distinction is crucial. While PMCs do lack clinical significance, thromboembolic phenomena can cause or contribute to death. Unfortunately, textbooks on pathology, autopsy, and forensic pathology often offer limited or no robust guidance on identifying clots and thrombi during autopsies. When addressed, these resources typically force the categorization of clots as either antemortem or postmortem.

PMCs were classically classified into two subtypes: soft, dark red "red cruor" clots and gelatinous, yellow-red "chicken fat" clots. These types are distinctly different from AMTs, characterized by their pale tan to dark red color, firm texture, and granular surface.[1] However, some authors recognize a third category, considering "chicken fat" clots as Agonal Thrombi (AgTs), which form as blood flow slows in a dying patient.[2,3]

The present study aimed to analyze intravascular formations detected at autopsy, correlating the macroscopic data to the circumstance of the death and applying special and immunohistochemical staining to evaluate their diagnostic sensitivity and specificity in distinguishing antemortem thrombi and PMCs.

The Section of Forensic Medicine of the University of Verona database was queried for cases between 2015 and 2019 (pre-COVID-19 pandemic) in which intravascular formations were detected during autopsies. A total of 37 cases with available paraffin-embedded intravascular formations were retrieved. Each case was reviewed for medical records, high-resolution pictures of the autopsy, autopsy findings, and cause of death to categorize each incident: 10 cases died from pulmonary thromboembolism, 10 cases died from agonal death ("slow" death), and 17 cases were rapid and sudden death (traumatic causes).

All paraffin-embedded intravascular formations were processed to prepare glass slides, and each case was stained with standard hematoxylin and eosin, special stain Masson, Alcian-PAS, Grocott, Perls, PTAH, Von Kossa, and immunohistochemical stain CD3, CD15, CD31, CD34, CD61, CD68, Glycophorin C, GLUT-1, and factor VIII.

Macroscopically, AMTs in cases of pulmonary thromboembolism were characterized by granular surface texture, complete filling of the vascular lumen, and scarce adhesion to the vessel wall. In contrast, PMCs appeared red or dark red, amorphous, crumbly, with smooth surfaces. Interestingly, AgTs were found in the heart with heterogeneous traits (yellow to red in color and soft to mild touch) and incomplete filling of the vascular lumen.

Microscopically, Zahn lines were unspecific, probably due to the presence of fibrine structures in PMCs and AgTs cases mimicking AMTs' actual Zahn lines. CD31, CD34, and GLUT-1 showed good sensitivity for thromboembolism (75%, 72%, and 82%, respectively), while Von Kossa and PTAH showed high specificity for thromboembolism (100% and 96%, respectively). CD3, CD15, CD61, and CD68 showed specifically different distribution patterns in AMTs, PMCs, and AgTs.

AMTs, PMCs, and AgTs often display suggestive gross features that can support the pathological diagnosis. Integrating macroscopic characteristics with standard histology and special and immunohistochemical staining methods provides further helpful morphological data. This methodological approach enhances the accuracy of categorizing endovascular formations found during autopsies.

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Autopsy; Thrombosis; Tissue Analysis

I106 How Technology Can Enhance the Reliability of Histopathological Forensic Evidence for Use in Court

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Learning Objectives: After attending this presentation, attendees will understand how technology can help forensic pathologists provide histopathologic and immunohistochemical analyses as valid evidence that can be objectively compared among specialists in a court.

Impact Statement: This presentation will impact the forensic science community by focusing on a new technological way to make an operator-dependent technique, as immunohistochemical or histologic analysis can be, objectively comparable, through the use of specific software.

Abstract Text: Histopathology and other special staining techniques (such as immunohistochemistry) can be crucial in the forensic field, permitting, for instance, the evaluation of the vitality of a lesion, the age of a wound, and even the pathological mechanism that has occurred in a tissue, such as hypoxia.

Immunohistochemistry is a staining method that uses antibodies to check for certain antigens (markers) in a tissue sample. The antibodies are usually linked to an enzyme or a fluorescent dye, so that, after the antibodies bind to the antigen in the tissue sample, the enzyme or dye is activated, and the antigen can then be seen under an optical microscope. A practical example is the use of an antibody anti-glycophorin A in immunohistochemical staining, on samples of decomposed bodies, to establish whether a lesion is vital or not: this can constitute substantial evidence to establish the truth in front of a court.

Another application of immunohistochemistry as forensic evidence can be with HIF-1α (Hypoxia-Inducible Factor 1-alpha), a key marker for diagnosing tissue oxygen deprivation. When detected in lung tissue, it suggests the involvement of hypoxia as a mechanism and can be particularly useful in clarifying ambiguous cases of mechanical asphyxia deaths.

Traditionally, the observer can directly identify and count the number of positive (i.e., colored) cells through a microscope, applying a scoring system from 0 to 3+ depending on the intensity of positivity. This interpretation of the samples could be influenced by the pathologist's expertise, so this technique needs validation to be used as evidence in a trial court.

Here is where technology can help pathologists. ImageJ is an open-source software for processing and analyzing scientific images and can be usefully used for automated digital analysis of immunohistochemical images, providing a reproducible, quantitative assessment of antibody staining intensity in sections of tissues. Standardization of analysis with ImageJ, across different experiments and operators, shows a significant reduction in inter-observer variability, with higher precision of results compared to manual methods.

The use of ImageJ is widely applied in the research field, but this software offers a useful solution for forensic laboratories, allowing for high-quality image analysis and reproducibility. Furthermore, ImageJ provides detailed documentation and traceability of the analytical procedures, which is essential for the chain of custody in forensic investigations. Every step of the analysis can be documented and verified.

By automating the quantification process and reducing the reliance on subjective human interpretation, ImageJ minimizes potential biases and errors, enhancing the credibility of the forensic evidence. ImageJ's open-source nature allows for full transparency in the analysis process, which is crucial for forensic applications. The ability to review and audit the software code ensures that the analysis can withstand scrutiny in a legal context. Indeed, using ImageJ, the pathologist could apply the results of scientific papers about forensic immunohistochemical markers in real court cases, with a validated and reproducible method.

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Histological; Forensic Analysis; Software

I107 Unraveling Sudden Unexpected Death: Metabolomic Insights From the Friuli-Venezia Giulia Sudden Cardiac Death Register in the Young

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Learning Objectives: Attendees should be able to explain how metabolomics can be used to identify specific biomarkers associated with different causes of Sudden Cardiac Death (SCD). Attendees should be able to describe the distinct metabolic profiles observed in cardiac tissue from individuals who died of ischemic heart disease versus toxic causes and discuss the potential pathophysiological mechanisms underlying these differences.

Impact Statement: The scientific community will recognize the value of metabolomics as a powerful tool for enhancing forensic investigations. Integrating metabolomic approaches can aid in the identification of specific biomarkers linked to various pathological processes, ultimately leading to a more precise determination of the cause of death.

Abstract Text: **Introduction:** SCD poses a diagnostic challenge, particularly when autopsies reveal no obvious cause of death or when findings are influenced by the pathologist's subjective assessment rather than objective, scientific measurements. One such challenge occurs when significant coronary artery blockage is observed without corresponding signs of heart muscle damage. This discrepancy raises questions about the true cause of death and the relationship between the observed blockage and the absence of myocardial injury. The standard threshold for significant coronary stenosis (exceeding 75% narrowing) may not always be reliable in these cases. Metabolomics, a field that studies small molecules involved in biological processes, offers a novel approach to identify biomarkers and shed light on the mechanisms of SCD.

Materials and Methods: Fresh frozen samples from the left ventricle of the heart were collected from six patients: three who died from coronary artery disease and three from toxic causes. Samples were acquired using a dermopunch from the anterior, lateral, and posterior walls of the mid-ventricular left ventricle prior to fixing the heart in formalin. Metabolomic analysis was performed using liquid chromatography coupled with mass spectrometry. The resulting data was processed using the online tool MetaboAnalyst. Various statistical analyses, including t-tests, Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLSDA), heatmap analysis, and correlation analysis, were conducted to evaluate the data.

Results: The PCA revealed that the ischemic and toxic groups show partial separation along PC1. Some overlap between confidence ellipses indicates variability within each group, as certain samples exhibit similar lipid profiles regardless of classification. PC1 accounts for 36.5% of total variance, while PC2 explains 19.4%, together capturing approximately 55.9% of total variance. The PCA loading analysis identified key metabolites contributing to group separation. Notably, PC 18:1_22:5, PE 18:1_22:6, and PC O-36:1_A were more abundant in ischemic samples, while PC O-28:0_A, PI 18:0_20:4, DG 16:1_16:1, and DG 16:0_18:0 were more prevalent in "Toxic" samples. The volcano plot, based on t-test results, confirmed significant differences in several metabolites between the groups. Heatmap analysis further supported these findings by showing distinct expression patterns for these metabolites and identifying additional potential biomarkers for the ischemic condition, including PC O-40:5_C, PC 16:0_16:2, and PC O-44:6. Lastly, the VIP scores from the PLS-DA model confirmed the importance of metabolites such as PC 16:0_16:2, SM 34:1;3O, PC O-40:5_C, SM 40:2;2O_A, and PC 16:0_18:1_B in differentiating between the groups.

Discussion: The results suggest that ischemia and toxic causes induce distinct metabolic alterations in cardiac tissue, supporting the hypothesis of different pathophysiological mechanisms. Ischemia appears to activate sphingomyelinases, leading to the accumulation of sphingomyelins and ceramides, which are mediators of apoptosis and cardiac dysfunction. The increase in triglycerides in the "toxic" group indicates an alteration in lipid metabolism induced by substance abuse.

Conclusions: This study demonstrates the potential of metabolomics in identifying specific biomarkers for postmortem diagnosis of SCD and in understanding the molecular mechanisms underlying different causes of sudden cardiac death. Further studies with larger sample sizes and diverse causes of death are necessary to validate these findings.

Metabolomics; Sudden Death; Biomarkers

I108 Procalcitonin as a Marker of Sepsis at Autopsy: A Reflective, Novel, Evidence-Based, Effective Point-of-Care Tool

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Learning Objectives: Attendees will learn about the importance of Procalcitonin (PCT) as a point-Of-Care Testing (POCT) tool in autopsies, serving as a biomarker for sepsis. PCT has the potential to be used as a rapid, low-cost screening test for sepsis in autopsy cases. The collaboration of autopsy findings, histopathology, and microbiological results can provide valuable insights into its utility, further corroborating the incidence of sepsis in deaths.

Impact Statement: This presentation will enhance the forensic community's ability to quickly identify sepsis during autopsies, as PCT through POCT requires minimal skill and uses a portable machine that provides information on infection or sepsis within 20 minutes. PCT can easily be incorporated as a routine investigation in autopsies, improving the quality of the investigation. This will help in identifying sepsis with evidence, ultimately contributing to more precise cause-of-death determinations and benefiting stakeholders in both forensic and clinical settings

Abstract Text: Sepsis, a systemic inflammatory response to infection, leads to unacceptably high mortality, and autopsy-based postmortem investigations are essential for improving understanding and better managements of diseases.[1,2] Detecting biomarkers linked to sepsis can aid early diagnosis, predict disease progression, and assess treatment effectiveness.[3]Microbiological cultures are the gold standard for diagnosing infections, but their low sensitivity and delays necessitate rapid biomarkers. PCT, released by thyroid C cells, shows promise for early detection of systemic bacterial infections.[4] Under normal conditions, PCT is low (≤ 0.1ng/mL) and stable with a half-life of 25–30 hours.[5] PCT was first described in 1984 by Le Moullec and its diagnostic value for bacterial infections and sepsis was established in 1993 by Assicot et al.[6,7] PCT more accurately distinguishes bacterial infections from non-bacterial ones compared to other inflammatory biomarkers like C-reactive protein.[8] Systemic inflammation and immune responses are involved in deaths due to trauma and disease, and postmortem diagnosis of sepsis in such death is difficult.[9] Studies show that PCT can distinguish between septic and non-septic fatalities postmortem. Stable PCT levels can persist up to 140 hours after death.[10,11] Levels above 10ng/mL suggest sepsis, though PCT can rise after surgery, transplantation, trauma, and burns.[12] So, in the current state of scientific research, PCT makes it possible to quantify septic inflammatory activity in a manner relevant to differential diagnostics.

A pilot study was conducted to assess whether the PCT (Standard F PCT FIA) test using Point of Care Testing (SD Biosensor F2400) indicate a rapid "tableside" diagnosis of sepsis. Postmortem PCT levels were examined for 21 medicolegal autopsy cases at the Department of Forensic Medicine and Toxicology, AIIMS Bhopal, using the SD Biosensor F2400 POCT. Blood samples were collected from the subclavian vein or heart, transferred into EDTA vials, and processed with PCT kits in a POCT analyzer. The analyzer automatically displays the test results within 15 minutes. The results were correlated with bacterial and fungal culture reports in each case using blood and spleen tissue. In these 21 cases, using POCT, 6 cases had PCT levels below 0.05ng/ml. Among these, the cause of death was hanging (n=3), head injury (n=2), and multiple injuries (n=1), and all had a short survival period and died before reaching the hospital. In 7 cases, PCT levels ranged between 0.05ng/ml - 2ng/ml in which 5 cases died before reaching the hospital, while 2 cases were hospitalized for 3 and 12 days, respectively, and showed the presence of bacteria. In 2 cases, PCT levels ranged between 2ng/ml-10ng/ml, both hospitalized for 9 and 12 days and died from multiple injuries and abdominal injury, respectively, both showing the presence of bacteria. In the remaining 6 cases, PCT levels were above 10ng/ml, all cases were hospitalized and cause of death was head injuries(n=4), multiple injuries (n=1), and poisoning (n=1), all showing the presence of bacteria. On correlating PCT levels with microbiology reports, 19 cases (90.47%) had at least one pathogenic organism either from blood or spleen, and in these, 9 (47.36%) cases showed PCT above 0.5ng/ml. Raised PCT levels correlate with positive bacterial culture reports. The PCT test as POCT may be used as screening to indicate sepsis. PCT as a POCT in medicolegal cases could be a less time consuming, effective, and reliable screening test for sepsis in the cases. Correlating PCT levels with culture reports and histopathological reports can further provide more corroborative evidence for the diagnosis of sepsis postmortem.

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Procalcitonin; Sepsis; Postmortem Microbiology

I109 Trying to Establish a New Paradigm With the Help of Genetic Analysis for Investigating Sudden Cardiac Death in India: A Preliminary Study

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WITHDRAWN

I110 Standards Development Activities in Medicolegal Death Investigation and Forensic Pathology

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Learning Objectives: 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.

Abstract Text: 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 has many discipline-specific subcommittees that conceive of and draft standards that would be useful to the forensic science community. OSAC, however, is not a Standards Development Organization (SDO) and thus cannot publish documentary standards on its own. Thus, draft standards are then submitted to appropriate SDOs that put them through defined, consensus-based processes to vet and publish them. One such SDO is the Academy Standards Board, which is working closely with OSAC on standards development in the fields of medicolegal death investigation and forensic pathology. 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; Medicolegal Death Investigation; Medical Examiner

I111 A Comprehensive Framework for Undergraduate Forensic Medicine Training in Turkish Medical Faculties

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Learning Objectives: Attendees will learn the essential components of forensic medicine education within Turkish medical schools, including theoretical knowledge, practical applications, and interdisciplinary integration. By the end of the presentation, attendees will have an academic understanding about how to implement and enhance forensic medicine training to meet global standards and effectively prepare graduates for handling forensic cases.

Impact Statement: This presentation will positively impact the Turkish academic community by offering a detailed framework for enhancing forensic medicine education in medical schools and will foster improved academic standards and practical training, ensuring that Turkish medical graduates are better prepared to handle forensic cases and contribute effectively to the legal system. Additionally, the international community will gain insights into the Turkish system, allowing for cross-cultural exchange and potential collaborations to further advance forensic medicine education globally.

Abstract Text: The Framework Program for Medical Education in Turkey determines the standards, scope, and requirements of education implemented in medical schools. This standard organizes educational activities that medical students must receive based on the global standards of The World Federation for Medical Education (WFME).[1] The program encompasses various areas, including Basic Medical Sciences, Clinical Sciences, Internships and Rotations, Professional Skills Training, Community Health and Family Medicine, Professional Development and Ethics, and Research and Scientific Studies. The program is established and supervised by institutions such as the Council of Higher Education (YÖK) and the Council of Deans of Medical Schools (TFDK) in Turkey, and it is regularly updated to align with international medical education standards. It typically lasts six years and the curriculum is divided into three phases: Basic Medical Sciences (first three years), Clinical Sciences (years four and five), and Internships (final year), which is comparable to European systems rather than the United States one. Students enter medical school directly after high school.

Forensic medicine education is an integral part of this program and is provided as a standard in medical schools to ensure that graduates can correctly and effectively intervene in forensic cases. Typically included as an internship in the fourth or fifth year of medical school, this education covers basic information about forensic science, forensic medicine practices, and the application of medical knowledge in legal processes. Key topics include the basic concepts of forensic medicine, death and postmortem processes, trauma and injuries, poisoning and toxicology, forensic psychiatry, sexual crimes and child abuse, forensic genetics and DNA analyses, medicolegal reporting, and legal processes.[2]

Learning methods include theoretical courses, practical applications, case studies, and rotations. The objectives of the forensic medicine internship are to provide training on the application of theoretical knowledge, forensic case studies, autopsy experience, reporting, and forensic laboratory studies. While the core elements of forensic medicine education have remained stable, there have been significant advances and improvements in the approach, integration, and technology used in teaching forensic medicine over the past decade. Interdisciplinary learning and updated knowledge and skills about current forensic medicine tools and techniques as well as topics are now emphasized, along with a greater focus on human rights and ethical education.[3,4]

In this study, we have examined the curricula and the specific approaches of all the medical faculties in delivering Forensic Sciences undergraduate courses. The information was gathered by questioners and personal communications as well. Our results indicate that 143 medical faculties, 97 state, and 46 foundation universities are actively providing education. While state universities generally have the necessary academic staff for Forensic Medicine studies, only 65% of the foundation universities have permanent faculty members, making it difficult to carry out the programs effectively. Additionally, we have found out that despite the ideal standards of the program on paper, the education must be completed within a very limited time, usually between 1 to 2 weeks, depending on the curricula and practices of the universities, which is almost impossible. It is challenging to convey the necessary knowledge and skills within this period. We suggest including theoretical courses that last approximately one semester (half a year) in the curriculum of the faculties of medicine for forensic medicine education before the internship period. This should be followed by two weeks of practical applications and internships. With such comprehensive training, graduating physicians will be better equipped to handle forensic medical problems they may encounter in their professional practice.

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Forensic Medicine Education; Medical Faculty Curriculum Türkiye; Interdisciplinary Learning

I112 Sudden Cardiac Death in the Balkan Area: A Multidisciplinary Approach and Multinational Cooperation

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Learning Objectives: Attendees will gain insights into the epidemiological patterns, diagnostic advancements, and collaborative strategies for addressing Sudden Cardiac Death (SCD) in the Balkan region. The session will emphasize multidisciplinary approaches and the importance of international cooperation in enhancing diagnostic accuracy and improving patient outcomes.

Impact Statement: This presentation aims to enhance the forensic and medical communities' understanding of SCD by emphasizing modern diagnostic tools and the benefits of multinational collaboration. By sharing knowledge and practices, the presentation will improve competence in identifying risk factors, ultimately leading to better preventive measures and management strategies.

Abstract Text: SCD remains a significant public health concern in the Balkans, requiring comprehensive strategies to reduce its incidence and improve patient outcomes. This session will present an extensive review of epidemiological data on SCD, examining rates and underlying causes across several Balkan countries, including Serbia, Greece, North Macedonia, Slovenia, Turkey, and Italy. The focus will be on recent advancements in Postmortem Cardiac-Magnetic Resonance (PMCMR) imaging, genetic testing, and forensic pathology, all of which are crucial in identifying underlying cardiac abnormalities that contribute to SCD.

Our study highlights the utility of PMCMR in detecting myocardial ischemic lesions, with findings showing ischemic changes in 53% of cases (32 out of 63). These findings were corroborated through autopsy and histological examination, confirming the effectiveness of PMCMR in identifying both acute (19 cases) and chronic (13 cases) myocardial ischemia. Such advancements underscore the growing role of imaging techniques in postmortem analysis.

The session will also explore key topics such as precision phenotyping in cardiomyopathies, cardiac genetic testing, and the challenges posed by Variants of Uncertain Significance (VUS). Genetic predispositions, particularly within specific ethnic groups, play a pivotal role in SCD, making it essential to understand how genetic factors differ across populations. Emerging technologies, such as artificial intelligence, hold promise in revolutionizing genetic testing and integrating complex data, which could significantly enhance the diagnosis and prevention of SCD.

Additionally, the session will summarize conclusions and future research directions from the 1st International Meeting "Sudden Cardiac Death in the Balkan Area." A major emphasis will be placed on the importance of multinational collaboration in advancing the understanding and prevention of SCD. By pooling resources and expertise across the region, Balkan countries can enhance both clinical and forensic practices, ultimately improving the accuracy of SCD detection and facilitating more effective prevention strategies.

In conclusion, the integration of new technologies and cross-border cooperation is vital for addressing the SCD challenge. Continued research and collaborative efforts in the Balkans and beyond will be crucial in reducing the burden of SCD on public health.

Sudden Death; Multidisciplinary; International

I113 Forensic Medical Evidence and the Assessment of Violence at the International Borders in the Balkan Area

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Learning Objectives: Attendees will learn about the methodologies and findings related to forensic medical evidence in assessing cases of violence at international borders in the Balkan region. The session will highlight the importance of cross-border monitoring and the integration of forensic evidence in documenting human rights violations, with a focus on the work of the European Committee for the Prevention of Torture and Inhuman or Degrading Treatment or Punishment (CPT).

Impact Statement: This presentation will enhance the forensic community's competence in handling cross-border forensic cases by providing a framework for systematic documentation and analysis. It will improve the performance of forensic experts in gathering and using medical evidence to support legal and humanitarian responses, ultimately influencing patient and stakeholder outcomes by ensuring accountability and justice.

Abstract Text: In the complex geopolitical landscape of the Balkan region, assessing violence at international borders presents significant challenges for forensic experts. This session explores the role of forensic medical evidence in documenting incidents of violence, focusing on cross-border monitoring activities led by the CPT. The presentation will draw on case studies from various Balkan countries, illustrating the procedures and challenges faced by forensic teams in gathering and utilizing evidence. Serious concerns about migrants being subjected to violence and other inhuman and degrading practices by police units and being pushed back across the borders without being given an opportunity to apply for asylum.[1]

Key topics will include methodologies employed in forensic investigations, types of injuries documented, and the legal and ethical considerations in reporting findings. Specialist forensic medicine teams, experienced in working with victims of mistreatment and applying the principles of the Istanbul Protocol, conducted physical examinations and compiled reports on the injuries.[2] These included conclusions about the consistency of the allegations of mistreatment with the observed medical findings.[3] The session will also discuss coordination between different countries in the region, highlighting the role of multinational cooperation in ensuring the integrity and reliability of forensic evidence. The CPT has identified and established clear patterns of physical ill treatment deployed against foreign nationals in the context of pushback operations across Council of Europe member states' borders.[4] The CPT has directly documented recognizable medical evidence, such as the classic "tram-line" hematomas on various parts of foreign nationals' bodies (which are consistent with truncheon blows) and typical dog-bite wounds on their limbs.[5]

The presentation will emphasize the need for standardization in forensic procedures and the use of advanced technologies in injury documentation. It will also address the impact of forensic findings on legal proceedings and the protection of human rights. A special team composed of lawyers, psychologists/psychiatrists, and forensic specialists was formed to document cases of mistreatment and collective expulsion of refugees and migrants from neighboring countries in accordance with the Istanbul Protocol.[4] The team's coordinator was responsible for connecting with Non-Governmental Organizations (NGOs) that come into contact with refugees and migrants who are victims of mistreatment, identifying suitable cases for documentation, preparing questionnaires, and ensuring the presence of translators and transportation to locations where affected individuals were located.[5] The methodology applied by the CPT in the scrutiny of violent pushback operations consists of identifying the alleged victim, collecting a detailed account of the allegations, documenting any medical evidence and psychological impact on the victim and assessing their compatibility with the allegation.[5] Further, to corroborate the findings, a triangulation of the information is pursued through separate and individual interviews, with the help of interpreters as required, with members of the same migrant group and the examination of available medical documentation and injury reports.[6] By examining specific cases, the session aims to provide practical insights into improving forensic practices in the context of border-related violence, contributing to the broader goal of safeguarding human rights in the region.

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Evidence; Migrants; Human Rights

I114 The 48-Hour Protocol on Deaths Following Traumatic Events at the Hospital of Novara in Eastern Piedmont, Italy: A Review of the Effectiveness of Reporting to the Judicial Authority

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Learning Objectives: After attending this presentation, attendees will understand the pivotal role of judicial autopsies in detecting potential hidden crimes. There is currently a significant worldwide decrease in judicial autopsies: a valuable tool for accurately determining the cause and manner of death, especially in cases of violent death.

Impact Statement: This presentation will impact the forensic science community by analyzing all the traumatic deaths occurred in three years (2017, 2020, 2023) at the hospital "Maggiore della Carità" of Novara in Eastern Piedmont, Italy, and evaluating how these deaths are taken into consideration by the competent territorial prosecutor's office. The autopsy rate has been declining worldwide for decades, including in Italy.

Abstract Text: In the past decades, the autopsy rate has been declining worldwide, including in Italy, especially of judicial autopsies, partially losing sight of the pivotal importance of autopsy in detecting the cause and the manner of death.[1-4] Indeed, discrepancies between clinical diagnosis and hospital autopsy are quite high.[5,6]

This study aims to review the effectiveness of the "48-hours protocol" in operation at the hospital "Maggiore della Carità" of Novara. This protocol states that after the death of a patient with a traumatic event in their past medical history, a notification of death must be sent to the Judicial Authority, which must be made aware that, after a time of 48-hours, a non-judicial autopsy is planned, giving the time to decide if the autopsy should be changed into a judicial one or not.

The review was made on all cases in which the "48h protocol" came into effect, in the years 2017, 2020, and 2023. Out of the 127 medical death records, 116 were correctly reported as 48-hour protocols. A total of 107 autopsies were carried out, among which 103 were hospital ones, and only 4 were judicial ones. The most documented traumatic events were falls at home, followed by road traffic accidents. Over 46% of the autopsy results totally or partially differed from clinical diagnosis made in the hospital.

The interpretation of the results of this study suggests three conclusions: first, the autopsy is pivotal to correctly detect the cause of death in traumatic deaths; second, the Judicial Authority does not make enough and proper use of the "48-hours protocol," underestimating the role of judicial autopsies; and third, that the protocol might be too superficially used.

Therefore, some changes to the protocol should be made in order to have a better communication to the Public Prosecutor's Office, maybe adding more specific information to the reports sent to hopefully increase the authorities' interest.

Once these details have been refined, and the usefulness of this protocol validated, it would also be desirable to disseminate it to other prosecutors' offices to raise awareness of the issue throughout Italy.

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Judicial Autopsy; Traumatic Death; Hidden Crimes

I115 Cognitive Bias in Forensic Pathology: Types, Error, and Mitigation

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Learning Objectives: The learning objectives of this presentation include increased recognition and awareness of an individual's cognitive biases related to decision making and information processing; recognition of systemic biasing factors in the forensic community; and identifying successful ways to mitigate cognitive biases within a forensic practice. Portions of the presentation will encompass real-time audience polling to provide immediate feedback regarding situations and questions related to cognitive biases as applied to forensic pathology.

Impact Statement: This presentation will impact the forensic science community by continuing the controversial discussion of cognitive bias in the forensic disciplines, particularly as applied to forensic pathology. The many misconceptions and conflations regarding cognitive bias in forensic pathology will be explained, including the assumption that error in forensic pathology is caused by cognitive bias. The forensic pathology community has a difficult time defining "error," partially due to the variations in practices between medical examiner offices. There is also no individual or institutional arbiter that holds the absolute truth, particularly in criminal investigations. The presentation will explain why withholding biasing information from the forensic pathologist, in a technique such as linear sequential unmasking, is largely untenable. The causes of erroneous outcomes related to forensic pathology will be explained as multifactorial and consist of multiple levels of error, often related to the biases inherent in our combative court system. The presentation will present authentic and independent peer-review as a best practice for mitigating cognitive bias.

Abstract Text: Cognitive bias continues to be a controversial topic in the scientific, medical, and forensic communities. The use of the proper definition of cognitive bias is essential as the general term "cognitive bias" is often used to refer to the systemic, repetitive negative effects of bias. It must be clarified that the concept of cognitive bias consists of the heuristic structure in which humans process and interpret all information. It is critical to understand what cognitive bias is and what it is not, especially in regard to error.

Twelve types of cognitive bias will be discussed in this presentation with examples applicable to forensic pathologists. The types of cognitive bias discussed in this presentation include: Contextual bias, Confirmation bias, Ascertainment bias, Overconfidence (Dunning-Kreuger effect), Affective bias, Anchoring bias, Availability bias, Attribution error, Groupthink, Premature closure, Unpacking failure, and Framing.

Forensic pathology "error" is a poorly defined concept in which much care must be taken in recognizing what "errors" are potentially caused by cognitive bias versus and what "errors" are related to other factors. The ability to quantify which errors are due to cognitive bias is very limited, as any error in any part of the decision-making process may be attributable to any one of the hundreds of types of cognitive biases. The altruistic notion that errors in forensics can be educational and establishing a positive error culture seems incompatible with the grave consequences of forensic error that include the wrongful conviction of an innocent person or the wrongful release of a guilty individual.[1] There are numerous systemic issues, some of which may or may not involve cognitive bias, that also lead to these erroneous outcomes, including the perpetuation of inaccurate, unscientific, and unjustified forensic pathology opinions.

The workflow of a forensic pathologist contains multiple decision points, any or all of which are influenced by experience, training, context, and inseparable cognitive bias. Recognizing cognitive bias in practice is essential to understanding and taking steps to mitigate it. Medical examiners must strive for objectivity in autopsy practice, autopsy reports, and expert witness testimony. They must seek out and implement best practices, such as generous peer review and case discussion conferences. They must also continue to fight for independence to be free of outside pressures and political influence. The role of higher level "independent review" panels at state or federal levels are controversial and possibly subject to political/personal influence. The technique of linear sequential unmasking, or removal of biasing context from forensic medicine, results in "black box" autopsies, which are dangerous and ill-advised.[2] Further limiting the already limited context and information we receive on cases does not apply well to the practice of medicine.

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Forensic Science; Error Rate; Medicolegal Death Investigation

I116 "To Err Is Human": AIn't? Medical and Forensic Perspectives on Artificial Intelligence Use in the Italian and European Context

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Learning Objectives: After attending this presentation, attendees will be aware of the main developments that are characterizing medical practice during the "Artificial Evolution Era" (such as the use of Artificial Intelligence (AI) and Generative-Artificial Intelligence (GenAI)). During this presentation, some of the main limitations related to the adoption of these innovative technologies will also be highlighted and analyzed.

Impact Statement: The impact brought to the forensic science community is the stimulation of a proactive dialog, to implement GenAI in medical practice, in a critical manner, without taking the risk of relegating doctor's skills as secondary to AI's conclusions. Furthermore, we want to underline the importance of knowing how to identify the righteous profiles of responsibility, in case of medical malpractice attributable to the use of GenAI.

Abstract Text: The historical period we live in, identifiable as the "Era of Artificial Evolution," is facing the introduction of numerous new technologies in the medical field.[1] Among others, it is worth mentioning AI in its narrow and GenAI forms, Machine Learning (ML), and Deep Learning (DL).

In the medical field employing AI, GenAI, powerful ML, and DL for their data analysis capabilities would enable rapid and efficient processing of huge volumes of medical information in order to: make early diagnoses; carry out targeted therapies; carry out scientific research, after identifying any causal relationship between those data and pathologies patients suffer from.

Nevertheless, we must underline the limitations that characterize these means, and the errors related to their use, as those sufficiently shown by numerous worthy-of-mention cases, in which unsupervised trained GenAI or GenAI trained on corrupted data, results in dangerous conclusions such as gender inequality and racial biases.[2]

Therefore, the possibility that AI may make mistakes when working alongside doctors makes this topic extremely relevant also in forensic medicine.

Twenty-five years after the report "To Err Is Human" was released by the United States Institute of Medicine, the ability of AI to act independently from human control, learn automatically through ML and DL, and autonomously produce results, raises questions about whether modern Forensic Medicine is still adequate to respond to new emerging medical-malpractice-related issues.[7,8]

In conclusion, in order not to slow down progress, this presentation aims to:

- Raise awareness over this crucial theme
- Advance a few questions (Who will train GenAI for health use? Which personal data are safe to use? Is it safe to sell private data belonging to patients to private companies to train AI algorithms? Would it be ethical? Who would be responsible for ensuring that GenAI does not fall into biases?)
- Offer proactive suggestions about one of forensic medicine's controversial subjects, particularly for the Italian and European Forensic context (Who should be responsible for a therapeutic or diagnostic mistake that occurs when a physician uses AI? Who would guarantee AI's trustworthiness when used for its manifold forensic applications?).

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Generative Artificial Intelligence; Artificial Intelligence Bias; Medical Malpractice

I117 Jay Dix Memorial Lecture Series

James R. Gill, MD, Connecticut Office of Chief Medical Examiner, Farmington, CT; Katherine Maloney, MD, Erie County Medical Examiner, Buffalo, NY; Andrew Baker, MD, Hennepin County Medical Examiner's Office, Minnetonka, MN; Susan F. Ely, MD, Office of Chief Medical Examiner of the City of New York, Pelham Manor, NY; Nicole R. Jackson, MD, MPH, University of Washington, Seattle, WA; Kanayo Tatsumi, MD, St Louis City Medical Examiner's Office, St. Louis, MO

Learning Objectives: A proper medicolegal death investigation is a multi-disciplinary 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 deaths related to thermal injury, intoxications, firearms, natural disease, pediatric death investigation, and death certification.

The attendees can expect to learn how and why deaths related to the previously specified topics occur. Attendees will learn a systematic approach to the evaluation of such deaths that can easily be implemented in their daily practices.

Impact Statement: The attendees will receive a comprehensive review of what causes and contributes to deaths related to the previously specified topics. They 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.

Program Description: We provide a comprehensive review of the following topics:

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. (Andrew Baker, MD)

Fires result in 3,800 civilian deaths a year. Smoke inhalation and thermal injuries are common causes of deaths in fires. Determining if the person was alive at the time of the fire is an important diagnosis. (Kanayo Tatsumi, MD)

In the past decade, we have faced the opioid crisis, which now involves fentanyl. Methamphetamine and cocaine deaths also have increased with regional variations. Novel psychoactive substances challenge toxicology labs and death investigators. (Nicole Jackson, MD)

Children are not just small adults. The accurate recognition of the cause and manner of death has important implications for investigative and health agencies. Recognition of factors involved in pediatric deaths can serve as a basis for formulating death prevention strategies.(Katherine Maloney, MD)

Natural diseases that result in the sudden unexpected deaths are often evaluated by the medicolegal death investigator. They may involve previously undiagnosed common conditions or conditions lacking anatomic manifestations. (Susan Ely, MD)

The cause and manner of death impact criminal investigation and public health policy. The determination of the cause of death is the practice of medicine and is based on integrating and interpreting information about the circumstances, physical examination, and ancillary studies. (James Gill, MD)

Medicolegal Death Investigation; Autopsy; Medical Examiner

J1 A Forensic Investigation of a Psychiatric Patient: Cause of Death Associated With Risperidone Usage

Vjola Fera, MD, Institute of Forensic Medicine of Albania, Tirana, Albania*

Learning Objectives: This presentation will inform attendees of the interests of government to take care of the health of prisoners.

Impact Statement: The judgement of the people in jail is also necessary for taking care of their health as free people of their state.

Abstract Text: This case report describes the autopsy findings in a psychiatric patient who was serving a sentence in a prison. The patient lost consciousness while eating his lunch and was sent to the emergency department of the hospital where he was pronounced dead. Afterward, the body was subjected to an autopsy where a foreign body was found in the nasopharynx, the remains of a sausage. Dysphagia in psychiatric patients requires comprehensive evaluation and management. Dysphagia in such a patient population should be evaluated for any potential medication side effects, especially from neuroleptics, which could be life-threatening due to the risk of aspiration. Dysphagia due to antipsychotic use is usually reversible and, in most cases, improves by lowering the dose, discontinuing, and/or changing the antipsychotic medication. Multidisciplinary collaboration, including speech and swallowing evaluation, medication adjustments, dietary modifications, and careful monitoring, is essential to optimizing the swallowing function and reducing the risk of aspiration. The cause of death was determined to be mechanical asphyxia from a foreign object. From the study of the hospital's medical record, it was noted that this patient was being treated with the diagnosis of paranoid schizophrenia, for which he received medication. The patient was on risperidone 4mg, initiated a month prior to treating acute psychosis.

The mechanism could have extrapyramidal side effects causing acute dystonia involving muscles of deglutition. Additionally, antipsychotics may cause sedation and impair the muscles' coordination in swallowing. The anticholinergic effects of antipsychotics can also impair the smooth coordination of the muscles of the pharynx. Another possible mechanism involves the blockade of dopamine receptors, which are present in the basal ganglia and have a role in regulating swallowing movements.

The autopsy finding was also pneumonia. Changes in medication from risperidone to aripiprazole, along with a short course of benztropine and dietary modifications, were implemented, gradually, in jail.[1-4]

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Asphyxiation; Autopsy; Cardiac Death

J2 Woman-in-Danger Thermometer: An AI-Driven Predictive Tool Designed to Assess the Risk of Femicide

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Learning Objectives: Attendees will learn about the development and application of the Woman-in-Danger Thermometer, an AI-driven predictive tool designed to assess the risk of femicide among women who have experienced violence. The presentation will cover the construction of the model, the variables used, data handling techniques, and evaluation metrics, providing a clear understanding of its potential implementation in forensic practice.

Impact Statement: This presentation will enhance the competence and ability of forensic professionals by introducing an innovative tool for assessing femicide risk. Implementing this tool will improve performance in identifying high-risk individuals, leading to better intervention strategies and potentially saving lives

Abstract Text: Despite being one of the safest states in Brazil, Santa Catarina has a relatively high femicide rate of 1.48 victims per 100,000 inhabitants, surpassing the national average of 1.38.[1] This highlights the need for effective predictive tools to prevent such incidents. The Woman-in-Danger Thermometer is an AI-based classification model designed to predict the risk of femicide among women who have experienced violence. This tool distinguishes between women who have survived violence and those who have been killed, based on various factors.

The model incorporates variables such as age, geographic location (latitude and longitude), previous records of bodily injury or sexual assault, and city-level environmental factors including population density, schooling index, the Municipal Human Development Index, child mortality rates, and gross domestic product.[2,3] Given the unbalanced nature of the dataset—only 0.24% of women attended by the Scientific Police were femicide victims—oversampling techniques, commonly used in bank fraud detection, were employed to enhance the model's classification performance within a random forest algorithm.[4]

The primary evaluation metric chosen for the model is Recall, emphasizing the importance of identifying true positive cases of women at high risk of femicide. The model achieved a recall score of 0.70 and an Area Under the Curve- Receiver Operator Curve (AUC-ROC) of 0.81, indicating a high ability to distinguish between high-risk and low-risk cases. However, the precision score was low at approximately 0.06, suggesting that while the model effectively identifies many true positives, it also generates a substantial number of false positives. This outcome underscores the need for additional variables to improve precision further. Potential enhancements could include the victim's occupation, number of children, and marital status.

The presentation will detail the methodology behind the Woman-in-Danger Thermometer, including data collection, preprocessing, the application of the random forest algorithm, and the use of oversampling techniques. Attendees will gain insights into the challenges of working with unbalanced data and the importance of selecting appropriate evaluation metrics. Real-world case studies will demonstrate the practical application of the model, showing how it can identify women at high risk and enable timely interventions such as social assistance and increased police surveillance.

In conclusion, the Woman-in-Danger Thermometer represents a significant advancement in forensic science, offering a data-driven approach to predicting and preventing femicide. While the current model shows promise, further research and refinement are needed to improve its precision. Expanding the dataset and incorporating additional variables will enhance the model's predictive capabilities, ultimately contributing to better protection and outcomes for women at risk of femicide.

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Femicide; Violence Against Women; Artificial Intelligence

J3 Medicolegal Approaches to the Analysis of Femicide Victims: Diagnosis, Autopsy, and Forensic Implications

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Learning Objectives: The objective is to analyze the characteristics of complex phenomenon of femicide, also through the comparison of Sicilian cases with international ones. By examining various parameters, it aims to identify differences between types of crimes and develop a shared medicolegal definition of femicide to inform preventive measures and awareness policies.

Impact Statement: These findings underscore the critical need for global research, standardized investigation protocols, and comprehensive preventive measures to address and ultimately eradicate femicide. The study highlights the importance of understanding risk factors and patterns "to counter this phenomenon."

Abstract Text: The concept of femicide was first coined in the 20th century by Diana Russell, who defined it as "the killing of a woman by a man simply because she is a woman." This definition was later adopted by the World Health Organization, allowing for a formal distinction between femicide and non-femicide cases.

Within the sphere of femicide, several universally recognized types exist:

- "Sexual murders," such as those resulting from rapes or sexually related actions.
- "Femicide of elderly women," where the victims are over 65 years of age.
- "Intimate partner femicide," where the perpetrators have an intimate relationship with the victim.

This study is a retrospective epidemiological analysis of female homicides extrapolated from all autopsies performed between 2018 and July 2024 at the Institute of Legal Medicine of the University of Catania. Various parameters were analyzed: temporal trends, sociodemographic characteristics, circumstances, and risk factors associated with these crimes. The main objective was to analyze the complex characteristics of this phenomenon to determine whether Sicilian cases exhibit similarities to international cases and, if possible, to identify differences between various types of crimes and achieve a shared medicolegal definition of femicide.

Inclusion criteria were: age of the victim, age of the perpetrator, type of relationship between the parties, means used, type and location of injuries, possible involvement of erogenous zones, and toxicological screening (drug test/alcohol levels). Based on the inclusion and exclusion criteria, 27 cases were included: of these, 15 were classified as femicides, and the remaining 12 as non-femicide female homicides. From the data analysis:

- No statistically significant differences emerged in the median age comparison between femicide perpetrators and non-femicide perpetrators. Conversely, analyzing the age of the victims, a statistically significant difference was found between femicide victims and non-femicide victims, with the latter group being older ($P < 0.05$). Further analysis of the "age delta" (age of the perpetrator and age of the victim) in the two groups using a t-test revealed a statistically significant age gap between victim and perpetrator, which was much higher in non-femicide cases compared to femicide cases ($P < 0.05$).
- Although no statistically significant difference was found between the type of homicide and the location of injuries, our study highlighted that the upper and lower limbs were more frequently affected in femicides, likely due to the younger age of the victims and thus a more vigorous defensive action.
- Contrary to what is reported in the literature, no injuries were found in erogenous zones.
- The most used means were sharp and firearms (19 cases out of 27 total); the remaining cases, in order of frequency, were asphyxiation mechanisms, use of fuel, and car crashes.
- Regarding the location of the homicide, it was found that 5 femicides and 7 non-femicide female homicides occurred in domestic environments, while 10 femicides and 5 non-femicide female homicides occurred in non-domestic environments.
- Additionally, it emerged that, of the 27 cases analyzed, 13 were committed by partners: 7 by current partners and 6 by former partners.
- Of the 15 femicide cases, in 86.67% of cases, the victim and perpetrator were in an intimate romantic relationship.

The results of this study underscore the urgent need to adopt preventive measures, including global awareness policies, aimed at closely monitoring the phenomenon and understanding its underlying causes to drastically reduce its incidence.

Femicide; Autopsy; Forensic Investigation

J4 A Psychiatrist's Criminal Liability: A Study of Italian Final Criminal Judgements on Suicide and Hetero-Aggressiveness Cases of the Mentally Ill

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Learning Objectives: Our poster will allow participants to understand the complexity of psychiatric liability evaluation in cases of self or hetero-aggressive behaviors of psychiatric patients. Through our analysis of judgements on the subject, lawyers and forensic physicians will be provided with insights that will guide the proper assessment of psychiatrists' professional conduct in these circumstances.

Impact Statement: Our work will have a favorable impact on the scientific community in general, as it will allow us to highlight the scientific tools concretely available to the psychiatrist to prevent the perpetration of violent acts by the mentally ill. A critical review of the psychiatrist's liability in these circumstances is a valid starting point for highlighting any critical issues, to identify common forensic criteria for forensic evaluation, and to reduce the use of defensive medicine by psychiatrists.

Abstract Text: In Italy, Law 180/78 (Voluntary and Compulsory Health Examinations and Treatments Law) repealed all measures of forced hospitalization of persons considered "criminally insane" and "socially dangerous," except for Involuntary Health Treatment (IHT) which, however, has a limited duration and requires strict applications' criteria.[1,]

Therefore, the management of these subjects is entirely entrusted to the psychiatrist who often, through his health care, becomes a guarantor of the safety of the patient and of the entire society.[3] While the responsibility of the psychiatrist is increasingly onerous, his legal duties are rather vague. In the case of self- or hetero-aggressive acts by a mentally ill, the assistance provided by the psychiatrist is subject to judgment.[4,5] In these circumstances, the medical-legal retrospective evaluation of the professional conduct is quite complex; in fact, the psychiatrist is not only required to assist the patient by a therapeutic plan, but also to foresee (and try to avert) the perpetration by the assisted person of violent gestures that are often, by their very nature, difficult to anticipate.

In order to understand how psychiatrists' liability is actually assessed in these cases, we analyzed 27 final judgments on psychiatric liability issued by various Italian criminal courts between 1983 and 2022. Among the various quantitative results, it is interesting that out of 27 psychiatrists accused, 13 were found guilty by the courts; inadequate management of suicide risk was the most frequent charge, and the most frequent motive of the psychiatrist's conviction was "omitted surveillance."

The qualitative results of our study have been integrated with each other to identify, point by point, which aspects of the psychiatrists' professional conduct have been most frequently the subject of evaluation and contestation. The results of our analysis are very useful to highlight the complex role of the psychiatrist in such circumstances and to guide a proper evaluation of any criminal liability profile arising from his conduct.

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Court; Medical Liability; Suicide

J5 "I Don't Remember . . . It Seems Like I Remember": The Erba Massacre Trial When False Testimony Precludes a Verdict of Innocence

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Learning Objectives: After attending this presentation, attendees will have a clearer understanding of the criminological and legal implications of the crime of perjury.

Impact Statement: This presentation will impact the forensic science community by providing an overview of the scientific and legal aspects of perjury.

Abstract Text: In Italy, the trial of Olindo Romano and Rosa Bazzi, who were found guilty of a terrible massacre committed in 2006 in Erba, Italy, has received significant attention. In this trial, the scientific-forensic theme of perjury became central, and the trial is currently under review. According to Rosa and Olindo's lawyers, the quantity of contradictory elements (over 300) is an indicator of perjury.[1]

The crime of perjury occurs when a person called to testify in a trial lies or denies the truth, or remains silent, in whole or in part, about what they know regarding the facts for which they are being interrogated, thus influencing the outcome of the trial. Perjury is considered a serious crime everywhere as it causes judicial errors, unjust convictions, and/or the acquittal of the guilty. In Italy, this crime is addressed in article 372 of the Penal Code, wherein the punishment is defined as imprisonment from two to six years, more if it has been decisive for the outcome of the trial.

From a scientific-forensic perspective, one of the most used criteria to identify who is lying is the detection of contradictions during the witness's testimony. However, studies show that inconsistency and contradictions do not always equate to lies, just as consistency and the absence of contradictions are not always indicators of honesty. Leins et al. demonstrated that the difference between liars and truth-tellers drastically decreases in realistic experimental situations.[2]

The authors compared the accounts of a couple who went to a restaurant and a couple of subjects guilty of a simulated theft who had to falsely describe an alibi that consisted of them going to the restaurant.[3] Both the honest couple and the guilty one were aware of being suspects, and they had to describe the same scene of having gone to the restaurant. In this case, the diagnosis of contradictions as a method to distinguish the integrity of the liar diminished significantly. Other studies show that although liars have levels of contradiction seven times higher than honest people, this figure decreases significantly if the liar has the opportunity to repeat the story several times. Other research has shown further criteria for detecting lies, such as the lengthening of reaction times when asking unexpected questions that the liar did not have the chance to prepare for. Moreover, forensic neuroscience could be indicative of the reliability of testimony through memory detection techniques (autobiographical Implicit Association Test [aIAT]), a procedure that is based on the subject's reaction times and verifies the existence of a memory trace within the subject's mind (Verona Court, February 23, 2012). In the context of perjury, the phenomenon of false confession, also a cause of judicial errors, must be addressed and differentiated. The confession, like the DNA test for identifying the culprit, is considered one of the fundamental pieces of evidence in criminal trials as it has a minimal error rate and is difficult to refute, yet false confessions account for 27% of wrongful convictions.[4] Among the main causes of false testimony are individual factors, like the subject's young age or cognitive issues, and situational factors, including the length of interrogations, sleep deprivation, and the subject's understanding and justification of the crime. This work aims to highlight the fundamental scientific and legal indicators of perjury with particular reference to the Erba case.

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Perjury; Homicide; Memory

J6 Attention Deficit Hyperactivity Disorder, Substance Use, and Driving

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Learning Objectives: After attending this presentation, attendees will understand some aspects of the relationship between Attention-Deficit/Hyperactivity Disorder (ADHD), unsafe driving behaviors, driving safety, and traffic medicine.

Impact Statement: This presentation will impact the forensic community by underscoring the possible relation between some specific risk behaviors (driving under the influence of alcohol and/or illicit drugs,) and the diagnosis of ADHD.

Abstract Text: ADHD is a chronic neurodevelopmental condition with symptoms such as inattentiveness, impulsivity, and hyperactivity. Although ADHD is commonly considered a childhood disorder, it can persist into adulthood and affect daily life performances of older adults. The reported prevalence of ADHD in adults has increased in recent years due to improved diagnosis. Major symptoms of ADHD may impair cognitive functioning, including complex executive functioning in activities of daily living, such as driving.

Previous research showed a positive association between ADHD and unsafe driving behaviors based on self reports, informants, and official reports. For on-road driving outcomes, drivers with ADHD were more prone to receive license suspensions and to be involved in vehicular crashes. Despite the aging driver population and the increasing prevalence of ADHD in adults, research on ADHD and driving safety is limited primarily to adolescents and young adults. Few studies have assessed the association of ADHD with crash risk among older adults.

This study was designed as a single-center trial and was conducted from January 2022 to December 2023. The aim was to examine the prevalence of ADHD (previous ADHD diagnosis) in older adult drivers who contacted our facility in order to undergo a fitness-to-drive medical assessment in the aforementioned period. Four hundred cases were reviewed, of which 12 per cent (48 cases, 46 males) reported having been diagnosed with ADHD in the past. Twenty cases contacted our facility for driving under the influence of alcohol, 22 cases for driving under the influence of illicit drugs (cocaine and/or amphetamine in 18 cases), and 6 cases for driving under the influence of both (alcohol and illicit drugs). Of the 48 cases considered, only 4 reported continuing to benefit from specific treatment for ADHD. The results of our study appear interesting in view of the specific context in which the participants were recruited (people with a documented traffic offence for driving under the influence of alcohol and/or illicit drugs) and the fact that the previous diagnosis of ADHD was confirmed by the participants themselves.

Taking into account the prevalence of ADHD in the general population and the number of undiagnosed cases, individuals for whom the competent authority requires a medical assessment of fitness to drive for driving under the influence of alcohol and/or illicit drugs represent a potentially very interesting sample to investigate some specific risk behaviors (driving under the influence of alcohol and/or illicit drugs, in this case) and the possible relationships with the diagnosis of ADHD, whether known or unknown.

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ADHD; Impaired Driving; Psychological Behavior

J7 Evaluation Issues on Imputability and Aggravating Circumstances in Crimes Caused by Pathological Jealousy: A Retrospective Study of 20 Judgments of the Italian Supreme Court of Cassation

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Learning Objectives: First, our poster will allow attendees to understand the blurred line between "physiological" and "pathological" jealousy. In crimes of passion, this distinction can be crucial to establish the defendant's imputability and therefore its punishability. In addition, through the examination of the most recent judgments on the subject issued by the Italian Supreme Court of Cassation, the crucial role of the forensic doctor and forensic psychiatrist in establishing the penalty of the offender will be deepened.

Impact Statement: This poster will have a positive impact on the forensic scientific community because it will shed light on a topical issue that is almost unexplored in the literature. The increasing frequency of crimes of passion requires a scientific study about jealousy. Our in-depth analysis can be a valid scientific support for all forensic professionals who are involved in assessing the mental state of the accused at the moment of the crime. In addition, this study may also be useful to raise awareness among judges about the importance of forensic assessments in such crimes.

Abstract Text: When faced with bodily injury or murder with a jealousy motive, it would be essential to investigate whether the offender's jealousy exhibits morbid characteristics (pathological jealousy) or whether it is the result of a cultural, character, or personological drive (physiological jealousy). Proper differentiation is essential to establish the imputability of the agent and the attribution of any aggravating circumstance (cruelty and premeditation). As we have pointed out in our study, pathological jealousy may be the extrinsic manifestation of numerous psychiatric and/or organic disorders and has the potential to impair the agent's ability to understand and/or will.[1-4]

Therefore, in these cases, the medical and psychiatric evaluation of the accused carried out by the forensic doctor and/or by the forensic psychiatrist plays a crucial role; in fact, these are the only professional figures able to scientifically prove the possible existence of pathological jealousy and to assess whether it was such as to have impaired judgment of the defendant.[5]

In our poster we will present a retrospective analysis of 20 judgments of the Italian Court of Cassation in the period 2004-2022; the judgments concerned cases of murder or personal injury motivated by the jealousy of the offender. The qualitative and quantitative results have been summarized to obtain a critical overview of the topic. Among the various quantitative findings, significant is the fact that in 15% of the cases the judges did not rely on a medical-legal or psychiatric evaluation report.

Moreover, based on our findings, the distinction between pathological and physiological jealousy cannot be rigidly framed; each individual case requires repeated expert investigations, which are essential for a complete assessment of the offender's social context, character and personality characteristics, and clinical–anamnestic and circumstantial elements. However, in our poster, we will provide a useful schematic guide for the forensic evaluation of these cases based on the analysis of the judgments.

The insufficient forensic-psychiatric deepening emerged by our study risks opening the way to contradictory judgments based on deductive reasoning without scientific rigor. This also considers that the crimes of passion motivated by jealousy resent many socio-cultural conditionings. Such conditioning can be contained only with a rigorous psychiatric-forensic assessment, a scientific tool essential to ascertain the actual imputability (and therefore punishability) of the offender.

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Femicide; Forensic Psychiatry; Court

J8 The Effect of Plaintiff Malingering, Deception, and Perceived Reprehensibility on Damage Awards

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Learning Objectives: This poster presentation will describe the impact of exaggerated testimony on juror decision-making in civil damages determinations. After attending this poster presentation, attendees will better understand the effect of malingering on jurors.

Impact Statement: Due to advances in technology, many people who once died from Traumatic Brain Injury (TBI) now survive. As a result, many cases that would have been wrongful death cases are now personal injury cases.[1,2] Forensic neuropsychologists are frequently called upon by the legal system to perform evaluations on plaintiffs in lawsuits involving compensation or litigation. Since a financial incentive is at stake, the assessment of malingering is an integral part of the evaluation process.[3-5]

Abstract Text: The present study measured the effects of plaintiff malingering and exaggeration on monetary damages awarded in a civil case. The *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*) defines malingering as "the intentional production of false or grossly exaggerated physical or psychological symptoms, motivated by external incentives such as avoiding military duty, avoiding work, obtaining financial compensation, evading criminal prosecution, or obtaining drugs."[6] The *DSM-5* also states that malingering should be suspected in: (1) a medicolegal context, (2) situations where there is a discrepancy between objective findings and the individual's claimed stress and disability, (3) patients who fail to cooperate with treatment or diagnostic procedures, and (4) cases where there is a presence of antisocial personality disorder. However, malingering is not a binary distinction. Rather, it is viewed along a continuum that can range from mild forms of exaggeration to the deliberate outright faking of symptoms.[7-10]

Participants in this study were jury-eligible undergraduate students from a university in the Northeastern United States. The sample consisted of 191 women and 38 (N = 229) with a mean age of 23.1 (SD = 2.3). Participants completed a demographic questionnaire, reviewed a case vignette containing the details of a factitious case involving a plaintiff who sustained a mild TB (mTBI) in a Motor Vehicle Accident (MVA) and a summary report from a neuropsychologist describing the plaintiff's injury. This report included the results of the evaluation and future prognosis, answered questions pertaining to the reprehensibility of both the plaintiff and defendant; and completed the Belief in Just World Scale (BJW) and the Revised Legal Attitudes Questionnaire (RLAQ).[11,12] Participants were presented with one of three conditions: Truthful, Exaggerated, or Malingered. After reading the details of each case, participants were instructed to award the plaintiff compensatory damages up to a maximum of $1 million dollars.

To examine the influence of plaintiff conduct on awards, a one-way Analysis of Variance (ANOVA) with three levels (honest, exaggerated, and malingering) was computed for each type of award. As expected, the honest condition differed from the exaggerated and malingered conditions ($F(2, 226) = 14.71$, $p < .01$, $\eta 2 = .086$). Post hoc tests revealed that the honest condition award (M = $80,090) differed from the exaggerated (M = $4,590) and malingered condition awards (M = $4,447). However, the exaggerated and malingered conditions did not differ from each other. Participants with a higher belief in a just world (r (227) = -.371, p = .036) and higher levels of legal authoritarianism (r =.419, p = .023), tended to award less compensation overall.

A significant difference was found among the level of plaintiff reprehensibility ($F(2, 226) = 6.40$, p = .002, $\eta 2$ = .006). Post hoc tests revealed that jurors found the malingering plaintiff most reprehensible (M = 7.07, SD = 2.68), followed by the exaggerating plaintiff (M = 6.25, SD = 2.24), and honest plaintiff (M = 5.63, SD = 2.60). No significant differences were found for defendant reprehensibility ($F(2, 226) = .209$, p = .811) Juror confidence in the amount awarded in each condition did not differ ($F(2, 226) = .2.40$, p = .093).

The results of the present study indicate that any amount of deception on the part of the plaintiff can influence jury decision-making. These results are consistent with previous findings that suggest juries are often skeptical of plaintiffs and become rigid when they feel the plaintiff has been deceptive.[13,14]. However, juries are rational in their decisions of compensation and tend to award monetary damages concurrent with the plaintiff's economic loss.[15,16]. In general, juries award compensation to plaintiffs based on the severity of their injuries; the more severe the injury, the larger the financial award.[17-20]

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Malingering; Civil Damages; Jurors

J9 Interdependency Between Alexithymia and Alcohol Use Disorder

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Learning Objectives: After attending this presentation, attendees will understand some aspects of the interdependency between alexithymia, characterized by impairments in emotional awareness, and alcohol use disorder.

Impact Statement: This presentation will impact the forensic science community by showing how close the relationship can be between alcohol use disorder and alexithymia, characterized by difficulties in identifying feelings.

Abstract Text: Alexithymia was originally defined as the inability to recognize and verbalize emotions. A poverty of imagination (or of a fantasy world) as well as a lack of positive emotions and a high prevalence of negative emotions have also been described as characteristic of alexithymia.

Alexithymia is often viewed as a personality trait with a normal distribution among the population. The most commonly used scale for assessing alexithymia is the self-reported Toronto Alexithymia Scale with 20 items (TAS-20). Several studies reported a high prevalence of alexithymia among subjects with illicit drug abuse and alcohol use disorder. Concerning this latter, some authors showed a higher prevalence rate of alexithymia in individuals suffering from alcohol use disorder compared with control groups, and a positive relationship between alexithymia, the amount of alcohol use, and the severity of alcohol problems among individuals suffering from alcohol use disorder.

In this study, the French version of the TAS-20 was proposed to people (males and females aged between 18 and 65 years) who contacted our facility in order to undergo a fitness-to-drive medical assessment from January 2022 to December 2023. Fitness-to-drive medical assessments were requested by the authorities following driving under the influence of alcohol. A total of 102 individuals were recruited in the study. Twenty-two of them received a diagnosis of alcohol use disorder, based on the results of all investigations, including toxicological analyses (ethyl glucuronide determination in hair samples and/or phosphatidylethanol determination in blood samples). Results of our study were consistent with previous findings of positive relationships of TAS-20 alexithymia scores with Alcohol Use Disorders Identification Test.

Numerous previous investigations found a relationship between severity of alexithymia and alcohol use disorder among patients suffering from this condition. Some other studies summarized that alexithymia might have the potential to interfere with treatment outcomes among these individuals. The reasons for this finding might be related to a bidirectional mechanism: alexithymia might increase alcohol use, and alcohol use might involve a tendency to affect personality, including alexithymic traits, in a negative way. Individuals suffering from alcohol use disorders might therefore benefit from psychotherapeutic interventions focused on ameliorating alexithymia in order to improve care and prognosis.

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Alcohol; Psychological Behavior; Driving Under the Influence

J10 The Role of Forensic Assessment/Investigation in the Protection of Incapable Adults

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Learning Objectives: After attending this presentation, attendees will better understand the controversy over mental manipulation and the investigation relating to the circumstance of incapacity and possible circumvention

Impact Statement: This presentation will impact the forensic science community by showing that the process of persuasion takes place through the selection of the "most likely" subjects, who can engage in indoctrination in a psychological and relational context; thus it is necessary to validate a proper scientific methodology for evaluating possible vulnerable subjects.

Abstract Text: In fact, it must be recognized that different degrees of incapacity may exist and may be due to a mental disability, a disease or, most frequently, victims of such crimes are people who conduct a normal, well-adapted existence, but who may be affected by some intrinsic fragility, traumas, unresolved problems that have not been processed, or a tendency for dependency or a highly suggestible personality, exposing the subjects to the risk of prejudicial consequences.[1,2] We report the case of a widowed, secular man in his 70s, who suddenly discovered he is suffering from an infectious hepatitis and begins interferon therapy. Following this condition and treatment, he develops a progressively worsening depressive disorder (with "thoughts" about death and euthanasia) and, at the same time, he relies on two female catechists who belong to a theocratic religious movement. The women begin to take care of him in an apparent philanthropic manner but, as time passes, he converts and donates his entire life savings to the movement, prompting his relatives to be concerned about an impairment of his personal faculties. This act was motivated by the man as a recognition for the care and assistance received from those who had introduced him to "the true faith."

From this peculiar case we can deduce an empirical methodology, based on some fundamental areas of investigation: (1) the previous state of the subject (i.e., his entire biography, with particular attention to the clinical and pathological traits, but also to the values of his existential dimension); (2) the mental state of the subject at the moment of the legal act, focusing not only on his intellectual-cognitive functioning, but also on his affective-emotional state; (3) the characteristics of the relationship between the person who donates and whoever becomes the beneficiary, with specific reference to the type of investment; and (4) the consequences that this act, in concrete terms, produces for the subject himself.

The context in which this case was examined and came under investigation is that of an expert report ordered by the Italian judicial authority. It is well known that some religious movements, presented as meaningful systems, offer shared beliefs, stories, and meanings to confer order to social reality; however, in some circumstances, it is not clear if mental manipulation led to indoctrination and donation.[3,4]

The process for clinical and forensic psychiatric evaluation followed the standards and method of rigorous forensic medical and psychological, forensic psychiatric investigation, which included examination of the victim's personalities, examination of the offenders, and examination of the relational and affective context in which the criminal event took place.

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Forensic Psychiatry; Elder Abuse; Fraud

J11 Misinformation in Forensic Sciences: False Statements Regarding Parental Alienation Theory

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Learning Objectives: Attendees will become aware of the vast amount of misinformation that has occurred in recent years in hard science and social science publications. Attendees will understand that misinformation does not occur evenly or randomly across many publications, but is focused in a small number of professional books and journals.

Impact Statement: Misinformation is particularly important in forensic sciences because false statements in professional publications can lead to faulty forensic investigations, which can lead to incorrect testimony and, ultimately, to erroneous legal conclusions. All members of the scientific and legal communities should strive to identify and correct misinformation in publications and in presentations at professional meetings.

Abstract Text: Participants in this session will understand the scope of misinformation in professional publications, which may influence forensic investigations, testimony, and legal decisions. In recent years, thousands of journal articles and book chapters have been corrected or retracted because of misinformation and grossly faulty research practices. Misinformation does not occur evenly or randomly across many publications, but is focused in a small number of professional books and journals.

Presenters will explain how to identify, track, and correct published misinformation. For example, one approach is to create and circulate a formal, scholarly rebuttal of a published document; that method was used to refute and correct a report distributed by the Human Rights Council of the United Nations. A second way to counteract misinformation is to create and publicize clever, animated videos that appeal to the general public; that approach was used to respond to a book that contained hundreds of false and misleading statements regarding parental alienation. Finally, a comprehensive research program in the form of citation analysis demonstrated how the same misinformation regarding parental alienation started in a law review article and spread over many years to legal and mental health journals and policy statements of professional organizations.

Misinformation regarding parental alienation and other forensic topics has found its way into legal briefs and testimony by expert witnesses. All members of the scientific and legal communities should strive to identify and correct misinformation that occurs in publications and in presentations at professional meetings.

Parental Alienation; Junk Science; Peer Review

J12 A Comparison of Virtual and In-Person Forensic Evaluations

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Learning Objectives: The goals of this presentation are to: (1) identify a useful tool for limiting time in detention, shortening evaluee waitlists, minimizing infective risks, and preserving an overtaxed workforce; (2) describe barriers to virtual evaluations; and (3) recognize any variation in outcomes between virtual and in-person forensic evaluations.

Impact Statement: The frequency at which mental health patients are interacting with the criminal justice system is on the rise.[1] Competency to stand trial evaluations represent the most common type of forensic mental health assessment in the American legal system, occurring in approximately five to eight percent of all cases.[2,3] The aftermath of the recent pandemic has further amplified the demand for forensic evaluations, highlighting the need for solutions that reduce infectious risk for both evaluators and defendants, minimize prolonged detention times, and ensure accessibility to defendants amid workforce shortages. Virtual platforms present a promising solution to address these challenges.[4]

Abstract Text: This investigation compares in-person and virtual evaluations in the inpatient forensic setting at Saint Elizabeths Hospital, Washington, DC. Utilizing competency to stand trial reports—the most common United States court evaluation—we conducted a retrospective chart analysis. Reports are reviewed with an anonymized checklist assessing competence outcomes, length of interview, refusals/no-shows, charges, and demographics. The study compares periods before and after the pandemic when virtual platforms gained popularity.

A power analysis using two related studies indicates that 40 reports in each sample are sufficient to pick up large differences between the groups, while samples of up to 110 reports are required to pick up smaller differences. Preliminary results do not yet distinguish the samples in any of the study's hypotheses:

1. There is no difference between virtual and in-person evaluations in the length and number of evaluations before a final recommendation.
2. There is no difference between virtual and in-person evaluations outcomes, (e.g., "competent," "incompetent").
3. The number of times technology is mentioned in virtual assessments (a proxy for barriers to virtual assessment) does not affect the kind and number of opinions required for a final recommendation.
4. Evaluee demographics and evaluators' years of experience do not affect assessment outcomes using the virtual platform.

We expect that ongoing data collection will continue to support virtual evaluations as an avenue for limiting time in detention and meeting the needs of vulnerable persons who require forensic assessment.

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Forensic Assessment; Competence to Stand Trial; Civil Rights

J13 Future-Proofing Forensic Psychiatry: Technological Advances Aiming to Shape Tomorrow

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Learning Objectives: This presentation will: examine how Artificial Intelligence (AI), Virtual Reality (VR), neuroprediction models, and genetic testing are currently being used in risk assessment, predicting recidivism, and enhancing personalized treatment; identify the shortcomings of conventional approaches in capturing the complex factors underlying psychiatric disorders and criminal behaviors and understand the need for innovative solutions; evaluate the impact of genetic testing and neuroprediction on risk assessment; assess how genetic testing can identify markers for aggressive behavior and recidivism risk, and how neuroprediction uses brain variables to predict prognosis, treatment outcomes, and behavioral forecasts; identify the use of VR in cognitive rehabilitation, resocialization of detainees, and crime scene simulations for courtroom scenarios; and help attendees understand, through our evaluation of the literature, the ethical implications and potential risks associated with the use of AI, VR, and other technologies in forensic psychiatry, and discuss the importance of regulation in preventing inaccuracies and errors.

Impact Statement: This paper explores the transformative potential of emerging technologies such as AI, Virtual Reality (VR), neuroprediction, and genetic testing in forensic psychiatry. By critically evaluating current literature, we aim to highlight how these innovations can improve the accuracy and objectivity of risk assessments, reduce human error, and offer new avenues for personalized treatment. We also address the ethical considerations and regulatory needs essential to safeguarding against the potential risks of premature implementation. Our research emphasizes the need for a careful, informed integration of these technologies to enhance the effectiveness of forensic mental health practices while ensuring justice and public safety.

Abstract Text: Technologies such as Artificial Intelligence (AI) and Virtual Reality (VR) are increasingly integrated into various aspects of society. Alongside neuroprediction models and genetic testing, these innovations hold transformative potential for forensic psychiatry by enhancing risk assessment accuracy, predicting recidivism, identifying genetic influences, and supporting personalized treatment approaches. These tools aim to reduce human error while improving the objectivity and inter-rater reliability of forensic evaluations.

Current violence risk assessments often fail to capture the complex interplay of factors contributing to psychiatric disorders and criminal behavior, resulting in limited predictive validity. This limitation underscores the need for innovative approaches to address these complexities and advance the field.

Genetic testing provides insights into an individual's predisposition to aggressive behavior and recidivism by identifying relevant biomarkers. Neuroprediction applies structural and functional brain variables to forecast behavior, aid in lie detection, and predict treatment outcomes and prognoses. By reducing biases, it aims to enhance predictive reliability. Meanwhile, VR offers innovative applications for cognitive rehabilitation and offender resocialization, further enhancing diagnostic and therapeutic strategies in forensic settings.

Collectively, these technologies seek to transform forensic psychiatry. They promise to reduce bias, enhance diagnostic accuracy, and improve therapeutic interventions. However, their integration requires careful ethical analysis and regulatory oversight to address potential challenges, such as inaccuracies or unintended consequences that could impact individuals and the broader justice system.

To explore these developments, a comprehensive literature review was conducted, analyzing peer-reviewed articles published within the past five years. Databases such as PubMed and Cochrane Reviews were searched using predefined keywords, including "forensic psychiatry," "artificial intelligence," "genetic testing," and "virtual reality." Key studies were selected for their relevance and contributions to the field.

This presentation examines the emerging impact of these technologies on forensic psychiatry, exploring both their potential benefits and challenges. By reviewing recent advancements, we aim to provide an overview of the intersection of these technologies in the field of forensic mental health.

Forensic Psychiatry; Risk Assessment; Artificial Intelligence

J14 Military Sexual Trauma, PTSD, and the Forensic Psychiatric Exam

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Learning Objectives: This presentation will: (1) outline the importance of Post-Traumatic Stress Disorder (PTSD) assessment for forensic psychiatrists in criminal and civil cases; (2) review the epidemiology of military sexual trauma; and (3) instruct the audience on how to conduct a succinct and thorough PTSD screen during forensic assessment.

Impact Statement: This presentation will increase awareness of military sexual trauma as a cause of PTSD, which is of utmost importance in civil forensic psychiatry practice. It will increase the ability to properly screen for PTSD in criminal and civil forensic psychiatric cases.

Abstract Text: PTSD is perhaps the most important diagnosis in civil forensic psychiatry, and certain populations are at especially high risk of PTSD, including military Service Members (SM). PTSD claims in civil cases require evaluation by a forensic psychiatrist for assessment of the validity of symptoms, severity of injury or disability, and establishment of a timeline of symptoms in relation to an index trauma. Index traumas can occur in any setting, but certain occupations or historical events are known risk factor for trauma, including refugee status, first responder jobs, and military SMs.

Knowledge of at-risk professions or individuals is intuitive and public perception of SM's accurately reflects an increased risk of PTSD in that population. However, public perception and the perception of civilian psychiatrists is incorrect in assuming the PTSD is strictly from deployment- or battlefield-related trauma. PTSD more commonly arises from other causes, including childhood trauma and sexual trauma. Sexual trauma that occurs during military service has been under increased scrutiny over the past two decades. It is a significant source of PTSD and a disintegrating force in military esprit de corps.

As a previous Army psychiatrist, graduate of a forensic psychiatry fellowship, and current consultation-liaison psychiatrist who has taught and lectured in many settings, I have found a strong demand from general psychiatrists and forensic psychiatrists for education on obtaining a military trauma history with skill and sensitivity. This presentation will examine military sexual trauma as an entity that all psychiatrists who evaluate military service members should understand and screen for. This presentation will offer practical instruction on conducting a thorough trauma screen in SMs and veterans, and the skills translate to any forensic evaluation. Included in this will be discussion of how technology, including "phrases" and artificial intelligence templates may benefit forensic evaluators in assessing an individual and preparing a work product.

Forensic Psychiatry; Post-Traumatic Stress Disorder; Traumatic Life Events

J15 Forensic Implications of Excited Delirium Syndrome: Diagnostic Challenges and A Case Series

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Learning Objectives: After attending this presentation, attendees will understand Excited Delirium Syndrome (ExDS), its forensic implications, and the necessity of precise and applicable diagnostic criteria in forensic practice.

Impact Statement: By presenting a case series of suspected ExDS-related deaths, we endeavor to spotlight the challenges associated with diagnosing this syndrome. The aim of this study is to encourage the forensic community to improve diagnostic tools, possibly by incorporating machine learning systems, given the current ambiguity and conflicting findings surrounding them.

Abstract Text: ExDS is a disputed and poorly understood condition that is characterized by extreme agitation, aggression, acute distress, and sudden death.[1] Although the American College of Emergency Physicians (ACEP) recognizes ExDS, it remains controversial among major medical organizations like the American Medical Association (AMA) and the American Psychiatric Association (APA) due to ambiguous diagnostic criteria, and it is not included in the *International Classification of Disease, 10th edition* (*ICD-10*) or *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*).[2,3]

This study examines ExDS through a forensic case series to highlight the clinical and forensic challenges associated with diagnosing this syndrome and advocate for more precise diagnostic tools. Only forensic investigations involving individuals with abnormal behavior observed before sudden death, a non-specific cause of death, and no pre-existing, established, or evolving medical condition were selected.

We collected consultations containing forensic investigations conducted at the Institute of Legal Medicine of the University of Rome "Tor Vergata," and at the Institute of Legal Medicine of the University of Bari "Aldo Moro" by two pathology experts. These reports were submitted in consultations on behalf of five different Italian public prosecutor's offices. Six consultations were selected and subsequently analyzed by two forensic pathology and forensic psychiatry experts to collect relevant information. The analysis focused on criteria established by the ACEP, including historical, scene, contact, clinical assessment, death, and autopsy features, to evaluate if the cases met ACEP guidelines for diagnosing ExDS [4].

The six cases reveal common patterns and significant variations in the presentation of the syndrome. All six subjects were male, five aged 25 to 36, aligning with known epidemiological data on the ExDS.[4-10] In half of the cases with documented information, symptoms emerged rapidly, partly explaining why this syndrome is frequently observed in specific settings, such as prehospital emergency medical services, police interventions, and forensic settings. Medical intervention was only seen in three out of six cases. It was impossible to assess for the presence of tachypnoea, tachycardia, hyperthermia, hypertension, acidosis, and rhabdomyolysis in all six cases. Cardiac rhythm information was available only in one case, showing pulseless electrical activity. This lack of clinical data affects any efforts to prevent and early clinically assess these patients, thus confirming the marginal role that health professionals currently have in managing ExDS cases.[11] Most reported cases described police intervention and resistance to physical restraint. Many authors have linked ExDS with the adoption of restraint maneuvers during arrests by law enforcement.[9,12] In the present case series, sudden collapse after restraint and respiratory arrest occurred in four cases each. Moreover, the cases varied in terms of substance use and mental health history, underscoring the complexity of ExDS. Autopsies frequently indicated asphyxia, highlighting the need for thorough forensic investigations to rule out other causes of death and address the contentious nature of ExDS as a diagnosis.

The heterogeneity and complexity of symptoms complicate the ExDs diagnostic process, and none of the proposed criteria provides a definitive diagnosis. A prospective approach should integrate comprehensive clinical, documentary, and manifestation-related information. In the absence of a pathognomonic marker for this syndrome, a multidisciplinary approach is imperative. Given the intricate nature and extensive data associated with ExDs, artificial intelligence and machine learning systems could serve as valuable tools, especially considering the low prevalence of these syndromes. Such technologies could enable a detailed analysis of each case.[13] Genetic and immunohistochemical investigations of dopamine circuit structures should also be considered despite the current uncertainty regarding their role,[14,15]

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Excited Delirium Syndrome; Agitation; Restraint-Related Deaths

J16 Recovered Memories and False Memories in Sexual Assault and Homicide Investigations

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Learning Objectives: After attending this presentation, attendees will have learned about general principles of memory—sensory memory, declarative and procedural memory, and short-term and long-term memory. Attendees will also learn of the role of memory in investigating violent crime, how memories can be "recovered," and the development of false memories. The attendees will also learn about why some individuals are more susceptible to developing false memories and the role of therapists in "assisting" in the "recovery" of memory. Experimental paradigms and case examples will be discussed.

Impact Statement: This presentation will impact the forensic science community, in particular the Behavioral and Psychiatry Section and police investigators in: (1) understanding how "normal" memory works, how false memories develop, and the role of therapists in causing iatrogenic recovered memories; (2) how forensic psychiatrists can assess these individuals; and (3) educating police investigators of violent crimes on how false memories can impact their investigations.

Abstract Text: After attending this presentation, attendees will have better understanding of how memory functions (sensory memory, procedural and declarative memory, short-term memory, and long-term memory). Although the "memory wars" of the 1980s and 1990s that impacted therapists and the legal system are over, skirmishes are still breaking out.

Memory is malleable. Details can be distorted, and wholly false memories can be "implanted." Just because memory is detailed, confidently expressed, and emotional, does not mean that it reflects a true experience. It is possible to remember events that never happened. One factor that contributes to memory distortions is that memory functions best in remembering a gist of the event, not the particulars from which the gist is derived.

Most false memories are iatrogenic; they are suggested, validated, encouraged, and "recovered" through unproven and pseudo-scientific practices and methods of "therapists." Gudjonsson states, however, that some individuals can also develop a belief and a memory regarding trauma that is internally motivated and generated rather than being the result of a suggestion in therapy.[1]

Loftus states that imagination can play an important role in producing false beliefs and false memory.[2] Her research has that asking individuals to imagine they had experienced a made-up event significantly increased the likelihood that a person believed the event had taken place. According to Geraerts and McNally, individuals who are susceptible to false memories may have source-monitoring deficits—incorrect judgements about the origin or source of information.[3]

Each and every case must be investigated on its own merit. The goal of this presentation is to discuss the role of memory, how memories can be influenced and developed, and how these false memories can impact violent sexual assault and homicide investigations. Experimental paradigm, of false memories and case examples will be presented and discussed.

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Forensic Psychiatry; Memory; Recovered Memory

J17 The Recent Acceptance of Borderline Personality Disorder for Pretrial Mental Health Diversion in California

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Learning Objectives: After attending this presentation, attendees will be familiar with California's Assembly Bill 1412, "Pretrial Diversion: Borderline Personality Disorder," which broadened the qualifying mental disorders for pretrial diversion to include Borderline Personality Disorder (BPD) while still maintaining the exclusion of Antisocial Personality Disorder (ASPD). This presentation will also highlight the potential challenges the bill poses for forensic mental health evaluators to differentiate between personality disorders contributing to the commission of a crime and provide recommendations for addressing these challenges.

Impact Statement: This presentation will impact the forensic science community by illuminating the difficulties of determining whether BPD or ASPD contributed to the alleged offense and by providing guidance on how to navigate these challenges.

Abstract Text: In 2018, the State of California introduced a pretrial diversion program under Penal Code 1001.36, allowing defendants with qualifying mental disorders to receive treatment and potentially have their charges dismissed. A mental disorder diagnosis would qualify if it significantly contributed to the commission of the alleged offense and would respond to treatment. Initially, BPD, ASPD, and pedophilia were excluded as qualifying diagnoses. However, in February 2023, advocacy efforts led to the introduction of Assembly Bill (AB) 1412 to include BPD as a qualifying diagnosis. This bill was signed into law on October 10, 2023. This legislative change aims to provide equitable access to pretrial diversion, reduce stigma, and lower recidivism rates among those with BPD. Notably, BPD accounts for 12-30% of individuals who are incarcerated, contrasting sharply with 1-2% prevalence in the community.

While most of the United States have pretrial mental health diversion programs, California is the first to specify which personality disorders qualify. New Jersey and Iowa exclude all personality disorders, and Florida excludes "antisocial behavior" without specifying personality disorder diagnoses. Globally, efforts to destigmatize personality disorders are gaining momentum.[1,2] Thus, California's AB 1412 reflects this movement and may potentially influence future legislative developments in other jurisdictions.

Despite these advancements, unique challenges arise with the new legislation. BPD and ASPD commonly occur together, especially in convicted offenders.[3] This comorbidity amplifies risks of violence and aggression due to traits such as hostility, impulsivity, and increased risk-taking behavior. Including BPD in pretrial diversion criteria while excluding ASPD poses challenges for forensic mental health evaluators in pinpointing the primary disorder that contributed significantly to an offense. The goal of California's pre-trial diversion is to encourage treatment for qualifying mental disorders linked to criminal behavior. Given the widely accepted and established treatments for BPD that reduce recidivism, forensic mental health evaluators must expose the reasoning for their opinion as to whether the mental health symptoms, if any, contributed to the crime and will respond to available treatments.

This presentation will highlight key diagnostic traits of ASPD and BPD relevant to criminal behavior, which will aid forensic mental health evaluators in distinguishing between the two disorders. Additionally, a treatment-oriented approach for cases where diagnoses overlap indistinguishably will be discussed.

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Borderline Personality Disorder; Criminal Behavior; Mental Health Diversion

J18 Cognitive Bias in Forensic Psychiatric Evaluation: A Scoping Review

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Learning Objectives: After attending this presentation, participants will understand the potential sources of cognitive bias in forensic psychiatric assessment, which could result in low inter-rater reliability and impact the forensic evaluation's objectivity.

Impact Statement: This presentation will impact the forensic science community by illustrating potential cognitive biases influencing forensic psychiatric assessment, discussing de-biasing strategies, and the implications of implementing artificial intelligence, which introduces its own specific cognitive biases.

Abstract Text: Cognitive bias is a systematic distortion pattern in human judgment that can affect human perceptions and decisions in many ways.[1] Several studies have shown that cognitive biases can significantly affect forensic sciences and arise from several sources.[2-4] In 2020, Dror proposed a model that categorizes primary sources of bias of forensic experts into three categories: "case-specific," "environmental, culture and experience," and "human nature." These categories were organized in a hierarchical eight-level pyramid model.[5] Moreover, to assess the performance between and within forensic experts, Dror introduced the concepts of "Biasability" and *"Reliability."*[6] "Biasability" pertains to the ability of the expert to make decisions based on relevant information without being biased by irrelevant contextual information, while "Reliability" pertains to the forensic expert decision's consistency, coherence, and reproducibility.[6] A forensic evaluation is considered unbiased if consistent within and between experts.

Research on the inter-rater agreement in forensic psychiatric evaluations has yielded mixed results, generally showing low levels of concordance and cognitive biases could have a relevant role.[7] Moreover, in forensic psychiatric practice, this issue becomes more common as patients may consciously or unconsciously manipulate symptoms, while experts may selectively focus on certain aspects of the patient's condition, contributing to low inter-rater reliability.[8,9]

Forensic psychiatrists must provide objective information to the court, and a biased forensic psychiatric assessment can lead to a miscarriage of justice and pose a safety risk for individuals and society.[10] The complexity of cognitive biases' influence in forensic psychiatric decision-making represents a challenge, and research in this field reveals notable gaps.[2]

The present study aims to describe and summarize scientific literature on cognitive bias in forensic psychiatrists to improve awareness of this field and de-biasing strategies. Following the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines, a scoping review was conducted on PubMed and Scopus until January 2024. The initial search yielded 773 records, and after applying the inclusion criteria, 89 papers were selected for analysis. The examination of these 89 papers identified 29 different biases, with confirmation bias and blind spot bias being the most frequently studied. A new model has been proposed based on assessing the adaptability of forensic psychiatry to a source bias model proposed by Dror in 2020.[5] Furthermore, the present scoping review highlighted possible de-biasing solutions and illustrates the complexity of cognitive bias influence in expert witness activities regarding decision-making within the forensic evaluation. Finally, the opportunities and challenges of employing Artificial Intelligence Tools (AIT) in forensic psychiatry assessment and the specific biases associated with using such technologies in the forensic field were discussed. Despite efforts, controlling inherent biases in forensic psychiatry assessments in criminal matters remains challenging. Identifying, controlling, and avoiding these biases may improve the quality of forensic expertise and reports.

References:

Cognitive Biases; Forensic Psychiatry; Inter-Rater Reliability

J19 Missing White Woman Syndrome: An Evaluation of Bias Surrounding the Presentation of Missing Persons' Cases in True Crime Podcasts

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Learning Objectives: This poster presentation will provide an analysis of missing persons cases presented through popular true crime podcasts in comparison to the national crime database. After viewing this presentation, attendees will better understand the concept of Missing White Woman Syndrome and how current media compares to national data on missing persons.

Impact Statement: Missing White Woman Syndrome suggests there is a disparity in the cases of missing minority groups reported in both print media and televised news programs.[1,2] There is an increasing popularity of hobby true crime reporting, such as podcasts, but their coverage still lacks the diversity of cases reported nationally.

Abstract Text: This pilot study evaluated the disparities in race and sex between podcast coverage of missing persons and numbers reported by the Federal Bureau of Investigation's (FBI's) National Crime Information Center (NCIC). Research has suggested the impact of the overrepresentation of missing White women in media coverage, dubbing this phenomenon as "Missing White Woman Syndrome."[1,2] Some podcasts, such as *Black Girl Gone*, focus on cases that are less likely to be covered in the news.[3] A question remains as to whether other podcasts choose cases that correlate with NCIC data or also overrepresent White women in their episodes.

The sample for this study included 633 podcast episodes from popular true crime podcasts, *Crime Junkie*, *The Vanished Podcast*, and *My Favorite Murder*, which reported on international missing persons cases.[4-6] This sample was reduced to include only United States cases, leaving 525 cases in the sample. These cases ranged from the year 1912 to 2024, and the ages of victims ranged from 1 month to 82 years old. Identifying demographic information from each missing person was gathered and compared to the NCIC 2022 data.[7]

Chi-squared goodness-of-fit tests were run to compare reports of race, X^2 (3, n = 525) = 139.628, p < .001, and sex, X^2 (1, n = 525) = 44.622, p < .001, from the United States cases from all podcast reports and the NCIC report. Results were significant and congruent with our hypothesis that Missing White Woman Syndrome would be present within our sample, despite collecting international cases and narrowing our focus to only the United States. White individuals accounted for 78.8% of the cases covered in comparison to 53.79% of the NCIC data. Black individuals were underrepresented, with only 10.7% of the cases including Black missing persons in comparison to 31.18% of the NCIC data. Regarding sex, males were also underrepresented, with only 41.1% of the cases covered involving male missing persons, in comparison to 55.61% of the NCIC data. Females were overrepresented, with 62.9% of the cases involving female missing persons in comparison to 44.37% of the NCIC data.

These results suggest that disparities exist in true crime podcast coverage of non-White [EB1] missing persons. Similar disparities exist for male and transgender missing persons. There were two transgender female United States cases covered in the podcasts but not included in this data set because there was not a category for comparison in the NCIC report. For ethnicity, the NCIC data report also ignores salient details such as a specific category for Hispanic- and Latinx-identifying individuals. Government reporting agencies excluding essential identities can lead to further perpetration of underrepresentation. Should this proposal be accepted, more data will be gathered from other podcasts, such as *And Then They Were Gone*, *Missing Persons*, and *Unfound*, to provide evidence to further support these findings.[8-10]

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Missing White Woman Syndrome; Missing Persons; True Crime Podcasts

J20 Safe and Sound: A Retrospective Analysis of Deaths in Custody in Rome, Italy, From 2000 to 2024

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Learning Objectives: Attendees will learn more about the epidemiological factors that contribute to deaths in custody, specifically in Italy, and eventually compare our results with their previous knowledge. By recognizing an at-risk detainee, they can translate this new information into their everyday practice to accurately evaluate deaths in custody, both from a forensic psychiatry point of view and a forensic pathology perspective in the postmortem investigation.

Impact Statement: This presentation will impact the forensic science community by showing our experience in one of the largest cities in the world and identifying the most common risk factors associated with deaths in custody, which are relevant both from a forensic and a public health perspective.

Abstract Text: Based on the fundamental principles of the Italian Constitution, and safeguarded by international jurisprudence and soft laws, every individual is entitled to the right to be treated humanely and to have their life protected during detention, regardless of the circumstances under which imprisonment occurs.[1]

According to the literature, several factors can influence the health status of the detainees, both intrinsic to the environment of the prison and to the personal clinical history of the subjects.[2-5] Nonetheless, detainees tend to be more exposed to chronic diseases, such as mental illnesses, infectious diseases, and substance abuse disorders when compared to the general population. For this reason, the health of these subjects represents a challenge from a public health perspective and therefore requires specific protocols and measures to be applied to avoid medical malpractice claims.

In Italy, just like most world countries, the death of any individual that occurs while in the custody of the authorities (i.e., the police, or in prison) undergoes a medicolegal investigation.[6] This study aims to retrospectively analyze the mortality in the prisons of the metropolitan area of Rome to highlight the most frequently encountered issues in this population and provide an effective profile to recognize at-risk individuals.

From 2000 to 2024, a total of 62 deaths in custody for which all the records could be retrieved were collected. The postmortem investigation was carried out in the morgue of Policlinico Umberto I Hospital in Rome and included full autopsy, histological analysis, and toxicological reports. The deaths were classified into deaths due to natural causes, suicides, accidental deaths with positive toxicology, and homicides. The study population was entirely composed of male subjects, with an age range of 21-81 years. Regarding the manner of death, most individuals died of natural causes (47%), while 26% were suicides by hanging and another 26% had positive toxicological reports. Only one case of homicide by manual strangulation from another detainee was recorded.

Among the natural causes, heart diseases were the most common (65.5%), followed by cases of cerebral damage like stroke and spontaneous subarachnoid hemorrhages (10.3%), and tumors or septic states (24.2%). Additionally, almost 10% of the subjects were known HCV-positive patients. Among the acute cardiorespiratory failures with positive toxicology, the most commonly involved substances were therapeutic drugs, such as antidepressants and benzodiazepines, followed by methadone. Most of the subjects had a positive psychiatric history, most of them related to substance abuse or to a difficult family history. Therefore, the typical "victim of death in custody" is represented by an at-risk profile (i.e., a male subject, aged 21 to 30, with a history of psychiatric disorder, including substance-related ones, that might have been treated with drugs that may ultimately play a role in the death of the detainee).

The present study underlines the importance of guaranteeing prisoners a competent and suitable health service both from a clinical point of view, since we are dealing with deaths in subjects at risk for cardiovascular and metabolic diseases in polypharmacy, and from a psychiatric point of view. It therefore becomes essential to carry out a correct diagnostic approach and set up a correct therapeutic procedure for the treatment of physical and mental illnesses affecting the prison population in order to reduce the overall number of deaths in custody, as they represent a population at high risk of early death. Nonetheless, a thorough postmortem evaluation, with a complete autopsy and toxicological analysis is crucial both from a forensic perspective and for public health prevention purposes.

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In-Custody Death; Forensic Psychiatry; Forensic Investigation

J21 The Role of the Psychological Autopsy in the Analysis of a Case of Fatal Intoxication

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Learning Objectives: After attending this presentation, attendees will know how psychological autopsy plays a vital role in clarifying the causes of death in cases of suspicious deaths at home

Impact Statement: This presentation will impact the forensic science community by demonstrating the utility of differential diagnosis of lethal intoxication.

Abstract Text: Suicide is the third leading cause of mortality.[1] Every year, almost one million people die from suicide worldwide. Mental disorders, depression ,and substance abuse are present in 90% of cases. Alcohol, together with caffeine and nicotine, is among the most commonly abused substance in the world. According to the Italian National Institute of Statistics (ISTAT) data, every year 17,000 people die due to alcohol and related pathologies.[2] Ethyl alcohol enhances the GABA system, causing progressive behavioral changes, first with a disinhibiting effect, and then as a sedative effect up to the depression of the respiratory centers. The drugs used for the treatment of depression (benzodiazepines, antidepressants, neuroleptics) can interact synergistically with the effect of alcohol. We report the case of a middle-aged woman who died following acute intoxication from alcohol, benzodiazepines, and antidepressants who was found in bed in her home. A careful analysis of the clinical history and health documentation was carried out. A judicial inspection was performed. Photographic albums in the home were analyzed. External examination, autops,y and organ sampling for histopathological examination and biological fluids sampling for toxicological examination were carried out. A psychological autopsy was performed with a retrospective investigation into the victim's life and her psychological context. Interviews with family members, relatives, and health care personnel were performed. The investigators asked about the woman's habits, whether she had undergone psychiatric checks, whether she had substance addictions, for how long, and whether she was correctly treated with adherence to therapy. Furthermore, the woman's relational context was evaluated regarding whether she had conflictual relationships with her family.

The woman was messy and in a poor hygienic condition. On the furniture, there were several blisters of anxiolytic and antidepressant drugs, some empty, others used. Under the bed, there was a photo album depicting scenes from the victim's wedding. The toxicological examination showed high levels of alcohol, benzodiazepines, and antidepressants. The psychological autopsy highlighted that the victim lived with her brother in an old, dilapidated house after her divorce from her husband, which occurred a few years earlier. Since then, she had been treated for major depressive disorder with psychotic manifestations, alcohol abuse, and intoxication from anxiolytics.

The discovery of the photo album, together with the data collected, allowed us to conclude that the death was due to acute voluntary intoxication mixing alcohol and psychotropic drugs. This case shows how the forensic pathologist has an important role, not only in carrying out the autopsy, but also for analysis of the circumstantial data found during the on-site inspection and psychological autopsy. Any data found during the inspection must be carefully analyzed to understand the nature of the event. The analysis of the wedding photo album opened near the body, although apparently of little importance, in reality assumed the role of a potential "suicide note" in the context of the suicide, considering that it suggested, as a circumstantial element, a plausible sentimental motive for the suicide. We therefore underline the importance of always carrying out the psychological autopsy investigation in these cases, through interviews but also through the search for circumstantial elements, present on the scene in the form of alternative suicide notes, which can offer information on the motive and dynamics for investigative purposes.

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Forensic Science; Intoxication; Suicide

J22 Forgotten Baby Syndrome: Society's Reaction to the Phenomenon Via a Presented Case

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Learning Objectives: In this presentation, society's approach to immigrants will be examined through the forgotten baby syndrome.

Impact Statement: This presentation will impact the forensic science community by discussing the forgotten baby syndrome, the cases encountered in Turkey, and the anger and aggression of the local people toward people coming to the country from abroad.

Abstract Text: Forgotten baby syndrome is a painful case that forensic experts all over the world encounter from time to time. In the case in question, a little child was forgotten in the car and the child who remained in the car on a hot summer day, died. There are studies in the literature on which age groups, which type of children, and which type of parents the cases in question are seen.

In Turkey, cases of childhood deaths due to children being forgotten in cars are occasionally encountered, especially on hot summer days. Among these, the cases of a 3-year-old boy in August 2022, a 4-year-old boy in July 2016, and a 4-year-old boy in June 2024 are noteworthy. There are debates around the world about whether the parents should be punished in such cases. However, no such debate has taken place in Turkey. When one of the cases in question is examined closely, it is seen that one of the deceased children and his parents were people who migrated from Syria to Turkey. This presentation will evaluate 130 comments made by Turkish citizens regarding the case in question. Below is a sample of those comments:

- Children are worthless because they have too many children
- They replace the dead child in the evenings
- The incident is actually murder, but they camouflaged it
- Syrians are not human
- I wish the mother had died
- Such mothers should not have children
- The child was saved instead of growing up in such a family
- The family must be punished
- This situation is medically possible
- I do not believe such an incident happened
- He could not bear to wake the child and did not realize what time it was
- What the Syrian did was not done by an animal

When these comments are examined, it becomes clear that there is a deep polarization between the local people in a country and those who have migrated to that country later, that the local people are angry with such newcomers, and that integration between the newcomers and the established population is not easy.

Turkey; Syrian Refugees; Forgotten Baby Syndrome

J23 General Self-Efficacy Belief as a Predictor of Job Satisfaction in the Crime Prevention Department: The Case of Addis Ababa Police

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Learning Objectives: The presentation attendees will learn the following main study findings:

- General Self-Efficacy Belief (GSEB) significantly predicts variance in job satisfaction. Moreover, among the two categories of job satisfaction, GSEB significantly predicts internal satisfaction rather than external satisfaction. The police chief should hire new officers based on their GSEB to have a police force that is a fit for professional roles.
- Training programs should be given to officers to improve their policing skills and mental resilience and increase their confidence in their ability to successfully execute the police role.

Impact Statement: I believe that my presentation impact forensic science community by guiding how to improve policing skills and mental resilience to successfully execute the police role in investigating complex crime cases.

Abstract Text: Problem: There are few studies worldwide that have tried to investigate that GSEB significantly predicts job satisfaction. However, most of the studies are conducted in a Western context, and there are few studies conducted in African setting that show the pattern of relationship between GSEB and job satisfaction. This study, therefore, aimed to test the effect of GSEB on job satisfaction of crime prevention officers in the Ethiopian context.

Approach: A cross-sectional correlational method was employed in this study. Using a simple random sampling, 240 crime prevention officers were selected and completed the survey. General Self Efficacy and a short version of Minnesota Job Satisfaction Survey instruments were used to collect data.

Findings: In the present study, the researcher failed to reject null hypothesis because the GSEB of crime prevention officers significantly predicted job satisfaction in Addis Ababa. Evidence from the present study is consistent with previous studies because there is a statistically significant relationship between GSEB and job satisfaction among crime prevention officers in Addis Ababa.3,4,7-11,13,22,23 Moreover, the present result is consistent with meta-analysis conducted by Judge and Bono, which discovered that self-efficacy exhibited a positive and significant correlation with job satisfaction (r = .45).9 In the present study, GSEB partially mediates the relationship between Self Efficacy and job satisfaction. This might account for work conditions, goal progress, and positive affect, which were found to mediate the relationship between self-efficacy belief with job satisfaction. Among the two job satisfaction categories, GSEB significantly predicted variance on internal job satisfaction than external satisfaction. This implies GSEB has a linkage with the ability to carry out police professional duties and responsibilities by coping with stressful situations. GSEB of Addis Ababa crime prevention officers significantly predicted variance on job satisfaction (β = 0.247, t (240) = 3.933, p =.000). Similarly, GSEB of officers significantly predicted internal job satisfaction (β =0 .307, t (240) = 4.954, p =.000). However, it has a non-significant prediction on external job satisfaction (β =.114, t(240) = 1.1770). p =.078).

Conclusion: The GSEB significantly predicts variance in job satisfaction. Moreover, among the two categories of job satisfaction, GSEB significantly predicts internal satisfaction rather than external satisfaction. The Addis Ababa Police Commission should hire new officers based on their GSEB to have a police force that is fit for professional roles. Training programs should be given to officers to improve their policing skills and mental resilience and increase their confidence in their ability to successfully execute the police role.

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General Self-Efficacy Belief; Job Satisfaction; Police

J24 The Role of Trauma in Shaping Antisocial Personality Disorder and Criminal Behavior Among Incarcerated Males

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Learning Objectives: Goals of this presentation are:

- **Understanding ASPD and Criminal Behavior:** Attendees will gain a comprehensive understanding of Antisocial Personality Disorder (ASPD) and its correlation with criminal behavior among incarcerated males.
- **Assessment Tools and Methodologies:** Participants will learn about the specific assessment tools and methodologies used in the study, including the Structured Clinical Interview for *DSM-IV* Dissociative Disorders (SCID-D), SCID's PTSD module, Dissociative Experiences Scale (DES), and Childhood Trauma Questionnaire-28 (CTQ-28).
- **Key Findings and Statistical Analysis:** Attendees will be introduced to the key findings of the study, focusing on the significant statistical differences between inmates with ASPD and those without, using chi-square and t-tests.
- **Impact of Trauma on Criminal Behavior:** Participants will explore how different types of childhood trauma, particularly physical neglect and abuse, are linked to the development of ASPD and subsequent criminal behavior.
- **Practical Implications for Forensic Settings:** The presentation will discuss the practical implications of these findings for forensic settings, including the development of targeted interventions and support mechanisms for individuals with ASPD.

Impact Statement: This presentation aims to contribute to the forensic science community by offering insights into the connection between trauma history and ASPD in incarcerated males. By examining how early trauma influences criminal behavior, the findings may help refine forensic evaluations and support the development of targeted interventions. Through the use of validated assessment tools and statistical analysis, this research provides a foundation for better understanding the complexities of ASPD. We hope this study will encourage further exploration and discussion, ultimately aiding in the improvement of prevention and rehabilitation efforts within the criminal justice system.

Abstract Text: This study investigates the relationship between ASPD, trauma history, and criminal behavior in a sample of 108 male inmates, convicted of various offenses including homicide (43), wounding (13), extortion (12), theft (8), and other miscellaneous crimes such as illicit drug abuse, political offenses, and traffic crashes. A psychiatrist conducted assessments for ASPD, using the SCID-D and the SCID's PTSD module. Additionally, participants completed the DES and the CTQ-28 as self-reports.

Results indicated significant differences between inmates diagnosed with ASPD and those without. Statistical analyses, employing chi-square and t-tests ($p< 0.05$), revealed that inmates with ASPD were younger, with a one-sided p-value of 0.026, and a mean age difference of 4.82 years. They also commenced criminal activities at a younger age, as indicated by a one-sided p-value of 0.011 and a mean difference of 5.93 years. The remaining time to serve in prison was longer for those with ASPD, with a one-sided p-value of 0.022 and a mean difference of 36.98 months. Furthermore, higher levels of physical neglect (one-sided $p=0.016$, mean difference=0.35) and physical abuse (one-sided $p=0.036$, mean difference=0.33) were reported among the ASPD group.

However, no significant differences were found in other types of childhood trauma, dissociative symptoms, type A trauma in adulthood, comorbid dissociative disorders, or family history of crime, physical, or psychiatric disorders. Notably, chi-square analysis revealed a significant difference in the commission of homicide, with inmates diagnosed with ASPD being more likely to have committed murder than their non-ASPD counterparts ($p=0.30$).

These findings suggest that the enactment of criminal behavior in individuals with ASPD can also be influenced by their physical childhood trauma history rather than solely by inherent traits. This study provides valuable insights into the behavioral patterns of antisocial criminals, highlighting the significance of early trauma and its impact on the timing and nature of criminal activities. Understanding these dynamics can inform the development of targeted interventions and support mechanisms for individuals with ASPD in forensic settings. This research sheds light on the complex interplay between trauma and antisocial behavior, offering a nuanced perspective on when and why individuals with ASPD engage in criminal acts.

Forensic Psychiatry; Traumatic Life Events; Murder

J25 Examining the Influence of Demographic Factors on Abuse Dynamics: Enhancing the Power Control Wheel Framework for Diverse Populations in VAWA Evaluations

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Learning Objectives: Attendees will learn how demographic variables such as gender, age, ethnicity/race, and socio-cultural background relate to the types of abuse experienced in intimate partner relationships. They will also gain insight into the intersectionality of these factors and how they compound vulnerability to various forms of abuse. This knowledge will enhance their ability to apply the Power Control Wheel (PCW) framework effectively in Violence Against Women Act (VAWA) evaluations.

Impact Statement: This presentation will enhance the forensic science community's competence in identifying and understanding the diverse experiences of abuse among different demographic groups. By offering empirically supported insights and integrating intersectional analysis into the use of the PCW, professionals will enhance their performance in conducting more accurate and culturally competent forensic evaluations. Ultimately, this will lead to better-informed intervention strategies and improved outcomes for individuals undergoing VAWA evaluations.

Abstract Text: The PCW is a tool that illustrates various tactics used to gain power and control over partners in abusive relationships, identifying physical, sexual, emotional, and economic abuse, as well as other controlling behaviors.[1] It provides a comprehensive framework for understanding these dynamics, particularly in VAWA evaluations, aiding professionals in recognizing and addressing different types of abuse effectively. Prior research indicates that the PCW, while valuable, may not fully capture the nuanced experiences of diverse populations, necessitating further exploration.[2,3]

The proposed study investigates the relationship between key demographic variables and other socio-cultural factors and the reported types of abuse, including physical, emotional, sexual, and economic abuse. The study seeks to identify differences in the prevalence of various forms of abuse among different demographic groups and to explore the intersectionality of these factors.

This study posits that demographic factors, including gender, age, ethnicity/race, nationality, and socio-cultural background, are significantly correlated with the types of abuse experienced in intimate partner relationships. Specifically, certain demographic groups are more likely to report specific types of abuse, reflecting the nuanced dynamics of power and control in diverse populations.

The study involved a diverse sample of 154 individuals who underwent psychological evaluations as part of their VAWA immigration cases, representing a broad population affected by abusive relationships. Data were collected through comprehensive psychological evaluations conducted by licensed forensic psychologists. Using the PCW framework, these evaluations assessed physical, sexual, emotional, and economic abuse as well as other controlling behaviors. Demographic information, such as key social and cultural factors, was gathered through structured interviews and standardized questionnaires. Descriptive data highlights the diversity of the sample and the prevalence of various types of abuse. Comparative analyses examined the relationships between demographic variables and types of abuse. Subsequently, logistic regression analyses were conducted to identify significant predictors of each type of abuse, considering the intersectionality of demographic factors. Interaction effects were explored to understand how combinations of demographic variables influence the experiences of abuse.

Preliminary findings indicate distinct patterns in abuse experiences based on gender, age, and cultural background with women reporting higher instances of emotional, sexual, and economic abuse compared to men. Younger individuals reported higher instances of physical abuse, while older individuals might report more economic and emotional abuse. The intersection of multiple demographic factors was a predictor of multiple forms of abuse, suggesting that overlapping social identities can compound vulnerability to abuse.

Overall, findings demonstrate the complexity of abusive dynamics and the importance of considering diverse demographic and intersectional factors in understanding and addressing intimate partner violence. Furthermore, the study has implications on enhancing the use of the PCW in VAWA evaluations, allowing professionals to recognize and address various forms of abuse more effectively. Understanding these variations can lead to more accurate evaluations and better-informed intervention strategies by providing a deeper insight into the diverse experiences of individuals in abusive relationships.

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Forensic Psychiatry; Immigration; Violence Against Women

J26 Medicolegal Ethics Associated With Access to Insanity Defense Reports

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Learning Objectives: The goals of this presentation are to: (1) explain the legal process in Maryland by which defendants plead Not Criminally Responsible (NCR); (2) explain the evaluation process in the state of Maryland; (3) explore differences among states regarding which parties have access to the NCR report (using a survey); and (4) explore medical and legal ethics associated with relevant parties having access to reports .

Impact Statement: This presentation will provide a nuanced discussion about criminal responsibility defenses while also exploring the medical and legal ethics associated with who has access to forensic reports and whether that access affects the fundamental fairness of trial proceedings. It will also provide a discussion of the role of forensic evaluators as operating for agents of the state versus agents for the defense.

Abstract Text: Criminal Responsibility (or "insanity") defenses are affirmative defenses chosen by the defendant. Maryland uses the American Law Institute (ALI) test, which states that a defendant is Not Criminally Responsible (NCR) if "as a result of a mental disease or defect, he lacks substantial capacity either to appreciate the criminality of his conduct or to conform his conduct to the requirements of the law."[1] When evaluations of criminal responsibility are conducted by a private expert hired by the defense, only the defense is privy to information obtained in that evaluation because it is protected via an extension of attorney-client privilege (*Pratt v. State*, *Harrison v. State*).[2,3] As a result, the prosecution and the finder(s) of fact are not privy to the results of that evaluation prior to a finding of guilt, unless the defense chooses to enter an NCR plea. This allows the defense to evaluate the utility of that evaluation without the prosecution being privy to potentially incriminating information.

In contrast, information obtained from evaluations conducted by a public expert is shared with the prosecution, the defense, and the judge prior to a finding of guilt. The defense does not have the opportunity to evaluate the risks and benefits of that potentially incriminating information before it is shared with the prosecution and the judge. This is true even if the defendant ultimately withdraws the plea of NCR.

We aim to examine various state laws and practices to understand how other states approach the issue of court-ordered evaluations and how these practices intersect with ethics in psychiatry and the law. After reviewing this presentation, attendees will understand: (1) how various states apply the insanity defense, (2) how those states manage the dissemination of insanity reports prior to a finding of guilt, and (3) principles of medical and legal ethics as they relate to criminal responsibility evaluations.

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Forensic Psychiatry; Ethics; Insanity

J27 A Novel and Implicit Measure of Self-Regulation: The Internal Moderation Questionnaire (IMQ)

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Learning Objectives: This presentation will present:

- Concept of Internal Moderation: Understand the theoretical basis and forensic implications of internal moderation as a self-regulatory mental mechanism
- IMQ Development, Reliability, and Validity: Gain insights into the methodologies used to create a novel questionnaire
- Forensic Applications: Discover how the IMQ can be used in forensic evaluations to uncover hidden truths and provide insights into criminal behavior.
- Future Research Directions: Explore the future research potential of the IMQ in identifying risk syndromes, assessing treatment responses, and predicting impulsive behaviors.

Impact Statement: The IMQ introduces a novel approach to forensic evaluations by implicitly measuring self-regulation, offering new insights into criminal behavior. This innovative tool enhances the accuracy of forensic assessments, identifies risk syndromes, and improves predictions of impulsive behaviors. By providing a reliable and valid method for uncovering hidden truths, the IMQ advances forensic science and contributes to more effective interventions.

Abstract Text: Internal moderation is suggested as a regulatory mental process that reduces the effect of conflicting inputs by producing a carefully adjusted internal response.[1] We conceptualized this inner process of response as successfully receiving an input and giving a dichotomous answer of turning internal mechanisms on or off. This study presents the IMQ, an innovative self-report tool developed to assess an individual's degree and manner of internal moderation, with potential applications in the field of forensic psychiatry and psychology, jurisprudence, and neuroscience. Based on the hypothesis, when there is a perception of stimulus, it prompts a reaction through both excitatory and inhibitory pathways. The IMQ seeks to detect these subtle self-regulatory reactions that may unveil concealed truths within the mind of a criminal.

The IMQ comprises inquiries regarding everyday routines and seemingly ordinary habits, such as whether an individual reserves the most enjoyable portion of a meal for the end or favors testing over speaking. These questions are intentionally designed to diverge significantly from conventional questioning methods, thereby diminishing the probability of receiving deceptive responses. During the development phase, an initial version of the IMQ was administered to 321 college students and 40 non-psychotic psychiatric outpatients. Additionally, the Dissociative Experiences Scale (DES), the Difficulties in Emotion Regulation Scale (DERS), and the Childhood Trauma Questionnaire (CTQ-33) were also administered. A test-retest examination was performed on a distinct cohort of 50 university students, with two administrations taking place over a period of seven days.

After conducting reliability and validity assessments, the IMQ was refined to a final version consisting of 28 items. The internal consistency of this version was found to be excellent. Strong associations were seen between IMQ scores and DES, DERS, and CTQ-33 total scores. The principal components analysis revealed a three-factor answer that represents the internal moderation modes of "dual," "on," and "off." The presence of these modes was linked to childhood adversity, dissociation, suicide attempts, and self-mutilation, demonstrating the questionnaire's ability to accurately detect and measure a range of psychiatric problems.

The initial results confirm the validity and reliability of the IMQ in a non-clinical sample of young adults and a convenient clinical sample. The findings indicate that the IMQ is capable of accurately assessing internal moderation patterns, offering a unique implicit measure of self-regulation. This metric has potential for forensic applications, potentially revealing concealed facts in the brains of criminals that conventional approaches could overlook.

Future study will investigate the IMQ's efficacy in larger and more diverse populations, with the goal of determining its importance in identifying prodromal risk syndromes, evaluating treatment outcomes, conducting forensic assessments, and predicting self-destructive and impulsive behaviors. The IMQ's capacity to generate veracious responses to harmless inquiries is an innovative approach in forensic psychology, providing a potent instrument for comprehending and evaluating criminal conduct.

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Decision Making; Forensic Psychiatry; Psychological Behavior

J28 Enhanced Trauma-Informed Policing: An Integrated Approach to Mental Health Safety in Law Enforcement Workplaces

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Learning Objectives: After attending this presentation, attendees will have an understanding of Enhanced Trauma-Informed Policing (ETIP) and its potential benefits to employees' health and wellness outcomes and resilience. After attending this presentation, attendees will also be able to appreciate the potential positive outcomes ETIP will have on police/community relations.

Impact Statement: This presentation will impact those in the forensic science community who are concerned about police reform and the ability of our police and public safety professionals to deliver services that are perceived to be organizationally and procedurally just.

Abstract Text: **Session Abstract.** ETIP provides a novel conceptual framework for police agencies by acknowledging and understanding that first-responders: (1) have disproportionate histories of Adverse Childhood Experiences (ACE); (2) are frequently exposed to secondary/vicarious trauma; (3) that historical trauma and other relevant lifetime experiences may have a negative impact on their mental and physiological health, work performance, and social functioning; and (4) are aware of the importance of strengthening their Emotional Intelligence (EI) abilities given its association with improved employee performance, better wellness outcomes, and increased resilience.[1-5]

Not often researched are the leadership skills that embody and promote trauma-informed practices, particularly in law enforcement agencies.[6-8] Organizations that are trauma Informed are perceived by its employees as being more organizationally just. A scaffolded trauma-informed approach that mobilizes skill- and perception-building at every rank can help agencies enhance officer engagement with the public. Notably, community policing, juvenile justice, and police reform advocates have highlighted Trauma-Informed Practices (TIP) in improving the level of trust between the police and the community.[9] TIP is a policing initiative that is perceived as being procedurally just, thereby giving the agency increased legitimacy.

Session Description: ETIP is introduced as a novel conceptual model of providing police services that infuses trauma-informed leadership skills and EI skill building to increase officer resilience, their perceptions of organizational justice, and reduce mental health issues. The ETIP approach specifically focuses on police officer mental health issues and behaviors, including adverse childhood experiences, secondary and vicarious trauma, suicide attempts, substance use disorders, and burnout. These traumatic events and circumstances include living at the poverty level, exposure (direct or secondary) to violence, exposure to racism, discrimination, homelessness, natural and man-made disasters, and pandemics. After attending this presentation, attendees will have a better understanding of ETIP and the potential benefits to employees' health and wellness outcomes and resilience.

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Enhanced Trauma-Informed Policing; Emotional Intelligence; Adverse Childhood Experience

J29 The Relevance of Ethnopsychiatric and Clinical Forensic Reports in the Medical Assessment of Asylum Seekers in the Metropolitan City of Milan, Italy

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Learning Objectives: Through this presentation, attendees will learn a better understanding of the potential role of ethnopsychiatric and clinical forensic reports in the asylum application process. This objective addresses the audience on how these reports influence the decisions of the Territorial Commission. Also, the attendees' knowledge of psychiatric disorders, particularly complex Post-Traumatic Stress Disorder (PTSD) and other trauma-related conditions, in migrant populations will be improved. Finally, the presentation aims at equipping attendees with the necessary skills to collaborate within a multidisciplinary framework involving ethnopsychiatry and clinical forensic medicine in order to effectively integrate diverse professional insights and, subsequently, improve patient outcomes.

Impact Statement: This presentation will significantly impact the forensic science community by highlighting the critical importance of integrating ethnopsychiatry and clinical forensic medicine in the asylum application process. It underscores the necessity of multidisciplinary approaches to effectively support asylum seekers with trauma histories, advocating for systemic changes that enhance the quality of care and protection offered to vulnerable migrant populations. By disseminating this knowledge, the presentation aims to improve professional practices and policymaking, ultimately fostering a more appropriate response to asylum seekers' needs.

Abstract Text: Asylum seekers and refugees are at an increased risk of developing psychiatric disorders due to the impact of various stressors present during the migration journey, including the legal status and access to services in the country of arrival.[1-3] Data show the influence that forensic reports have on the type of protection granted to asylum seekers, but there is still no evidence on the role that psychiatric reports can have together with the forensic ones.[4]

In the present research, 46 patients of the Ethnopsychiatry Service of Niguarda Hospital were analyzed. They were interviewed for their asylum application between January 1, 2018, and December 31, 2023. Among these patients, 13 also underwent a medicolegal evaluation of scars and/or lesions at the Institute of Legal Medicine of the University of Milan to ascertain their compatibility with allegations of torture.

The elements of patients' history that showed a positive correlation with the type of protection granted were discrimination based on sexual orientation and/or gender identity (87.5% of asylum grant rate), gender-based violence (90.9% of asylum grant rate), and forced marriage (100% asylum grant rate). Disorders Specifically Associated with Stress were the most represented psychiatric diagnoses with Complex PTSD, an *International Classification of Disease, 11th edition* (*ICD-11*) diagnosis, being the most frequently diagnosed pathology. When PTSD and complex PTSD were taken together, their prevalence in males was almost identical to that among females. However, upon dividing the two diagnoses, it was observable that there was a higher prevalence of complex PTSD among females (78.6% of those with a Disease Associated with Stress had the complex form of PTSD) compared to males (43.5%). The patients that reported having transited through Libya were at higher risk of developing a form of PTSD (100%) with respect to those who didn't (72.7%). All the patients that underwent a medicolegal evaluation for torture were diagnosed with one between PTSD or complex PTSD. Asylum and subsidiary protection, the two best protection forms, appeared to increase in percentage when thermal injury was reported more than when there was sharp force injury, and even more so compared to when blunt force injury was mentioned. Out of the 13 patients who underwent a medicolegal evaluation, the conclusions, given according to the Istanbul Protocol, pointed out 4 (30.77%) consistent, 7 (53.85%) highly consistent, and 2 (15.38%) typical. The patients evaluated had an average of 11.5 signs of injuries each, from 4 lesions to a maximum of 33 lesions/scars in the same individual. The mean value of different types of trauma in the same individual was 2. With medical reports, only one of the 46 patients received denial. Thirteen of the patients had already applied for asylum in the past. Of these, 11, after being denied, appealed against the decision and two cases, instead, after some time from the denial received, decided to submit a subsequent asylum. In no case was the previous denial confirmed.

This work highlighted the utility that medical certifications can have in the asylum application process.[4] All the alleged victims of torture present with a form of PTSD, and these extremely fragile patients are at huge risk of retraumatization during the physical evaluation. Therefore, it is important that properly trained physicians interact with them; forensic pathologists expert on migration, torture, and PTSD should be involved. The example set by the city of Milan should be replicated throughout Italy and beyond, but it must also be protected and adequately funded.

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Asylum Seekers; Clinical Forensic Medicine; Ethnopsychiatry

J30 The Economical Cost of Forensic Mental Health: Are We Saving Some Money?

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Learning Objectives: The learning objectives are related to understand how a program dedicated to mentally ill offenders can have an impact long term for the individual, the society, and, overall, the taxpayers. It will show that it is important to further fight stigma to ensure that these supports are offered to people in need and at risk of reoffending.

Impact Statement: This presentation will make attendees aware of the cost dedicated to individuals with mental illness who are involved with the justice system. It will make them aware of the cost saving when adequate programs are in place and will show the importance of advocating for more money being spent in forensic mental health.

Abstract Text: Forensic mental health care is widely implemented to rehabilitate individuals who combine involvement with the criminal justice system and mental health issues. This multifaceted approach serves not only to ensure that anyone requiring mental health care benefits from it but also to mitigate inappropriate behavior and enhance community safety. By addressing the mental health needs of offenders, forensic mental health care aims to reduce recidivism rates and promote rehabilitation, ultimately benefiting society as a whole.

From an ethical, humanistic, and social perspective, forensic mental health care is essential. Ethically, it aligns with the principle of providing adequate care to all individuals, regardless of their legal status. Humanistically, it recognizes the inherent dignity and worth of every person, advocating for treatment rather than punishment for those with mental health issues. Socially, it acknowledges the broader impact of untreated mental health problems on communities, including increased crime rates and societal costs. However, the economic implications of these programs cannot be ignored. Implementing and maintaining forensic mental health services requires substantial financial investment, which can be a significant burden on public budgets.

This presentation will delve into three critical areas of forensic mental health: forensic psychiatry units, correctional units, and drug rehabilitation programs. Forensic psychiatry units are specialized hospital wards designed to assess and treat individuals with severe mental health issues within the criminal justice system. These units play a pivotal role in stabilizing patients, conducting risk assessments, and planning long-term care strategies that facilitate their reintegration into society. Correctional units, on the other hand, integrate mental health care within the prison environment. These units provide ongoing psychiatric care, counseling, and support services aimed at improving inmates' mental well-being and reducing the likelihood of reoffending upon release.

Drug rehabilitation programs within the forensic mental health framework address the dual challenges of substance abuse and mental health disorders. These programs offer comprehensive treatment plans that include medical detoxification, therapy, and support groups. By targeting the root causes of substance abuse, such programs not only aid in the recovery of individuals but also contribute to a decrease in drug-related crimes and associated social costs.

Despite the initial high costs of implementing these programs, the overall benefit is substantial, extending beyond the individuals directly involved to society at large. When decision-makers follow expert recommendations, understand best practices, and actively work to combat the stigma surrounding mental health, the positive outcomes become even more pronounced. For instance, reduced recidivism translates into lower incarceration rates and decreased law enforcement and judicial expenses. Additionally, rehabilitated individuals are more likely to become productive members of society, contributing to the economy rather than draining public resources.[1-3]

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Forensic Psychiatry; Cost; Stigma

J31 Electronic Health Records and the Forensic Examiner: Pitfalls and Implications

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Learning Objectives: The objectives of this presentation are two-fold. First, we will define the electronic medical record and describe its history and purpose from both a legislative as well as a clinical perspective. We will examine the ways in which electronic medical records can lead to inaccurate documentation of medical history and outcome. We will then apply this knowledge to the forensic psychiatric setting in order to understand how these records can and do influence our forensic opinions and eventually the outcomes of legal cases. Lawyers and non-psychiatrists will especially benefit from this presentation. Practicing forensic psychiatrists will gain a deeper understanding of how to carefully examine these records and use their contents and particularly errors to support or refute their psychiatric-legal opinions.

Impact Statement: This presentation will impact the forensic science community by identifying the areas in which Electronic Health Records (EHRs), which are legal documents, can influence the content and outcome of forensic psychiatric evaluations. As forensic psychiatrists we must be acutely aware of the legal requirements of the EHR as well as the ramifications of uncorrected errors and missing information. We cannot assume that an error is electronic and not medical, or vice versa. This gray area can lead to significant impacts on our forensic psychiatric evaluations and psychiatric-legal opinions. Non-psychiatrists, including psychologists, who are asked to review medical records must be aware of the dangers and misinterpretation of these records, and attorneys must be educated on the legal meaning of these records. This presentation will address all of these issues, which are critical for accurate and ethical forensic psychiatric evaluations.

Abstract Text: The EHR was originally called an Electronic Medical Record (EMR) and has been in existence in the United States since the 1960s in various forms. In 1996, the Health Insurance Portability and Accountability Act (HIPAA) legislated certain requirements for medical providers who billed their patients or insurance companies electronically. In 2004, President George W. Bush created the Office of the National Coordinator for Health Information Technology, which outlined a plan to ensure that most Americans had EHRs within the next ten years. In 2011, the Centers for Medicare and Medicaid Services (CMS) established requirements and incentive programs for EHRs and created the concept of meaningful use. Subsequently, the private insurance companies followed suit. Today, virtually all hospitals and most independent medical providers use some version of an EMR for documentation of medical history, treatment, and billing.

While the original plan to utilize an electronic system was meant to make medical information more easily accessible and understandable across various treatment settings, the current system is not centrally managed and providers and hospitals have a huge range of EHRs to choose from. Additionally, the way that records are kept and managed is inconsistent. When we look at the various platforms and how they actually work, we can see that the system is rife for problems, particularly in the context of the psychiatric-legal setting.

In this presentation, we will outline this history and then dive right into the nitty gritty of how these records are organized, utilized, and saved. We will examine the many areas in which mistakes can be made and then we will look at the many areas in which these areas impact the psychiatric-legal opinions that forensic evaluators generate based upon reliance on these records.

Is EHR technology really a tool for transformation? Or is it a form of tyranny—a requirement that defeats its own purpose and lays open vulnerabilities and errors that can cost patients their lives and result in huge civil issues? We will look at real-life examples and you, the audience, can decide.[1-3]

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Electronic Medical Records; EHR; Ethics

J32 Do Beliefs About Science Matter? Evidence of the CSI Effect in the United States

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Learning Objectives: Presentation attendees will learn about current evidence regarding the impact of forensic-themed media—dubbed the "CSI Effect"—in the United States. They will also learn about the current state of public perception of forensic science from recent national survey data.

Impact Statement: This presentation will impact the forensic science community by fostering a better understanding of public perception regarding forensic science. In turn, it will improve scientists' communication competency with public audiences.

Abstract Text: Public understanding of forensic science is paramount for a fair and just criminal justice system. Current evidence suggests public perception of forensic science may not be in line with current scientific consensus for a variety of forensic techniques.[1] Media consumption has been shown to influence public perception on a wide variety of issues relevant to the criminal justice system, including crime incident rates and law enforcement officers.[2-4] Given the demonstrated effect media consumption can have on public perception, it is reasonable to expect forensic-themed media to influence public perception of forensic science. This is especially true following the emergence and growing popularity of forensic-themed media over the past several decades.[5] Dubbed the CSI Effect, the potential influence of forensic-themed media has been described as a kind of pretrial publicity effect that purportedly distorts public perception of forensic evidence. Concern over the potential influence of the CSI Effect on American jurors is widespread among those working in the criminal justice system and has been discussed widely in the popular press.[6-9] However, there is not a broad scholarly consensus regarding the existence or ramifications of the CSI Effect.[10]

This study extends and improves upon our understanding of the CSI Effect in several ways. First, it controls for variables known to influence bias toward forensic science to better isolate the unique impact of forensic media consumption. Second, it accounts for both forensic media consumption rates *and* perceptions of forensic media accuracy, as both have been shown to influence bias toward forensic science. Third, it examines how epistemic beliefs about science influence forensic science bias above and beyond variables already identified by prior literature, which, to date, remains underexplored. Fourth and finally, it utilizes a large, national, and statistically representative sample of participants, allowing for more robust and reliable statistical analyses.

Results show strong statistical support for the CSI Effect and demonstrate that frequency of forensic media consumption and estimations of forensic media accuracy both positively predict forensic science bias. Results also show that epistemic beliefs about science offer additional and significant explanatory power for public bias toward forensic science. Recommendations and strategies for forensic scientists' communication with public audiences based on study results will be provided.

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Public Opinion; CSI Effect; Media

J33 Internet Gaming Disorder, Substance Addictions, and Criminal Behavior: An Exploratory Study Among Italian Adolescents

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WITHDRAWN

J34 The Potential Role of AI in the Process of Radicalization

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Learning Objectives: This presentation will allow attendees to learn about the new process of radicalization that we are witnessing today and the role that Artificial Intelligence (AI) may play in it. They will learn how comparative psychology models are used for the development of AI technologies and how these are being constantly improved to mimic human intelligence more and more. Finally, they will learn about the process of radicalization and how online platforms are used to recruit members of extremist groups.

Impact Statement: The content of this presentation can have an impact on the way AI is implemented in social media and online communities to counteract the radicalization process. Moreover, it can impact the way online content is analyzed, filtered, and deemed appropriate for young audiences. Finally, this presentation may impact the general perception of radicalization by explaining how the process has evolved throughout the years.

Abstract Text: In recent years, AI has grown significantly, aiming to simplify, accelerate, and streamline processes and to reduce human errors often caused by tiredness or distraction.[1] The machine learning process is based on a structure similar to the human cognitive process and developers have used these notions to refine their product.

Comparative psychology compares behavior, instinct, and the emotional dynamics of various animal species, including humans.[2] The same comparative methodology can be used to understand how AI can improve itself exponentially over time and how this could be exploited by extremist groups trying to recruit followers. Based on what we know about AI and about the structure of cognition, we present the dynamics that can be established between AI systems and young users, which can ultimately lead to influencing their behavior (indoctrination). Artificial systems can impact our understanding of the world in general and our cognitive activities; therefore, it is possible to use this technology to steer consumers toward specific products as much as it can be used to recruit followers for radicalized groups.[3]

The goal of this presentation is to illustrate the strategies that can be used for the radicalization of youths ,which we are likely to observe soon through AI technologies; we will also discuss real examples and communication markers that have been associated with radicalization and recruitment techniques.

Extreme ideological groups used to recruit new members through action policies established by their organization. Nowadays, youth radicalization has changed, and it often occurs through guided self-indoctrination. Currently, we are witnessing an online radicalization process that utilizes models with whom youths can identify. These online models are generic enough that they can virtually speak to all young people whose identity is still being formed. The content includes apparently neutral elements able to make everyone feel unique and feed their sense of belonging. These methodologies facilitate total involvement with the group without significant mental effort.

Games, online communities, and, more broadly, the web are commonly used to create an alter-ego.[4,5] This is generally modeled after a successful individual, who has overcome problems and who, despite them, was able to make it on their own.[6] This model allows people to become followers without feeling any guilt and with a complete lack of responsibility even when it comes to deviant behaviors, which are shared across the other members and become therefore "normalized." Gender stereotypes and other polarizing views are also subtly introduced by these, reinforcing the concept of absolute good vs. absolute evil; the latter in the context of radicalization is usually represented by Western civilization, and it is presented to the followers as an enemy to defeat, just like the alter-ego models defeated their own problems to become the successful individuals they are today.[7]

A person who has fought for their success is a powerful motivator for people who feel purposeless and frees them from isolation allowing them to become active in their life. Success is presented not as mere radicalization, but as the possibility to finally have resources to take care of their families and become the glorious hero we all may feel the need to be.

AI can be effectively used in online radicalization by creating a machine-learning process that recognizes users' responses to certain messages and stimuli and creates content accordingly to keep users engaged and subtly introduce easy-to-embrace extreme ideas.[8,9] The role of social networks and online communities in radicalization has already been noted.[10-12] In the long term, AI-created realities could facilitate indoctrination by reducing the human effort behind recruitment, eliminating the need for physical movement, reducing costs, and increasing their reach.

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Artificial Intelligence; Radicalization; Psychological Behavior

J35 A Review of Preteen Suicides: The Child Death Review Committee of Oakland County, Michigan

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Learning Objectives: After attending this presentation, attendees will learn about the demographic, social, psychiatric, personal, and familial characteristics of preteen suicides which occurred in Oakland County, MI.

Impact Statement: This presentation will impact the forensic community by providing information and experiences about preteen suicide from the perspective of a forensic psychiatric consultant to the Child Death Review (CDR) committee.

Abstract Text: Societies have an emotional investment in children and a sense of responsibility to ensure their well-being. When a child suicide occurs, there is a need to determine what caused the event and how it could have been prevented. Suicide in general is a function of several factors: socioeconomic, intrapersonal and interpersonal stress, cultural mores, social forces, and physical and mental disorders. At the level of the CDR committee, which by statutory function reviews cases of child suicides, accidental deaths, cases of undetermined manners of death, and homicides, a group of specialists convenes to discuss referred child deaths in a closed, confidential manner that is restricted from public view.

Recent research indicates that the suicide rate for preteen children in the United States has increased in the past several years, increased more in girls than boys, and with increased use of a firearm as lethal means.[1] Such troubling developments necessitate better understanding of suicide in this age group to foster preventative and educational efforts in the community, important goals of the collaborative CDR process. This effort is, however, hampered by the relative lack of knowledge of psychiatric and psychological concerns regarding preteen suicides, because the majority of young people who die by suicide have no prior psychiatric diagnosis, which suggests that mental health problems might be going missed or undiagnosed and, thus, untreated in some young persons.[2]

In summary, the interdisciplinary members of the CDR, which represent areas of medicine, law, social services, and law enforcement, endeavor to provide a more complete understanding of factors that contribute to child deaths. The presentation will review data obtained from index cases involving preteen children aged approximately eight to twelve years. By presenting data from specific preteen suicides cases, we intend to demonstrate how the interdisciplinary approach of the CDR process can improve measures to better understand and prevent preteen suicides.

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Preteen; Suicide; Interdisciplinary

J36 Pregnant Inmates: An Examination of Reproductive Justice in Correctional Settings

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Learning Objectives: After attending this presentation, attendees will understand: (1) an overview of the national prenatal and postpartum care guidelines; (2) the current federal and state codes and statutes regarding care for pregnant inmates; and (3) the need for standardized prenatal and postpartum care guidelines in correctional settings.

Impact Statement: This presentation will impact the forensic science community by: (1) focusing on the needs of a highly vulnerable population, and (2) highlighting areas of improvement regarding reproductive justice in correctional settings.

Abstract Text: In recent decades, women have seen a dramatic increase in incarceration rates, rising 5-6 times since 1980.[1] Additionally, most of these women are of reproductive age and many studies estimate that anywhere from 3-10% of these women are pregnant or recently postpartum at the time of incarceration.[2,3] Typically, the Centers for Disease Control and Prevention (CDC) tracks data on pregnant women for the United States general population annually, which includes both maternal, fetal, and neonatal outcomes.[4] However, incarceration status is not usually denoted, and there is no uniform method for tracking or collecting data on pregnancy outcomes in the incarcerated population.[5]

Under the 1976 U.S. Supreme Court landmark case of *Estelle v. Gamble*, it was established that incarcerated individuals have a constitutional right to receive medical care.[6] Further, access to prenatal care and reproductive counseling and screening services are considered a reproductive right in the United States. However, a preliminary review of federal and state codes and statutes revealed about half of the states do not have any published codes or statutes related to pregnant inmates at all. Moreover, only about one-third of the states have codes published establishing that some form of prenatal and postpartum care be provided for pregnant inmates. Without explicit guidelines in place or transparent monitoring in correctional settings, an already vulnerable population is at risk for poor outcomes. Could the states without any codes or statutes in place be at risk for potentially violating the eighth amendment by not ensuring access to proper maternal care in correctional settings? Equally, could correctional settings be leaving themselves susceptible to legal action if guidelines are not published?

The American College of Obstetricians and Gynecologists (ACOG) and the American Academy of Pediatrics (AAP) published their most recent prenatal guidelines in 2017, with recommendations regarding education, counseling, screening, tests and diagnostics, prenatal care, and postpartum care.[7] These guidelines are considered the gold standard for pregnant patients' care. In 2024, ACOG updated their 2011 guidelines on prenatal care specifically for pregnant inmates because women are more likely to have a history of physical or sexual abuse, mental illness, substance use disorders, and chronic medical conditions compared to the general non-incarcerated population.[3-5] These risk factors, in addition to being incarcerated, place the pregnant inmate and the child at higher risk for poor perinatal outcomes. For example, studies have found that pregnant inmates who received prenatal care were more likely to have sustained reductions in long-term recidivism rates compared to those who did not.[8]

The United States Department of Justice's Bureau of Justice Statistics recently published a study in January 2024 concluding it is feasible to collect data on maternal health from state prisons, the Bureau of Prisons, and local jails.[9] Furthermore, with recent attention toward reproductive rights nationwide, addressing this topic within incarcerated populations is necessary.

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Reproductive Justice; Pregnant Inmates; Medical Care Accessibility

J37 Crime and Brain Disorders: The Legal Maze of Neuroimaging Evidence

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Learning Objectives: A variety of brain diseases may affect personality and lead to significant behavioral alterations that may represent the first manifestation of the disease and also lead to the commission of illicit demeanors. In these cases, it is challenging to assess the degree to which the neurological disorder has "influenced" criminal behavior. Within the forensic context, this assessment is the mandatory prerequisite for establishing whether and to what degree a defendant may be responsible for his or her unlawful acting. Such an assessment may be challenging, as the mere neuroradiological evidence of a brain structural and/or functional alteration may not be sufficient to prove the causal link with the criminal act, especially in those individuals who retain a general condition of well-being and good cognitive functioning.

Impact Statement: Assessing the effective impact of a brain structural or functional alteration on the individual's mental capability may become even more challenging, as, in the majority of the cases, the expert's evaluation takes place several months, or even years, after the unlawful act. Documenting the presence of brain pathology in these cases, by also using imaging and functional studies, may not be sufficient to resolve the question. This may have social and legal consequences for the patient and their family, as well as for the whole community, which may be affected by the criminal act.

Abstract Text: It is well known that several brain disorders can cause cognitive impairment as well as personality and behavioral changes. These alterations may occur as the result of structural and/or metabolic changes in those regions of the brain, including frontal lobes, amygdala, and hypothalamus, that are involved in executive functions, self-awareness, emotional processing, language, and control of aggressive, social, and sexual behaviors.[1,2]

In addition to traumatic injuries or acute vascular events, whose effects may be more immediately evident, personality and behavioral changes may appear in the course of several neurodegenerative disorders, such as frontotemporal dementia, Alzheimer's disease, Parkinson's disease, vascular dementia, and Huntington's chorea as well as epilepsy and multiple sclerosis.[3-8] Furthermore, neoplastic formations or invasive brain surgery may cause dramatic personality and behavioral alterations.[9,10]

In some cases, behavioral changes represent the very first clinical manifestation of the disease, in the absence of any other detectable symptom or sign, and can also lead to illicit and criminal behavior.[11] Although such cases are not frequent, they still represent a major issue because of the social and legal consequences that may entail for the patients and their families as well as for the whole community.

Here is reported the case of a 76-year-old male gynecologist who was sued by a young female patient for sexual violence during a medical examination. The doctor did not have any premorbid medical history. Several months after the episode, he began to manifest symptoms of cognitive impairment, which prompted a neurological examination and led to further investigation with Magnetic Resonance Imaging (MRI). MRI scans revealed two meningiomas in the left frontal hemisphere, with mass effects and vasogenic edema on the surrounding brain parenchyma. Excision of both tumors led to the disappearance of the neurological symptoms.

From a forensic and legal perspective, the challenge is to determine, *ex post*, to what extent the meningiomas' mass effects compromised the individual capability to properly behave within such a sensitive situation, like that of a gynecological examination. The question, indeed, is to establish whether such a dramatic change in the doctor's behavior is to blame exclusively or predominantly on a meningioma-induced disinhibition and loss of critical judgment, or not. The mere subsequent detection of the tumors, even if documented by imaging studies, may not be sufficient to settle the issue.

As a matter of fact, in a recent study, different subjects who had committed an illegal act and who had a structural and/or functional brain lesion, demonstrated by neuroradiological examinations, received different verdicts: some of them were convicted, others were exonerated because of their mental insanity. However, upon comparison, all subjects had the same clinical characteristics, neural activation, and similar intellectual performance.[12]

This shows that neuroimaging and functional investigations alone do not have sufficient evidentiary weight to be considered more than a mere piece of documentation, yet. In such cases, the final verdict is still provided following an assessment carried out on a case-by-case basis and on the ability of the defendant's appointed experts and lawyers.

In conclusion, unlike all the other medical disciplines, at present (forensic) psychiatry still suffers from the lack of accepted and validated objective measures, with the consequence that forensic psychiatric assessments are, most of the time, highly subjective and controversial, with a dramatically different impact on the defendant's legal course. We propose that a multidisciplinary integrated approach that combines medical history, clinical evaluation, and neuroradiological and neuroscientific exams may reduce subjective variance, thus enabling forensic psychiatry to assume the same objective status as the remaining medical disciplines.

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Criminal Behavior; Imputability; Neuroimaging

J38 The Murder of Serena Mollicone: The Need for an Interdisciplinary Approach and the Use of Bayesian Network in a Cold Case

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Learning Objectives: Participants will be able to learn the importance of interdisciplinary work, applied to cold cases and complex murder cases, also through tools such as Bayesian networks and the analysis of the accuracy of testimony.

Impact Statement: After the presentation, participants will be able to learn the main steps of interdisciplinary work in murder cases through the description of the work carried out in an unsolved murder case in Italy.

Abstract Text: After attending this presentation, participants will understand the main stages of an interdisciplinary scientific work that, over the course of 14 years, has been used in a well-known unsolved Italian case involving the murder of a young woman.

The case refers to the murder of Serena Mollicone, who was found dead on June 3, 2001, in the Fonte Cupa grove, which is located 8km from Arce (Frosinone, Italy), where the victim lived together with her father and sister. The body was found lying on its back among the local vegetation, hidden by a large metal container. Whoever killed her had wrapped her head in a plastic bag, securing it with packing tape, and bound her hands and feet with the same type of tape and wire. Death was caused by mechanical asphyxiation.

All the technical and scientific investigations carried out over the years will be briefly discussed, alongside the reconstruction of the psychological profile of the victim and the probabilistic approach used in the case.

Scientific evidence as encountered in criminal cases is often incomplete. This leads to some uncertainty that forensic scientists have to deal with. Graphical methods, such as Bayesian networks, have been found to provide valuable assistance for the representation of the relationships among characteristics of interest in situations of uncertainty.

Bayesian networks were used to enable the correlation of the most significant testimonial contributions and the most relevant scientific evidence. By leveraging this interdependence through a mathematical inferential process, a probability has been derived that could provide significant answers to the murder of Serena Mollicone. Special emphasis will be placed on the accuracy of the various testimonies provided by a key witness in the case, who later committed suicide during the investigation.

The presentation aims to inform attendees about the importance of applying an interdisciplinary analytical strategy that fully leverages all available data. It will also highlight the significance of improving the use of Bayesian networks to provide the judge with objective scientific contributions. This approach is crucial in complex homicide cases, such as the one in question, to achieve a reliable and truthful reconstruction of the facts.

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Interdisciplinary Work; Bayesian Networks; Psychology of Testimony

K1 How to Do Less With More: Screening 101 Known Writers Down to a Manageable Number

Mark Goff, BA, Michigan State Police, Lansing, MI*

Learning Objectives: This presentation will examine a method for screening a large number of writers down to a manageable number before conducting a handwriting examination. Additionally, attendees of this presentation will be provided with information on how this relates to the recent American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 70, Standard for Examination of Handwritten Items 2022, and proposed language used to address examinations where screening is used.[1]

Impact Statement: This presentation will impact the forensic science community by increasing awareness of a method for managing a large number of known writers and providing results that are descriptive of this process.

Abstract Text: This presentation will illustrate a case study of threats written in a public area. Because of the nature of the school threats being in a public area, the pool of potential writers was large. In this case, the pool of potential writers included the entire female sophomore population of a high school. This amounted to 101 writers that needed to be compared to threats written on a bathroom wall in the girls' restroom. The ANSI/ASB Standard 70, Standard for Examination of Handwritten Items 2022, allows for the screening of large numbers of known writers. In this presentation, I will demonstrate how the writers were screened using characteristics that can quickly be identified and used to differentiate writers. The characteristics used were selected for ease of evaluation and typically consisted of a binary, yes/no decision regarding whether a writing characteristic was present in the body of the known writing or not.

Screening writers out of an examination removes them from a full comparison. Therefore, the language used to express results for an examination where screening is used needs to address the use of screening in the examination and the limitations associated with it. In this presentation, I will provide examples of the limitation language that can be used.

Additionally, this presentation will provide an overview of the ANSI/ASB Standard 70, Standard for Examination of Handwritten Items 2022, and other relevant standards relating screening known writers in handwriting examinations.

Reference:

1. ANSI/ASB Standard 70, *Standard for Examination of Handwritten Items* 2022.

Questioned Documents; Handwriting; Screening Methods

K2 A Comparison of Documents Written With Different Typewriters and a Determination of Which Typewriter the Document Subject to Examination Is Written From With Reasons

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WITHDRAWN

K3 Academy Standardization: Progress and Goals for Forensic Document Examination

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Learning Objectives: After attending this presentation, attendees will have knowledge of the history of the American Academy of Forensic Sciences Academy Standards Board (AAFS ASB) and its creation and an appreciation of the role that the Forensic Documentation Consensus Body (CB) plays in the creation of standards and a technical report. These standards represent experiences from different individuals from various educational backgrounds and even different countries.

Impact Statement: The impact to the forensic science community would be the benefit of learning the history of the ASB and the importance of the forensic document examination community's association with the ASB, an American National Standards Institute (ANSI) -approved organization. Younger examiners will learn how they can contribute to the ASB process.

Abstract Text: The forensic document examination community has been involved in the creation of standards and terminologies since the late 1990s, first with the creation of the Technical Working Group on Digital Evidence (TWGDOC), which reorganized as **Scientific Working Group** for Forensic **Document Examination (**SWGDOC), sponsored by the Federal Bureau of Investigation (FBI). During this time, the Questioned Document Subcommittee of the American Society of Testing and Materials (ASTM) was created, and this resulted in over 20 published Forensic Document Examiner (FDE) standards.

In 2015, the AAFS Board of Directors approved the creation of the ASB and in that same year, the program received accreditation by the ANSI, an International Organization for Standardization (ISO) member body. The Forensic Document Examination Consensus Body (FDE CB) was then formed and proceeded to update the previously published standards.

This presentation will relay the progress of the FDE CB and will provide some of the ways in which the CB has relied on the community in improving and updating the methods that those in the discipline use every day. One important goal of the body has been to create a terminology, which is classified as a technical report in the ASB. Through the work of a working group of four members, a thorough compendium of terms has been developed and approved by the CB. The CB is currently reviewing public comments about the terms, and it is hoped that it will be published by the time of the presentation. Complete with images, the technical report covers all aspects of forensic document examination, and, through thoughtful comments, the working group feels confident that the final product will provide a valuable resource for all document examiners in their daily work.

Contributions to the standardization process by the forensic document examiner community have been realized for almost 30 years, and the results have created foundational works that will stand the test of time though the ASB development and review processes.

Questioned Documents; Standardization; Terminology

K4 An Introduction to the Forensic Handwriting Analysis Software Handwriter

Stephanie Reinders, PhD, CSAFE, Ames, IA; Alicia L. Carriquiry, PhD, Iowa State University, Ames, IA; Danica M. Ommen, PhD, Iowa State University, Ames, IA*

Learning Objectives: After attending this presentation, attendees will have a better understanding of the Center for Statistics and Applications in Forensic Evidence (CSAFE) handwriting analysis software called handwriter. They will learn how to use it and its capabilities and limitations.

Impact Statement: This presentation will impact the forensic science community by showing them how to install and use handwriter. Attendees will learn about the current capabilities of handwriter and future directions for the software.

Abstract Text: The CSAFE created free, open-source handwriting analysis software called handwriter to aid forensic document examiners. The software is accessible through a downloadable user interface or as an R package for the advanced user.

The handwriter software compares a questioned handwritten document Q to handwriting samples from a *closed set* of potential writers or *Persons of Interest (POIs)*. A closed set refers to the case where Q had to have been written by one of the POIs, as opposed to the open set case where Q might have been written by one of the POIs or it might have been written by someone else. The software imports scans of handwritten documents and splits the writing into component shapes called *graphs*.[1] Graphs capture shapes, not necessarily individual letters. Graphs might be a part of a letter or contain parts of multiple letters. The software compares graphs by looking at the number of edges, vertices, and loops, as well as the angle and the distances between parts of the shapes.[2]

The key idea behind the software is that the rate at which a writer produces different types of graphs serves as an estimate of a *writer profile*. The software applies a statistical model called a Bayesian hierarchical model to estimate the writer profiles of the POIs from known writing samples collected from them. The statistical model is used to calculate the posterior probability that each POI wrote Q. For example, handwriter enables the user to make a statement like, "The posterior probability that writer C wrote the questioned document is 98%." In the closed set scenario, it is possible to toggle between a likelihood ratio and the posterior odds of writership. If there are N potential writers in the closed set, the prior probability of writership for any potential writer would be 1/N, unless other information is available. If p C is the posterior probability that C wrote Q, and p D is the posterior probability that D wrote Q, then the LR for C relative to D is just the ratio of p C/p D because the prior probabilities cancel out. If writer C has the highest posterior probability of writership of Q, then every pairwise LR comparing C to all other potential writers will be larger than 1.

The presentation will show how to download handwriter and demonstrate the software on handwriting samples from the CSAFE Handwriting database.[3] Current capabilities and limitations of the software will be presented as well as plans for expanding the software to address the *open-set* case and analysis of signatures.

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Handwriting; Statistics; Software

K5 Analyzing Toner Particle Distribution to Sequence Ink Lines and Laser Printing

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Learning Objectives: Attendees will gain a basic understanding of toner particle distribution analysis and its relationship to sequencing non-intersecting pen lines and laser printing.

Impact Statement: This research and presentation will impact the Forensic Document Examination community by detailing the trial of a system that addresses current and past issues in document examination and provides a quick, cost-effective, and simple analysis method and uses low-cost equipment for preliminary assessments that can be done before more in-depth investigations.

Abstract Text: This study presents an innovative approach to sequencing non-intersecting pen lines and laser printer writings through toner particle distribution analysis under infrared imaging. Building on previous research on sequence determination of electrophotographic print and handwritten entries, we re-evaluated the process using a statistical and Bayesian approach instead of a deterministic one. Our system analyzes images to detect differences in toner density, validating previous conclusions while exploring additional variables, such as reprinting and indentation effects.

Traditional methods for determining the sequence of writing involved microscopic observation of toner particle morphology, which is time-consuming and requires expensive equipment. In contrast, our approach uses a low-cost Near-Infrared (NIR) microscope at 200x magnification, allowing for quick and easy observation with a computer. This method is fast, cost-effective, and straightforward, providing clear numerical data that can be easily explained and used to calculate probabilities while still being easy to explain at court.

Our study demonstrates that it is possible to distinguish the sequence between writing and printing, evaluate hypotheses without known samples, and potentially automate the measurement process. We also explored factors such as reprinting effects, the orientation of writing, and the sampling area on the paper. Our results show that this method can provide reliable preliminary assessments before more in-depth investigations, making it a valuable tool for forensic document examination. Recommendations are provided for the practical application of this method, emphasizing the benefits of using the questioned equipment, the importance of capturing multiple images for analysis, and the recommended number of samples considering the level of confidence you would prefer by following a simple sample equation.

Field testing this project using a defined methodology with printers and paper from various global sources could solidify its validity. By systematically analyzing toner particle distribution across diverse conditions, we can ensure the method's robustness and reliability. This involves standardizing the printing procedure using specific word documents and recommending consistent pen types for writing. Such a comprehensive approach allows for the identification of potential variables and inconsistencies, leading to refined protocols. Ultimately, this extensive testing will enhance the method's applicability and credibility in forensic science, ensuring accurate sequence determination in a wide range of real-world scenarios.

Toner; Ink Line; Questioned Documents

K6 An Overview and Comparison of Software Tools for Quantifying the Value of Handwriting Evidence

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Learning Objectives: Attendees will understand the differences between the currently available software tools for handwriting and learn where they can go to find out more information about these tools for assisting examiners in the objective analysis of handwriting.

Impact Statement: The forensic document examiner community will have the opportunity to test, evaluate, and improve the next generation of software tools for writer analysis of and quantification of evidential value for handwritten materials.

Abstract Text: For years, reviewers of the forensic science system have implored researchers in the community to develop tools for objective quantification of evidential value. In 2009, the National Research Council (NRC) issued a Report saying, "In most forensic science disciplines, no studies have been conducted of large populations to establish the uniqueness of marks or features. Yet, despite the lack of a statistical foundation, examiners make probabilistic claims based on their experience. A statistical framework that allows quantification of these claims is greatly needed."[1] In 2016, the **President's Council of Advisors on Science and Technology** (PCAST) released a Report advocating for the "improvement of current forensic methods, including converting subjective methods into objective methods," for all feature-comparison disciplines.[2] Luckily, the questioned document community was ahead of the game. *FISH* was developed in the 1990s, and *CEDAR-FOX* and *FlashID* were developed shortly thereafter in the early 2000s.[3-8] More recently, CSAFE developed the *handwriter* tool.[9,10] The main goal of each of these programs is to aid the examiner toward a more objective analysis of handwritten materials. Unfortunately, *FISH* is used exclusively within the German BKA, the Dutch NFI, and the United States Secret Service. Similarly, *FlashID* was developed in conjunction with and used most prominently by the FBI. *FlashID* is licensed, sold, and maintained by Sciometrics. *CEDAR-FOX* appears to have a broader range of users, but is, sadly, no longer maintained by the Center of Excellence for Document Analysis and Recognition at the University of Buffalo due to the passing of its pioneer, Professor Srihari. Both *CEDAR-FOX* and *FlashID* utilize patent-protected, proprietary algorithms.

The goal of *handwriter* is to provide examiners with a free, open-source handwriting tool for writer analysis. In 2021, the National Institute of Standards and Technology (NIST) Expert Working Group for Human Factors in Handwriting Examination published their report recommending that "the forensic document examiner community should collaborate with the computer science and engineering communities to develop and validate applicable, user-friendly automated systems."[11] CSAFE plans to enlist examiners to pilot test its *handwriter* tool with the aims to improve its user interface and applicability to casework. Furthermore, CSAFE hopes to compare *handwriter*'s performance with the other available software tools and on a wider range of writing samples.

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Software; Handwriting; Statistics

K7 An Update on Standards Development Activities Related to Forensic Document Examination

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Learning Objectives: 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.

Abstract Text: During this presentation, an update will be provided on the standards development activities related to FDE. 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.

Fourth, this presentation will discuss completed ASB Standards. As of the writing of this abstract, the ASB has published eight Forensic Document Examination Standards, including 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, and the Standard for Examination of Mechanical Checkwriters and Their Impressions. The ASB Forensic Document Examination Consensus Body is also currently working on seven 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; Questioned Documents; OSAC and ASB

K8 Current Status and Future Trends: Computer Science and AI in Forensic Handwriting Casework

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Learning Objectives: In this presentation, participants will learn about the extensive work conducted to date by a group of forensic document examiners collaborating with Pattern Recognition (PR) experts with the aim of automating handwriting comparisons. Past and present work will be reviewed, and future projects outlined.

Impact Statement: This presentation will provide attendees with information about previous and ongoing work between the forensic handwriting and the computer science research communities to address needs within the forensic handwriting experts discipline with respect to the use of automated handwriting comparison and identification systems.

Abstract Text: This presentation will show an overview of the various computer science competitions on signature verification and writer identification that were conducted using realistic forensic casework materials. The competitions were organized in unique collaboration between computer/PR scientists and Forensic Handwriting Experts (FHEs). The aim was to bridge the gap between technological developments of the time and forensic casework.

In the early years (2007-2010), pre-conference tutorials were provided at PR conferences in order to reach out to the computer scientist community to inform about the field of FHE, our methods, decision-making processes, and conclusions.[1-3] The future needs of FHEs were advocated, specifically to objectify and verify the subjectively formed interpretations of the comparison process that lead to a range of conclusions about the authorship of disputed handwriting or signatures. Realistic casework scenarios were presented in tutorials during which PR scientists examined representative casework-related samples to show the complexity of handwriting comparison work and how conclusions are drawn by FHEs.

In successive conference years of the International Conference on Document Analysis and Recognition (ICDAR): 2009, 2011, 2013, 2015, with changing compositions of a large number of authors, new test and training sets in either signatures or handwritten text were provided on Dutch, Chinese, Japanese, Bengali, Italian, and English, collected mainly by FHEs.[4-9]

The major aim of all tutorial and competition work was to bring the fields of PR and FHE closer together, borrowing on technological developments and applying them to forensic casework. Up until that point, the field of automated signature verification and writer identification had progressed without the benefit of FHE expertise and so could not and did not take the real needs of FHEs into account. FHE casework is comprised of handwriting and/or signatures produced in various real-world environments. These traces are more difficult to analyze compared to the texts produced in controlled environments for PR research. Adding to the complexity of our casework is that real work requires the FHE to consider simulations and disguise. These competitions introduced PRs to the questions asked of FHE during casework. Results of all competitions indicated that automated methods might be able to support FHEs in their formulation of the strength of evidence to be reported in court.

Another important aspect of this work that has not yet been highlighted is the usability and application of automated handwriting identification/ranking systems in real forensic casework. This next step will be the subject of future work—to evaluate the usability of these automated systems in coming ICDAR or other computer science competitions.

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6. 2010: *Competition 4NSigComp2010: Forensic Signature Verification Competition* at the 12th ICFHR, Kolkata, India.
7. 2011: *First International Workshop on Automated Forensic Handwriting Analysis (AFHA2011)* at the 11th International Conference on Document Analysis and Recognition (ICDAR), Beijing, China.
8. 2011: *Competition SigComp2011: Signature Verification Competition for On- and Offline Skilled Forgeries* at the 11th ICDAR, Beijing, China.
9. 2013: *Competitions on Signature Verification and Writer Identification for On- and Offline Skilled Forgeries (SigWiComp2013)* at the 12th ICDAR, Washington, DC, USA.

Handwriting; Automated; Comparison

K9 Blocked Photographs: An Exploration of Methods of Separating Photographs Adhered as a Mass by Moisture

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Learning Objectives: This presentation will examine methods of separating photographs that have been exposed to liquid and adhered as a mass when dried. Additionally, attendees of this presentation will be provided with information on how this relates to the recent Organization of Scientific Area Committees (OSAC) Registry entry American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard for the Preservation and Examination of Liquid-Soaked Documents, 2022, and how to preserve and report the results of this examination.[1]

Impact Statement: This presentation will impact the forensic science community by increasing awareness of a rare examination request that is increasing in frequency with the increased interest in cold case investigations. Forensic Document Examiners (FDEs) will have a resource to use when conducting examinations on dried, liquid-damaged photographs.

Abstract Text: With the advent of new forensic examination techniques, students, legal professionals, and investigators are increasingly looking into previously unsolved cases or cases that warrant a re-evaluation. Many of these cases date back to the 1970s, 1980s, and 1990s when crime scenes and criminal investigations were documented with physical photography. Through improper storage, or accidental exposure to moisture or liquids, some of these photographs can end up blocked as a solid mass. Separation of these photographs, while minimizing the loss of the images contained therein, is important to preserve evidence in cold case investigations. This presentation will examine multiple methods of separating photographs that have dried together. These methods can vary based upon the type of photography, black and white or color, and the substrate the photograph was recorded on.

Many of these photographs may be damaged during separation but the image information can be reassembled from the image fragments using image editing software. Caution needs to be exercised when reassembling these images and reporting the results to ensure that the photograph image is not altered from the original image or any alterations to the image to minimize damage are properly reported with the appropriate limitations. To this end, this presentation will provide examples of reassembled photographs and their relevant limitations.

Additionally, this presentation will provide an overview of the ANSI/ASB Standard for the Preservation and Examination of Liquid-Soaked Documents, 2022, and other relevant standards relating to the recovery of damaged photographs.[1]

Reference:

1. ANSI/ASB *Standard for the Preservation and Examination of Liquid-Soaked Documents*, 2022.

Questioned Documents; Photography; Cold Case

K10 Defining Contemporaneous(ly)

Meg OBrien, MS, United States Secret Service, Ashburn, VA*

Learning Objectives: After attending this presentation, attendees will have an understanding of the definitions used by document examiners for "contemporaneous" examination notes and "contemporaneous" dates of handwritten documents submitted for examination and comparison.

Impact Statement: This presentation will impact the forensic science community by presenting on definition(s) of "contemporaneous" used by forensic document examiners in reference to: (1) examination notes, and (2) dates of handwritten document submitted for examination and comparison.

Abstract Text: Forensic Document Examiners (FDEs) were polled to determine whether or not there is a consensus on the use of "contemporaneous" when used in a variety of ways (contemporaneously, contemporaneousness, contemporaneous); in particular, the three of the four uses and variations of "contemporaneous" in American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 070, *Standard for Examination of Handwritten Items*.[1]

This presentation reviews the use of "contemporaneous" in ANSI/ASB Standard 070 *Standard for Examination of Handwritten Items*, the content of the poll questions, and the demographic information of the FDE respondents. The qualitative data collected from the poll results and the resulting themes from the responses of the FDEs will be presented. Anonymity of respondents was a condition provided in the poll.

Twenty-four forensic document examiners responded to a two-question poll in January and February 2024. The first question posed was, "Does the definition of 'contemporaneous(ly)' mean the same thing to you when applied to: (1) examination notes, and (2) documents submitted for examination, per the statements from ASB Standard 070?" The second poll question was, "What is the time gap between writings that you consider to be a 'lack of contemporaneousness' that could hamper an examination or result?"

ANSI/ASB Standard 070, *Standard for Examination of Handwritten Items,* was included as an attachment to the poll for reference, if needed, for the respondents.

Respondents were also asked to provide their years' of experience as an FDE and their use of ANSI/ASB Standard 070, *Standard for Examination of Handwritten Items,* (does use ANSI/ASB Standard 70 in whole, does use ANSI/ASB Standard 70 in part, does use ANSI/ASB Standard 70 as a guidance document, does not use ANSI/ASB Standard 70). Responses were submitted in short-answer or essay format. Responses were compiled for review and identifying information of the respondent was deleted. Three themes emerged from Question 1 answers and four themes emerged from Question 2 answers.

Reference:

1. ANSI/ASB Standard 070, *Standard for Examination of Handwritten Items*.

Questioned Documents; Contemporaneous; Handwriting

K11 Definitive Conclusions: Should They Be Defenestrated?

Linton Mohammed, PhD, Forensic Science Consultants, Inc., Poway, CA*

Learning Objectives: This presentation discusses the use and strength of conclusions in handwriting examination based on current scales. Attendees will be made aware of different conclusion scales, and information will be presented on weaknesses in the scales. **Impact Statement**: This presentation will enable the forensic science community to be aware of conclusion scales used in forensic handwriting examination and possible issues with definitive levels of conclusions.

Abstract Text: In 1991, McAlexander, Dick, and Beck published a paper in which they described nine levels of conclusions that Forensic Document Examiners (FDEs) can express.[1] The paper formed the basis for the **Scientific Working Group** for Forensic **Document Examination (**SWGDOC) Standard Terminology for Expressing Conclusions of Forensic Document Examiners, which has been used by FDEs in North America and other parts of the world for many years. A lab may use 9, 7, or 5 levels, depending on its policy.

In Europe, the European Network of Forensic Science Institutes (ENFSI) proposed a framework for evaluative reporting in which the strength of support for one proposition was stated against the strength of support for a competing proposition. This can be expressed in the form of a likelihood ratio.[2] In the ENFSI guideline, no definitive conclusion is expressed.

This paper is concerned with handwriting and signature examinations only. Definitive conclusions in non-handwriting examinations can certainly be made. For example, this inkjet printer did not print this toner-printed document.

Signatures and handwriting have a very great issue of variation. To determine whether an individual did not write a questioned signature or handwritten entries, the examiner must be certain that there are differences between the questioned and specimen sets that cannot be reasonably explained. To identify a writer, the examiner must be certain that the specimen set is an excellent representation of the writer and that the writing is of such complexity that the possibility of simulation can be ignored.

To say definitively, in the case of signatures, for example, that an individual wrote a signature, the examiner has to discount completely the possibility of a skillful simulation or a machine-generated signature. Dewhurst, et al. have shown that there are skillful simulators that can fool handwriting examiners.[3] For the purposes of this presentation, original documents will be assumed so the copy/cut and paste option can be ignored.

To say definitively that an individual did not write a signature, the examiner must be certain that the specimen set covers the writer's range of variation. The examiner has to exclude the possibility of alternate signature styles. Huber and Headrick list 20 sources of variation that the examiner should consider.[4] Can the examiner really assess all 20 of these possibilities?

Is it time for examiners who currently use the SWGDOC Conclusions standard to defenestrate the identification and elimination levels?

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Forensic Science; Document Analysis; Scientific Evidence

K12 Distinguishing Dominant and Non-Dominant Handwriting Using General and Individual Characteristics

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Learning Objectives: After attending this presentation, the participants will gain knowledge about differences in handwriting characteristics when writing with the dominant and non-dominant hands. Participants will get insight into the potential application of statistical models when identifying text written by a non-dominant hand.

Impact Statement: This presentation will contribute to forensic handwriting examiners by expanding their knowledge in identifying disguised handwriting and attributing importance of particular class and individual characteristics while examining handwriting samples written by the non-dominant hand. By implementing the statistical model developed in this study, forensic experts can improve their performance in identifying non-dominant handwriting, leading to more evidence-based decisions.

Abstract Text: Handwriting is a relatively stable and individualized trait, directly resulting from its automatization after years of practice. For this reason, individuals sometimes attempt to disguise their identity by writing with their non-dominant hand when planning and committing criminal activities (e.g., fraud, threats, anonymous letters, etc.). While such handwriting is less controlled and skillful, it still maintains its individuality, often enabling forensic experts to identify the writer.[1,2]

However, in the forensic examination of such samples, it is crucial for a handwriting expert to recognize that such an attempt might be made so that an adequate analytical approach could be employed. Therefore, the present study aimed to explore within-individual differences in class and individual characteristics when writing with the dominant and non-dominant hand and to develop a statistical model that can identify handwriting written by the non-dominant hand.

The study included 94 adult right-handed participants from Croatia and Bosnia and Herzegovina who were asked to copy the sentence ("Ništa veliko neće biti postignuto bez velikih ljudi, a čovjek može biti veliki samo ukoliko je odlučan da to i postane.") twice, first using their dominant and then their non-dominant hand. The handwritings were analyzed for 13 general and 18 individual characteristics.[3-5]

Differences between the characteristics coded by categorical scores were compared using McNemar's test. For the characteristics defined by clock positions, the values were converted to angles and radians and compared using a paired samples t-test. To develop a classification model for distinguishing dominant and non-dominant handwriting, the two samples of the same participants were treated independently, thus forming the sample of 188 texts. The sample was split into the training (70%) and the testing set (30%), while 5-fold cross-validation was employed to ensure the model's robustness. The classification model was developed using logistic regression in conjunction with a recursive feature elimination algorithm that selected the optimal number and combination of variables among all class and individual characteristics that could be scored in all individuals.

In total, 7/13 class characteristics showed statistically significant differences between dominant and non-dominant writing ($P < 0.05$), which included overall handwriting quality, legibility, neatness, spacing, and fluency and dynamic of writing. Among 18 individual characteristics, only 5 variables showed significant differences ($P < 0.05$): t higher cross stroke, e loop, n arched, s loop, and o angle. In the classification model, the optimal combination of variables (handwriting quality legibility, neatness, and fluency and dynamic of writing) had an accuracy of 0.89 in cross-validation and 0.91 in the test set. The achieved Positive Predictive Value (PPV) of 0.85 indicates that when the model predicts handwriting as non-dominant, there is an 85% chance that this is correct. The Negative Predictive Value (NPV) of 1.00 suggests that when the model predicts handwriting as dominant, it is correct in all cases.

The statistical tests and relatively accurate classification model imply that class characteristics might be sufficient for distinguishing handwriting produced by a dominant and non-dominant hand and that most specific characteristics keep the handwriting's individuality. This means that after identifying the text "as potentially written by non-dominant hand," the analysis of remaining class characteristics and most of the individual characteristics considered in the study might not be remarkably aggravated. However, specific caution should be exercised in analyzing and interpreting the characteristics that demonstrated significant differences. As the present study considered right-handed individuals only, future studies should also include left-handed individuals to secure the generalizability of findings and validate the classification model on the greater number of handwriting samples.

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Handwriting; Questioned Documents; Regression Analysis

K13 Font Flexin' With Aptos: Is This Next-Gen Typeface Slayin' or Playin'?

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Learning Objectives: This presentation compares the character differences between fonts.

Impact Statement: The Aptos font is likely to be observed on more documents in the near future. This is something Questioned Document (QD) examiners should be aware of.

Abstract Text: The Aptos font, originally named Bierstadt, became available for public use in 2023. Developed by typeface designer Steve Matteson, Aptos is a contemporary sans-serif typeface known for its clean lines, balanced proportions, and modern aesthetic. The Aptos font emerges as a compelling contender in the realm of typefaces, drawing comparisons to the longstanding favorites Calibri and Arial. While Calibri and Arial have dominated digital communication for years with their widespread adoption and readability, Aptos brings a fresh perspective to the typographic landscape.

Aptos and Calibri are two modern sans-serif fonts, each with distinct historical backgrounds. Calibri, designed by Lucas de Groot in 2002, was released in 2007 as part of the ClearType Font Collection with Microsoft Office 2007. It replaced Times New Roman as the default font in Office, reflecting a shift toward clearer, more readable typefaces optimized for screen use. Aptos' clean lines and sharp edges provide a contemporary look, suitable for digital interfaces and sleek branding. While Calibri became widely recognized as a standard for business and professional documents, Aptos is seen as a font for forward-thinking and modern design projects.

The comparison of fonts within a document and among several documents is a common practice of a Forensic Document Examiner. Knowing how this new default font differs from those like it is something a Forensic Document Examiner should be aware of.

Some of the design elements of the Times New Roman font, the Ariel font, the Calibri font, and the new Aptos font will be shared in this presentation. The unexpected emotion from customers following Microsoft's change to Aptos as its default font has been compiled for discussion and entrainment. Finally, this presentation will also walk through a detailed overlay comparison of several characters of the Calibri and Aptos fonts. Attendees should leave this presentation with an understanding of the obvious and slight changes that Aptos brings as the new default font.

Questioned Documents; Examination; Document Analysis

K14 A Forensic Analysis and Comparison of the Degradation of Cristal Violet in Blue Ballpoint Pen Inks Under Room Conditions and Accelerated Aging Conditions

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Learning Objectives: Attendees will learn the best way to create experimental design for simulation of room conditions and accelerated aging conditions, assess the effect of different environmental conditions on ink stability and degradation, and quantify changes in the dye. Result comparison from different testing conditions will contribute to making conclusions easier about the longevity and authenticity of the ink in the following analyses.

Impact Statement: The forensic applications of this presentation are: (1) learn how to write detailed forensic reports documenting test methods, results, and result interpretation; (2) preparation of expert testimony based on scientific analyses and results, and (3) help with ink identification and comparison.

Abstract Text: Given the importance of forensic ink analysis, especially in the identification and dating of documents, research focused on the degradation of Cristal Violet (CV) under different conditions may reveal valuable insights.[1] CV is a synthetic colorant used in the pen ink industry, and analysis of its degradation can provide useful information about ink durability and document authenticity.[2] Room conditions simulate typical exposure of samples to sunlight through window glass and the conditions in which documents are expected to be stored.[3] The samples were exhibited in May 2024 at middle European latitudes (Zagreb, Croatia).

In addition to the real conditions, the samples were also subjected to conditions of accelerated aging according to International Organization for Standardization **(**ISO) 12040 (Graphic technology, Prints and printing inks, Assessment of light fastness using filtered xenon arc light), which includes exposing the samples to conditions of 50°C, 35% relative humidity, and 42W/m² irradiation intensity.[4] ISO 12040 uses a filtered xenon arc to simulate sunlight and assess color fastness. Samples are exposed to defined light cycles to evaluate color stability over time.[4] These conditions lead to accelerated degradation of the ink, affecting its chemical stability.[4]

Using this method, it is possible to simulate real environmental conditions and predict how well the ink will retain its characteristics and quality over time, which is important for the reliable evaluation of forensic results.[5] By measuring and analyzing the quantitative indicator of CV in the blue inks of ballpoint pens, using the High-Performance Liquid Chromatography (HPLC) method, depending on the passage of time from 0 to 10 days and different exposure conditions, it was confirmed that samples subjected to natural and accelerated aging processes exhibit a reduction in the relative share of CV.[1,2]

By comparing the measurement results with two sample exposure methods, it can be concluded that the methods do not yield identical results.[3] It should be emphasized that the samples were not exposed to different types of room light sources that could have additional effects on the samples or ink, such as fluorescent lamps or LED lights.[6]

This research not only provides insights into chemical degradation processes but also encourages the development of strategies for the preservation and protection of forensic evidence in practice.[7]

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Cristal Violet; Pen Inks; HPLC

K15 Leopold-Loeb Revisited and Reprised: Document Examiners Help Unravel "The Perfect Crime"

Larry A. Olson, MFS, Lake Barrington, IL*

Learning Objectives: By attending this presentation, attendees will become acquainted with the facts of this historic 1924 case from the forensic document examiner's point of view.

Impact Statement: This presentation will impact the forensic document examiner community by bringing to light little-seen court displays that have not been in print since 1930, and not on public display since 2004, and presenting case information from a previously ignored forensic point of view.

Abstract Text: The year 2024 marks the 100th anniversary of the kidnapping/thrill-killing of Robert E. "Bobby" Franks by Nathan F. Leopold, Jr., and Richard A. Loeb, two wealthy, teenage geniuses in the Kenwood neighborhood in Chicago, IL. Although they had attempted to commit "the Perfect Crime," through a few careless mistakes, they became persons of interest almost immediately, and once their joint alibi was destroyed, confessed to the crime.

Due to the sensational elements of the crime and the presence of Clarence Darrow, opponent of the death penalty, for the defense, it was perhaps the first case to be dubbed "the Crime of the Century" and "the Trial of the Century." With the boys having confessed and pleading guilty, the trial became a sentencing hearing before a single judge, although the Prosecution presented the evidence and witnesses in full, as though before a jury, to prove their premeditation and planning.

The physical evidence in the case included handwritten, hand-printed, and typewritten documents. In the absence of a local crime laboratory at the time, the prosecutors allowed experts hired by newspapers to examine the evidence and give opinions to satisfy their readers. There were at least six daily newspapers in Chicago at the time, and according to newspaper accounts, as many as nine "experts" in handwriting and typewriting may have been consulted at the behest of various parties, although only two questioned document examiners testified at trial. Although the more sensational aspects of the case have been told and retold in works of fiction as well as non-fiction over the past 100 years, who these persons were and the role they played has been ignored in virtually all modern published accounts of the case.

The author will present a summary of the case, and what he learned about the experts involved, in conjunction with a poster session that displays the documentary evidence.

Questioned Documents; Typewriting; Handwriting

K16 The Application of Imaging in the Analysis of the Strokes Sequencing on Documents

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Learning Objectives: After attending this presentation, participants will learn about the possibilities of the multispectral imaging technique and image processing in the analysis of the strokes sequencing on documents based on the application of stochastic algorithms such as Principal Component Analysis (PCA) and Independent Component Analysis (ICA), which is unconventional in the context of forensic document review.

Impact Statement: Nowadays, various techniques such as Scanning Electron Microscopy (SEM), stereomicroscope, Video Spectral Comparator (VSC), Electrostatic Detection Apparatus (ESDA), and digital imaging systems are used in the examination of stroke sequencing, which is an integral part of forensic questioned document analysis. The main goal of the study is to present multispectral imaging as an accessible, non-destructive, portable, and cost-effective technique for determining the sequence of intersections of ink strokes.[1]

Abstract Text: This study investigates the application of multispectral imaging combined with PCA and ICA to determine the sequence of intersecting ink strokes.[2] The main objective is to enhance forensic document examination, specifically in identifying the order in which lines were written with different pens, which is critical for verifying the authenticity of various documents such as wills, contracts, and letters. The research utilized a laboratory multispectral imaging system to capture images of document samples with intersecting lines made by different types of pens (e.g., ballpoint pens, liquid ink pens, and gel pens).[3] The samples were analyzed for various combinations of overlapping order, including different writing pressures. The multispectral images were processed using PCA and ICA to identify distinct spectral signatures and determine the sequence of ink deposition. This technique proved effective in distinguishing between different ink types and their sequences. The PCA helped reduce data dimensionality, highlighting significant variations in the ink spectra, while ICA isolated independent components correlating to the order of strokes. The analysis showed that the multispectral system could reliably identify the sequence of crossing lines for most ink types, except for some combinations of similar inks and pens where results were inconclusive. Multispectral imaging, combined with PCA and ICA, offers a robust, non-destructive method for forensic document examination.[4] This approach can accurately determine the sequence of intersecting ink strokes, aiding in the verification of document authenticity and the investigation of fraudulent documents. Future research will focus on refining this technique and expanding its application to a broader range of writing instruments and printing methods.

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Document Examination; Sequence of Intersecting Lines; Multispectral Imaging

K17 The Availability and Detection of Signature and Handwriting Fonts

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Learning Objectives: Participants will become aware of issues involving handwriting and signature fonts, including the increasing challenge of distinguishing between human written content vs. machine-generated content.

Impact Statement: By increasing the understanding of the changing technological environment regarding both physical and digital documents, Forensic Document Examiners (FDEs) and digital and multimedia science analysts will become more aware of approaches to evidence examination.

Abstract Text: From rudimentary static characters to limited variation based on letter pairs to complex algorithms or AI-produced versions, computer-generated signatures and handwriting pose an ever-increasing challenge. With knowledge of how handwriting and signatures are executed, FDEs have an advantage over non-FDEs, but this advantage will wane. In addition to assessing hard copies of documents that include fonts or generated writing, there may be clues in the original digital files.

This comprehensive presentation will include the history of handwriting fonts, traditional methods of detection, real-time computer-generated handwriting (a.k.a. "generators"), and possible methods of detection. The possible methods will include techniques utilized in the examinations performed by Digital And Multimedia Sciences (DMS) analysts.

There have been script styles in printed text since the late 18th century and later incorporated into typewriters. Script fonts were available early in the personal computer environment, evolving into the ability to create a custom signature. The product of this process often included telltale signs due to the use of a limited character set; the result was frequently an unnatural, uniform appearance. More recently, the ability to generate variable fonts (either holistically or by reference to an individual's writing) has developed.

Based on the uniformity in earlier output, FDEs could readily identify the use of handwriting fonts. The subsequent addition of minor variability (2–3 versions of each character) was also not an impediment for FDEs in assessing content. However, the recent technological developments are presenting new challenges, including significant variability of the characters and a more natural appearance.

These challenges will require FDEs to innovate, developing new and novel ways to assess the content in digitally produced or captured documents. This should include greater collaboration between FDEs and DMS practitioners. This presentation will include an introduction to digital media authentication and its role in evaluation digital documents.

Handwriting; Document Analysis; Digital Evidence

K18 An Investigation of Burnt and Unburnt Papers Scribbled With Whiteboard Pen by Destructive and Non-Destructive Methods

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WITHDRAWN

K19 Counterfeit Cannabis Packaging: Detecting Fakes Through Forensic Document Examination

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Learning Objectives: Attendees will understand the Forensic Document Examination (FDE) methodology applied to the examination of four different types of counterfeit cannabis packages (low quality, high quality, "diverted," and "burner distro") and its role in safeguarding consumer's health.

Impact Statement: This presentation will impact the forensic science community by providing optical methods for the examination of counterfeit cannabis packages. Three levels of analysis are explored: (1) visual inspection at low magnification; (2) microscopic analysis; (3) and high-intensity (narrowband) light sources examination.

Abstract Text: The proliferation of legalized cannabis markets in the United States has inadvertently given rise to the counterfeit cannabis industry. Counterfeit cannabis products mimic legitimate brands, leading to consumer deception and potential exposure to unregulated and unsafe substances.[1] This poses a significant public health threat to consumers and, in instances regarding packaging, a violation of intellectual property with significant economic implications for producers and legitimate businesses.[2,3]

Medically and recreationally, cannabis edibles are the preferred and most frequently consumed form of cannabis for the 55+ demographic due to their standardized dosing, increased convenience, and the variety of products available, including gummies, chocolates, cookies, and more.[4,5] Unlike other counterfeit items, some illegitimate cannabis products are produced by legal manufacturers and then intentionally circulated to the black market via illegal distribution centers. These "burner distro" items add a distinct layer of complexity to the legal distribution, sale, and purchase routes of products beyond the traditional low- and high-quality counterfeit packages. Additionally, a fourth category of counterfeits includes "diverted" products, which are produced in legitimate facilities, distributed, and sold legally to dispensaries but are then resold on the black market. In all instances, the absence of controlled standards for transport, storage, and product expiration, which enables relabeling of the product, can alter the chemical properties of the product, posing a health threat to consumers. The effective identification and differentiation of counterfeit packaging is critical for law enforcement, businesses, and regulatory agencies. Forensic Document Examination (FDE), a discipline traditionally applied to currency, passports, and other secure documents, offers a fast, easy, and non-destructive approach to potentially characterize different categories of counterfeits and provide information for detecting counterfeit networks through forensic intelligence.

For this study, two different cannabis edible gummy products were purchased from four counterfeit sources (low quality, high quality, diverted, and burner distro) and compared with the genuine product. A blind examination of the items explored the efficacy of a multi-faceted FDE method in identifying different counterfeit packaging with a video spectral comparator: (1) visual inspection; (2) microscopic analysis; and (3) high-intensity (narrowband) light sources examination. The visual inspection was performed at low magnification (2x - 5x) under four wavelengths of Ultraviolet (UV) light (400nm - 254nm) and five Infrared (IR) lights (700nm - 1030nm). Microscopic analysis used higher magnifications (6x - 10x) to observe the quality of the printing techniques under the visible spectrum (370-700nm). Lastly, the high-intensity lights (400nm - 640nm), in tandem with certain filters, provided information on the inks' IR luminescence properties. These methods identified discrepancies in printing quality, color consistency, and ink absorbance/reflectance features.

Preliminary results have detected several common indicators of counterfeit cannabis packaging. Authentic cannabis packages exhibited consistent printing quality, uniform coloration, and precise alignment of graphical elements. In contrast, counterfeit packages often showed signs of poorer printing quality, such as misalignment, and color variations. UV light examination, but not IR light, further underlined discrepancies, with authentic items showing consistent fluorescence patterns, while counterfeits displayed irregular fluorescence.

The integration of these FDE methods has provided a comprehensive assessment of the packaging authenticity and highlighted a few limitations. FDE provides a powerful tool for the enforcement of cannabis packaging standards, aiding in the detection and prevention of, as well as fight against, counterfeit products. By ensuring the authenticity of cannabis packaging, regulatory agencies can protect consumers from potentially harmful products and maintain the integrity of the legal cannabis market.

Future research should focus on refining these techniques, exploring additional methods, expanding the database of authentic and counterfeit samples, improving portability of detection technologies, increasing awareness among consumers and businesses, and exploring applications in other industries.

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Cannabis; Questioned Documents; Fake

K20 Leopold-Loeb Revisited and Reprised: The Documents in the Case

Larry A. Olson, MFS, Lake Barrington, IL*

Learning Objectives: By attending this presentation, attendees will be able to view little-seen photographs and charts presented in evidence in this historic case from July 1924.

Impact Statement: This presentation will impact the forensic document examination community by allowing examiners to view photographs and court displays that have not been in print since 1930, and not on public display since 2004, so that they may view the practices of document examiners from 100 years ago and make their own conclusions about the exhibits' suitability and narrative value.

Abstract Text: The year 2024 marks the 100th anniversary of the kidnapping/thrill-killing of Robert E. "Bobby" Franks by Nathan F. Leopold, Jr., and Richard A. Loeb, two wealthy teenaged geniuses in the Kenwood neighborhood in Chicago. Although they had attempted to commit "the Perfect Crime", through a few careless mistakes, they became persons of interest almost immediately, and once their joint alibi was destroyed, confessed to the crime.

With the boys having confessed and pleading guilty, the trial became a sentencing hearing before a single judge, although the Prosecution presented the evidence and witnesses in full, as though before a jury, to prove their premeditation and planning. The trial was held at the old Cook County Courthouse in Chicago on Hubbard Street.

The physical evidence in the case included handwritten, hand-printed, and typewritten documents, which, through an administrative fluke, ended up in the Archives of Northwestern University, in Evanston, IL. Although, according to newspaper accounts, as many as nine "experts" in handwriting and typewriting may have been consulted at the behest of various parties, only two questioned document examiners, John F. Tyrrell of Milwaukee, and Jay Fordyce Wood of Chicago, testified at the trial on July 28, 1924. Court exhibits and photographs of J.F. Wood were preserved in the Northwestern Archives, while the exhibits of John F. Tyrrell were obtained from his protégé, Donald Doud of Milwaukee, and are now in the Author's collection.

This poster is a complement to the live presentation and displays many of the documents in the case and some of the charts created for use in court, including: (1) documentary evidence images (the hand-printed ransom note envelope, addressed to "Mr. Jacob Franks;" the two-page typed ransom note, signed "George Johnson," which was one of the two aliases the boys used, and the typed envelope and ransom delivery instructions left for Jacob Franks on the train); (2) Typewriting – Leopold's Underwood - J. F. Wood chart comparing the questioned typewritten ransom notes with specimen S4 and a law class "crib sheet" known to have been prepared on Leopold's portable Underwood typewriter (note defects in the "s" and "m"); (3) Hand printing – Leopold's - J. F. Wood chart comparing hand printing from the ransom note envelope with known Leopold "pen printing;" (4) J. F. Tyrrell chart comparing the Leopold printing with the ransom note envelope; (5) Handwriting – Leopold's - J. F. Tyrrell chart of the various questioned "Morton D. Ballard" signatures (written by Leopold); (6) A letter written by Leopold for the police on 25 May, describing the circumstances of the loss of his glasses at the murder site. This is the only extended writing of Leopold's in the Wood photo collection, although the trial transcript indicates letters from Leopold to Loeb were also used in the experts' examinations; (7) Handwriting – Loeb's - J. F. Wood chart comparing the handwritten "Morton D. Ballard" and R. A. Loeb entries with known Loeb writing ("Ballard" was the boys' other alias, and was used to open a bank account, establish residence at a hotel, and, thereby, rent the "murder car"); (8) J. F. Tyrrell chart comparing the "Morton D. Ballard" entries with known Loeb writing.[1,2]

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Questioned Documents; Photography; Expert Testimony

K21 The Distribution and Detectability of Overcharging on Bills by Using the Same Kind of Different Brand Pens and Different Kinds of Different Brand Pens

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WITHDRAWN

K22 The Advantages of a Trial Lawyer Also Being an Expert in the Same Subject Matter as the Experts to Be Examined and Cross-Examined: The Ideal Scenario in Questioned Document Litigation

Andrew Sulner, JD, Forensic Document Examinations, LLC/Sulner Law Offices, New York, NY*

Learning Objectives: Attendees of this presentation will learn about the significant benefits of employing a trial lawyer who is also a subject matter expert in the same subject matter that experts will be testifying about at trial. Of particular interest is the subject matter of handwriting analysis in cases involving conflicting expert opinions concerning authorship.

Impact Statement: This presentation will impact the forensic science community by highlighting the benefits and importance of employing a trial lawyer who is also a subject matter expert in the same subject matter that experts will be testifying about at trial.

Abstract Text: The advantage of a trial lawyer being an expert in the same subject matter as the expert he/she is tasked with proffering or discrediting cannot be overstated. This became self-evident to all who viewed attorney Barry Scheck's cross-examination of the government's DNA expert in the televised Los Angeles County murder prosecution of O.J. Simpson.

In medical malpractice cases, the advantage of the trial lawyer also being trained in medicine, and perhaps in the very same specialty as the defendant doctor, is well known to medical malpractice lawyers representing both plaintiffs and defendants. Similarly, lawyers with subject matter expertise in mechanical, electrical, or chemical engineering are oftentimes hired as special trial counsel in tort or patent litigation involving such subject matter expert testimony. However, such occurrences are much rarer when it comes to forensic science testimony, especially in the pattern recognition disciplines. For example, in the field of document examination, there is only one known instance of a lawyer who was also a forensic document examiner tasked with examining or cross-examining another document examiner. That lawyer, Andrew Sulner, will discuss the significance and advantages of his role as special trial counsel in any case involving conflicting opinions of handwriting experts and why his participation as special trial counsel truly serves the interests of justice. Mr. Sulner is a board-certified forensic document examiner (D-BFDE) and an attorney who earned a Master of Science degree in Forensic Science and a Juris Doctorate degree (with Honors) from The George Washington University in 1975. The Sulner name is associated with three generations of document examiners, and Andrew Sulner has over 40 years of experience in examining questioned and disputed documents on behalf of major law firms, banks, insurance companies, and financial institutions, as well as federal and state law enforcement and regulatory agencies. Mr. Sulner, who is also a certified fraud examiner and former state prosecutor, has been consulted nationally and internationally as an expert in determining the authenticity of documents, and his testimony as a forensic document examiner has been favorably cited in numerous federal and state court decisions.

Expert Testimony; Attorneys; Court

K23 The Physical and Digital Reassembly, Analysis, and Comparison of Fragmented Documents

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WITHDRAWN

K24 The Odyssey of Building a Functional Digital Forensic Research Library

Timothy Campbell, BS, Canada Border Services Agency, Ottawa, ON, Canada*

Learning Objectives: Attendees will recognize the obstacles of moving from a purely physical to a digital research library. They will learn of the processes and software that a forensic lab encountered in organizing a digital research library that previously existed in a state of disarray for many years but is now more easily navigable.

Impact Statement: The forensic science community will benefit from the experience of one laboratory having gone through the process of evaluating commonly available software packages in order to organize their own digital research materials collection. It will highlight the importance of the forensic laboratory librarian who is a specialist in this field, and should be supported, so as to alleviate the work that has been downloaded to the bench-level analyst as a result of cost-cutting measures.

Abstract Text: A collection of digital and physical reference files had been amassed over decades and became an unmanageable chaotic mess where a desired publication could not be easily found. A Forensic Document Examination Section started out in the mid 1990s with a single experienced examiner obtained from another government department. It grew significantly over the years, primarily by way of externally trained examiners, each bringing with them a different knowledge base and therefore a separate amount of familiar research and training material. Moreover, as newer examiners were trained internally, and research was conducted, additional materials were acquired in both physical and digital forms.

This work contributed to a massive collection, but without the requisite information organization that a dedicated library is usually known for. As a profession with a century of back-catalog publications, the collection ballooned to almost 38,000 digital files consuming 40 GB of space and with little logical structure. In the absence of an actual librarian due to funding cuts, the onus fell to individual examiners to "just know" where to look for desired publications on the laboratory's computer network.

A Library and Information Sciences term student was employed to evaluate the situation and determine a path forward that would both impose an organizational structure to the digital assets as well as aid in the ability to rapidly search for and, more importantly, find relevant but otherwise inaccessible information. Software programs that could aid in this task were evaluated based on their relative strengths and weaknesses, with one being chosen for organization of the holdings using deduplication, Optical Character Recognition (OCR) processing, the addition of modern metadata, and full-text indexing, with all processes ongoing into the future as newer publications are acquired.

Although not yet perfected, all section members, from newly minted trainees to experienced examiners conducting research, now have an equal ability to access the digital library collection and the ability to contribute their own acquisitions to it in an orderly manner, thereby saving time and decreasing stress.

Automation; Software; Research

K25 The Use of Natural Resources as Invisible Inks on Different Papers: Visualization With VSC8000 and Ink Production Using Natural Resources

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WITHDRAWN

K26 Which Came First? The Copy? Or the Other Copy?

Carolyn Bayer-Broring, MFS, Homeland Security Investigations Forensic Laboratory, McLean, VA*

Learning Objectives: Attendees will learn about the methods for differentiating genuine or original documents from copies or reproductions, and further, differentiating unaltered documents from those bearing alterations.

Impact Statement: This presentation will demonstrate for attendees a specific instance of casework encountered in the Homeland Security Investigations (HSI) Forensic Laboratory where reproductions of documents were submitted for examination, although at the time of submission, the case submitter was not aware they were reproductions. Standard laboratory equipment utilizing different light sources and magnification was utilized to assist with the visualization of the print processes used to prepare the documents. The standard laboratory equipment also allowed for comparison of the questioned/suspect documents to one another, as well as to genuine documents on file in the laboratory's reference library.

Abstract Text: In the course of a Questioned Document Section examination at the HSI Forensic Laboratory, a case was submitted to the laboratory containing three documents: an obviously altered United States Social Security Card with the data obliterated off the front, as well as two purported birth certificates from the state of Iowa (one a larger-sized document and the other a comparatively smaller-sized document). The obviously altered United States Social Security Card did not pose any issues during examination and was easily addressed as an altered genuine document. The remaining documents, on the other hand, were not as easily dealt with.

Early in the examination, it was determined that the two purported birth certificates were not original documents, but rather were copies or reproductions. The case submitter was not aware of this information and had submitted them under the assumption that they were original documents. The HSI Forensic Laboratory possesses an extensive library of genuine documents and happened in this instance to have genuine standard birth certificates from the state of Iowa (both a larger-sized document as well as a smaller-sized document that was slightly different in appearance and format from the larger one) that would allow for the examiner to conduct a direct comparative examination to the suspect documents.

As the examination progressed, mysteries arose with the suspect documents. These included questions as to whether the smaller-sized suspect document was attempting to mimic the genuine smaller-sized one; did one of the suspect reproductions serve as the source document for the other; and how many iterations of reproductions possibly existed? The suspect documents were found to have numerous print anomalies as well as multiple different print fonts, further adding to the mystery of the document's sources. Ultimately, some of these questions could not be answered in the forensic report that was written without going outside the scope of the Laboratory's examination protocols. But that doesn't stop us from treating them as a curiosity to be looked at here among other Questioned Document colleagues and possibly addressing those questions in a bit more detail.

Printing; Questioned Documents; Casework

K27 Rocketbook: The Notebook of the Future, Part 2

Stephanie A. Kingsbury, MFS, United States Secret Service, Brambleton, VA*

Learning Objectives: During this presentation, attendees will learn about the Rocketbook, an alternative to traditional paper notebooks. Techniques to analyze a Rocketbook in a casework setting will be discussed. This presentation will build upon prior research to include additional considerations for analysis, technological components of Rocketbook capabilities, and instrumental evaluation of previous entries that are unable to be visualized with other techniques.

Impact Statement: The forensic science community will be impacted by this presentation by learning tips and techniques pertinent to analyzing a Rocketbook containing prior entries that have been "erased." The field of forensic document examination will be directly influenced by understanding the most optimal execution of scientific procedure in conceptualizing and imaging handwriting in a Rocketbook notebook.

Abstract Text: The Rocketbook notebook is an alternative to the traditional-style paper notebook in that it contains pages of a plastic-type origin. This type of notebook is reusable and sustainable, in that it allows the user to write entries onto the pages, scan the sheets into an app for preservation, and then wipe the sheet clean for use in the future. Specific pens, markers, and highlighters must be utilized to write entries in the notebook, as not all writing instruments will be successfully eradicated with the application of water on the sheet.

In the field of Forensic Document Examination, the question of whether the handwriting previously written on the "clean" sheets in a Rocketbook could be visualized under an alternate light source is of primary consideration. Prior research conducted ascertained that a variety of light sources outside of what the unaided eye can see could be used to envisage entries that had been cleaned from the surface of the notebook. Specifically, a variety of Pilot Frixion pens and highlighters were studied to understand how the writing instrument could affect the visualization of previous entries. Additionally, other variables such as ink color, the time that the entry remained on the page before being cleaned, and the temperature of the sheet affected the clarity of the cleaned entry. The ability of indented impressions to be visualized on the entry sheet was also analyzed.

This presentation expands on previous research of the Rocketbook to include further discussion and analysis of the ability of a Forensic Document Examiner (FDE) to visualize previously cleaned entries on sheets below the origin sheet for indented impressions. The effect of the application of heat and cold on the sheets will also be discussed in regard to the analysis of the cleaned sheet. Finally, the ability to visualize Pilot Frixion markers that have been erased will be considered. Additional discussion will include the updates to the Rocketbook app and the ability of examiners to not only potentially analyze previous entries on the notebook itself, but also to non-original documents uploaded into the digital realm.

Questioned Documents; Document Analysis; Ink Analysis

K28 The Forensic Document Examiner Forum

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Learning Objectives: After attending this presentation, attendees will be exposed, via a group discussion, 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 examiners from different countries and cultures.

Impact Statement: The impact to the forensic science community would be the benefit gained by the attendees through 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 texts and other sources. Younger examiners can benefit from the experiences of the more-experienced examiners and those experienced examiners can benefit from hearing new and novel viewpoints.

Abstract Text: The concept of the Forensic Document Examiner Forum is not new to the Questioned Document community. In the authors' experience, this format was first utilized at the Southeastern Association of Forensic Document Examiners (SAFDE) meetings in the early 1990s as a method of open discussion on certain topics of interest to the members. At those 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, including opinion terminology, methods and percentages of peer-review requirements, and bias. However, not all discussions must necessarily be centered on difficult issues facing forensic document examiners. Others may also focus on the attendee's thoughts on and interpretations of quotes from recognized texts in the field. Still others begin with the conclusions and opinions of some of these recognized authors in criminal and civil cases of notoriety in which they were involved, both past and present. This type of forum, one which results in 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. Standards development in the forensic document examination community could be explored, including methods and terminology. Trainees in the field can gain knowledge about all sorts of topics that can range from individual laboratory requirements such as case file structure (paper or paperless) to topics such as administrative documentation and other subjects pertinent to accreditation to what types of equipment are the optimum for a particular exam.

Typically, the presenters request that prospective attendees submit ideas for topics they would like to have discussed. Any registered member or guest may submit a topic or specific question they would like to hear discussed to Carl McClary at crmcclary@gmail.com, Karen Nobles at karenjnobles@gmail.com, or Samiah Ibrahim at fdesamiah@gmail.com.

Questioned Documents; Discussion; Conclusions

L1 A Risk Assessment of Lead Exposure in Glass Industry Workers: Evaluating Occupational Toxicity

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Learning Objectives: Attendees will learn about the health risks of lead (Pb) exposure in glass industry workers. The study found that workers had Blood Lead Levels (BLL) above safety limits, leading to inhibited delta-Aminolevulinic Acid Dehydratase (ALAD) synthesis and increased oxidative stress markers. The findings emphasize the need for regular BLL monitoring to mitigate health risks. The session will highlight the importance of regular BLL monitoring to reduce Pb exposure and its harmful health effects.

Impact Statement: Our presentation will impact the forensic science community by providing crucial insights into the occupational hazards of Pb exposure in the glass industry. By demonstrating the biological effects of elevated BLL and their correlation with oxidative stress markers, we will underscore the importance of incorporating regular BLL monitoring into occupational health protocols. This will aid forensic scientists in understanding the toxicological impacts of Pb, improve workplace safety standards, and enhance the interpretation of forensic evidence related to occupational exposure.

Abstract Text: Occupational exposure to Pb occurs from industries emitting Pb such as smelting and glass manufacturing industries, battery manufacturing plants, steel welding and cutting operations, printing industries, radiator repair shops, and refining industries. Environmental and occupational Pb exposure poses significant health hazards in developing countries. In the glass industry, Pb is added to glass products, leading to harmful health effects in workers. The present study is designed to examine strategic health survey and biological monitoring of workers involved in glass industry. We also determine oxidative stress and elucidate the role of oxidative stress in mechanisms of Pb-induced toxicity.

The study included 100 exposed subjects and 100 control subjects. The socio-demographic data of the subjects groups (control and exposed) are presented, mean age was found significantly lower in the exposed group (39.54±12.89) when we compared with the control group (45.7±7.86). Our results showed that BLL in occupational workers was higher than the safety limits. Elevated BLL in exposed subjects significantly inhibited ALAD synthesis compared to controls, and a significant negative correlation was observed. Biochemical studies revealed that biomarkers of oxidative stress, such as Lipid Peroxidation (LPO), Superoxide Dismutase (SOD), and catalase, were significantly increased, while reduced GLUTAThione (GSH) levels were significantly decreased in exposed subjects compared to controls. Our findings suggest that elevated BLL causes negative health effects due to oxidative stress in glass industry workers. We emphasize the need for regular BLL monitoring of glass industry workers to mitigate further Pb exposure in recognition of the seriousness of the problem of Pb among large populations the world over and that there is no effective treatment. Our finding proposes that proper precautions, using automatic furnaces instead of manual bhatties (furnace), exposure monitoring, and risk assessment, especially estimated risk in the future at certain levels of exposure, may help in the reduction in further exposure of Pb.[1-3]

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Toxicity; Occupational Incidents; Lead

L2 An Alcohol Impairment Evaluation in Cases of Severe Injury

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Learning Objectives: Following this presentation, attendees will understand how data on muscle alcohol concentration could be useful to estimate blood levels and thus help in the evaluation of an impairment state of the deceased.

Impact Statement: Participation will have an effect on people understanding the need for integrating the "classical" forensic approach, based on data from blood, with data from muscle tissue to determine potential surrogate specimens for analysis.

Abstract Text: Ethanol represents the most abused drug worldwide, and among the most frequently evidenced in toxicological analysis on drug-induced/related deaths. Ethanol intakes alone or in combination with other drugs of abuse represent a risk factor for severe injury, mostly roadside.[1,2] Ethanol impairment is among the top ten causes of death in all countries, except for high income countries.[3,4] In 2020, there was an increase of approximately 14% of people killed in motor vehicle crashes in respect to 2019, accounting for 30% of all traffic-related deaths in the United States.[5] The risk of being involved in a road traffic crash starts at low levels of Blood Alcohol Concentration (BAC) and increases significantly when the driver's BAC is higher than 0.4g/L, with a remarkable positive correlation between BAC and injury severity in road accident victims[3,6]

In cases characterized by a high *vis lesiva* (injurious force) and in which the decomposition phenomenon has not occurred, there could be the need to identify an alternative matrix for toxicological analyses. With respect to ethanol, the candidate matrix should give information about BAC. Presented is the correlation of postmortem blood and rectus abdominis muscle ethanol concentration.

Ethanol concentrations in both blood and muscle were determined by Headspace/Gas Chromatography/Mass Spectrometry (HS/GC/MS). BAC was determined according to a previously published method.[7] The HS/GC/MS method for muscle ethanol quantification was validated according to the American National Standards Institute/Academy Standards Board (ANSI/ASB) guidelines. Analytical responses were linear within the range 0.1 to 3.0g/g ethanol in muscle. Analytical response of six matrix-based calibrators was compared with aqueous one by using three QC samples (2.0, 1.0 and 0.5g/g ethanol in muscle). QC quantification through aqueous calibrators was superimposable to matrix-based one. The method was validated by assessing specificity, recovery from biological matrix (92.5%), sensitivity (Limit Of Detection [LOD] 0.052g/g ethanol in muscle, Lower **Limit of Quantification** [LLOQ] 0.086g/g ethanol in muscle), bias of quantitative analyses was between 3.5% and 10.0%. The Analysis of Variance (ANOVA) F-test was used to determine within-run and between-run precision, both resulted less than 20%.

Thirty-four forensic cases with positive BAC were selected for the correlation study. Circumstances and time since death were known in all selected cases. A linear correlation between BAC and ethanol muscle levels was obtained ($y = 0.9928x + 0.3346$; $R^2 = 0.845$). The correlation equation was used for BAC estimation in three forensic cases for which abdominal muscle was the only matrix available.

Rectus abdominis muscle was demonstrated as a good candidate to estimate blood alcohol concentration at the time of fatality.

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Toxicological Analyses; Postmortem Alcohol Concentration; Muscle

L3 Biofluid Sampler: A Green and Robust Sampling and Sample Preparation Platform for Forensic Toxicological Analysis

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Learning Objectives: This presentation will report a novel forensic toxicological sampling and sample preparation platform, Biofluid Sampler (BFS) that combines classical equilibrium-driven sample preparation techniques (e.g., solid phase microextraction) and exhaustive sample preparation techniques (e.g., solid phase extraction) by design.[1] BFSs can selectively extract the target analytes directly from 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 sampling and sample preparation technique, its potential applications, and its underlying advantages over classical Dried Blood Spot (DBS) cards.

Impact Statement: This presentation will impact the forensic science community by demonstrating the advantages of 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.

Abstract Text: Most forensic toxicological investigations involve the analysis of different biological fluids, including whole blood, urine, saliva, and cerebrospinal fluid, although whole blood is the most preferred one. Due to the distinctive complexity of whole blood as the sample matrix, either plasma or serum is used as the primary sample in toxicological investigations as the proxy for whole blood. During the transformation of whole blood into plasma or serum followed by extraction of target analytes and their metabolites using conventional sample preparation techniques, including Solid Phase Extraction (SPE) and Liquid-Liquid Extraction (LLE), a significant portion of the analytical information disappears, resulting in questionable data in these critical studies. BFS, a new-generation sampling and sample preparation technology, has offered a paradigm shift approach in sampling and sample preparation for forensic toxicological analysis. BFS innovatively combines the benefits of SPE (works under exhaustive extraction principle) and solid phase microextraction (works under equilibrium extraction principle) into a single sample preparation technology platform. BFS utilizes a flexible and permeable fabric substrate, coated with high-performance sol-gel sorbents as the extraction membrane. This uniquely designed sampling and analytes extraction platform is capable of extracting target analyte(s) directly from whole blood and other biological fluids. Due to the special geometry of the BFS membrane (flexible, flat, and permeable) and sponge-like porous architecture of sol-gel sorbents, rapid analyte mass transfer occurs between the biological sample and the extraction sorbent, resulting in an exhaustive extraction within a fraction of the time required for other comparable sample preparation techniques.

BFS is particularly suitable for analyzing target analytes (e.g., drug residues, metabolites, and biomarkers) directly from whole blood without requiring any protein precipitation or other pre-extraction sample cleaning/manipulation. After extracting the target analyte(s) directly from the whole blood sample and drying it in the air to remove water from the blood, the BFS is exposed to a small volume of organic/organo-aqueous solvent for eluting the extracted analyte(s). The low viscosity of the organic solvent, capillary force of the fabric support, and sponge-like porous sol-gel network allow fast diffusion of the organic solvent into the BFS membrane for quick and complete recovery of the extracted analyte(s). As a result, BFS eliminates time-consuming and error-prone solvent evaporation and sample reconstitution steps often considered as an integral part of solid phase extraction/liquid-liquid workflow. During the solvent-mediated elution/back-extraction, any protein or matrix interferents adhered to the BFS membrane precipitate out and a final centrifugation of the resulting solution before injecting into the analytical instrument ensures clean particle-free highly concentrated target analyte(s).

In a most recent article, BFS has been deployed to monitor 11 basic drugs in human blood and urine samples, including fluoxetine, amitriptyline, chlorpheniramine, chlordiazepoxide, pheniramine, diazepam, clozapine, chlorpromazine, dothiepin, and tramadol with Limit Of Quantitation (LOQ) ranging from 72ng/mL to 95ng/mL in blood and 50ng/mL to 69ng/mL urine sample in Gas Chromatograph/Mass Spectrometry (GC/MS) analysis.[7] The matrix effects were very minimal (6.9-11.2%). Relative recoveries were reported between 81.2-98.5% in blood and 91.5-106.9% for urine. Extraction efficiencies were reported between 7-.0-79.7% in blood and 89.1-99.0% in urine. The performance superiority and operational convenience of BFS have already been presented in many published articles. For example, Fabric Phase Sorptive Extraction (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 postmortem samples, and adamantine analogs in human urine.[2-6]

In the current talk, some new and fascinating data on bioanalytical sample preparation using BFS and a comparison between BFS and conventional sample preparation techniques will be presented.

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Absorption; Biomarkers; Chromatography

L4 Cannabis-Based Medicines and Driving: Current Legal Issues in Switzerland

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Learning Objectives: After attending this presentation, attendees will understand current legal issues pertaining to cannabis-based medicines and medical fitness-to-drive.

Impact Statement: This presentation will impact the forensic science community by showing current Swiss legal issues affecting drivers that might receive medicines containing cannabis.

Abstract Text: Interest in cannabis-based medicines has risen importantly in recent years due to the wide range of potential uses. The reasons are many and can be difficult to summarize. Among those most mentioned, it deserved to be mentioned a greater request from patients for so-called "alternative" remedies to traditional pharmacological treatments, as well as a different perspective on the disease and the solution that a physician should propose for the treatment. However, delta-9-Tetrahydrocannabinol (THC) impairs driving performance and other safety-sensitive tasks.[1-5]

Under the Swiss Narcotics Act, use of cannabis with a THC content of at least 1% is generally prohibited. The Swiss Parliament has however decided to lift the ban on cannabis-based medicines from August 1, 2022. Exceptional authorization from the Federal Office of Public Health is therefore no longer required for cannabis-based medicine prescription. Accordingly, general practitioners may prescribe cannabis-based medicines irrespective of the medical diagnosis. Prescribing physicians must still inform their patients that these medicines may affect momentary and general fitness-to-drive.[6]

The positioning of cannabis as a legitimate medical treatment produces some tensions with other regulatory frameworks. A notable example of this is the so-called "zero tolerance" drug driving legal frameworks, which criminalize the presence of THC in a driver's bodily fluids, irrespective of impairment. Indeed, it has been observed that there is little evidence to legitimate the differential treatment of patients taking cannabis-based medicines compared with those taking other psychotropic medications potentially impairing fitness-to-drive.[1-5,7,8]

Patients using cannabis-based medicine should be advised to avoid driving during the initiation of treatment and in the hours immediately following each dose. Patients using THC-containing preparations should also be informed that they are at risk of testing positive for cannabis in oral fluid even if they are not impaired.[6]

The aim of this paper is to briefly discuss current Swiss legal issues concerning cannabis-based medicines and fitness-to-drive medical assessments.

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Tetrahydrocannabinol; Driving Under the Influence; Traffic Accident

L5 Cocaine and Aortic Dissection: The Need to Overcome the Underreporting Bias

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Learning Objectives: Following this presentation, attendees will understand how data on correlation between cocaine use and aortic dissection can be influenced by the underreporting of this situation.

Impact Statement: Participation will have an effect on people understanding the need for integration between clinical and forensic data, eventually collected in a register, in order to contribute to comprehensive knowledge on the phenomenon.

Abstract Text: The Dissection of the Aorta (AD) is a serious and potentially fatal consequence of cocaine use.[1,2] Nonetheless, the underlying mechanisms and characteristics of this phenomenon remain to be deeply studied.[3]

The case of a 46-year-old White male found unresponsive and unconscious in his house with a history of strong cocaine abuse is presented. Autopsy findings showed aortic arch and thoracic aorta of regular caliber, with evidence of a mid-adventitial dissecting aneurysm of the ascending intrapericardial portion of the aortic arch. Forensic toxicological analyses evidenced a positivity to cocaine and its main metabolite, benzoylecgonine, in all fluids (peripheral blood, bile, and urine), liver, and brain homogenates. Histopathological findings together with toxicological results led us to conclude for a correlation between cocaine abuse and AD (the cause of death).

In general, difficulties in outlining a correlation between cocaine abuse and AD on an epidemiological base are mainly related to the low prevalence of AD in cocaine abusers with respect to the general population, accounted for 1.8% (63 cocaine positive out of 3584 patients) according to the International Registry of Acute Aortic Dissection (IRAD) data collected from 1996 to 2012.[4] A reliable explanation of such a low prevalence could be a significative underestimation, since forensic deaths are not included in the IRAD registry and data from patients' habits are mainly derived from self-reports in clinical history collection.[5] Consequently, the so-called "self-reported bias" could represent a serious issue in limiting the accuracy of the exact prevalence of drug abuse among patients with AD. Moreover, a complete toxicological screening is not always routinely performed in clinical practice, with possible errors in highlighting the real patient habits, due to his/her reticence to self-admit the use. The correct estimation of aortic dissection prevalence could also benefit from forensic reports about cases not related to hospitalization.

The here-presented case study, the only one reported in our experience, provides additional support for an anatomopathological association about cocaine toxicity, specifically in relation to the cardiovascular district. This is particularly concerning as it could indicate the highly dangerous possibility of a dissecting aneurysm or AD. While the most dreaded outcome—the patient's death—often coincides with the suddenness and severity of this event, a further description of real cases will help understand the phenomenon and potentially prevent it.

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Cocaine Abuse; Forensic Toxicology; Aortic Dissection

L6 Comparative Postmortem Blood Alcohol Concentrations in Cardiac and Peripheral Blood: A Case Series

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Learning Objectives: After this presentation, participants will be able to understand the concept of postmortem ethanol redistribution, factors that may influence it, and the importance of sampling from two different sites to ensure the accuracy of postmortem toxicological assessments.

Impact Statement: This presentation will impact the forensic science community by showing how factors such as cause of death, chest trauma, cardiopulmonary resuscitation, and postmortem interval can alter alcohol blood concentrations.

Abstract Text: Postmortem redistribution is a widely recognized phenomenon. There are numerous studies investigating the mechanisms behind drugs' postmortem redistribution, but when it comes to alcohol, the number of studies decreases. In general, postmortem redistribution is a phenomenon in which the levels of exogenous substances in the body undergo modifications in mode and timing that differ depending on the substance and on the anatomical regions involved. Due to the proximity to the adjacent blood vessels, passive drug diffusion from reservoir organs is believed to be the primary source of drug postmortem distribution. When conducting toxicological analyses, it is always advisable to take a venous blood sample (taken, for example, from the femoral region) and a cardiac blood sample. Peripheral blood samples are anatomically isolated from the thoracic cavity and, consequently, are considered more indicative of the actual concentrations of substances in the period immediately preceding death.

This report will present a study conducted to assess the variation in blood alcohol concentration levels between peripheral blood and cardiac blood samples collected from 18 cadavers. No inclusion and exclusion criteria were posed regarding the cause of death. However, these were placed regarding the samples: the samples used had been properly preserved and had never previously been aliquoted for further analysis. The blood samples were subjected to concurrent analysis using Headspace/Gas Chromatograph/**Flame Ionization Detector** (HS/GC/FID). For this purpose, a rapid and sensitive HS/GC/FID method was validated for the evaluation and quantification of ethanol in blood using a minimal amount of starting sample (100µL) without diluting it. This method was internally validated in our laboratory according to the guidelines of the *Standard Practices for Method Validation in Forensic Toxicology* of the American Academy of Forensic Sciences. Evaluated parameters included bias, precision (% CV), linearity of calibration model, Limits Of Detection (LODs), Lower Limit Of Quantification (LLOQ), and interference studies. Bias and precision were determined for three different concentrations, low (0.1g/L), medium (0.8g/L), and high (3g/L), through the one-way Analysis of Variance (ANOVA) approach and were both lower than ±10%: -8.39% for low concentration, 6.32% for medium concentration, and 7.38% for high concentration. Analysis revealed significant differences in concentrations between cardiac blood and peripheral blood in 9 out of the 18 samples (55.6%). Four out of the 9 samples with significant differences belonged to victims of road accidents, resulting in thoracic trauma with probable consequent contamination of the cardiac blood sample. Another 4 of these 9 samples belonged to people who underwent cardiopulmonary resuscitation. Only one sample showed these remarkable differences without having suffered chest trauma or cardiopulmonary resuscitation.

With the obtained results and the existing literature on the topic, we tried to explain the noted discrepancies.[1,2] The presence of thoracic trauma and the practice of cardiopulmonary resuscitation, with the typical chest compressions associated with the maneuver, may have facilitated postmortem redistribution or led to a subsequent rupture of the heart and nearby organs, causing contamination of cardiac blood, consequently altering its blood alcohol concentration.

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Ethanol; Blood Alcohol Concentration; Forensic Investigation

L7 Ethanol Stability Over Time in Blood Samples Undergoing Headspace Analysis

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Learning Objectives: This presentation will allow the attendees to learn about the potential impact that time can have on the quantification of ethanol in blood samples. Attendees will also learn how the data was assessed and compared across the trials, and how the differences observed in the data were measured and interpreted. Finally, the attendees will learn the impact this research can have on the law enforcement community and the applicable forensic science professionals.

Impact Statement: This presentation may influence the protocols currently in use by forensic toxicology laboratories for the analysis of ethanol in blood samples using Headspace/Gas Chromatography with a Flame Ionization Detector (HS/GC/FID). The results show that the time samples are left undisturbed or analyzed after repeated injections may affect the ethanol concentration in the samples. This may, in turn, influence the impact that those results have on Driving Under the Influence (DUI) casework and on the subsequent outcome of the associated investigations. Therefore, this presentation may persuade laboratories to adopt standardized protocols that also account for post-sampling time.

Abstract Text: A "buzz," euphoria, and a wave of dopamine mixed with neurocognitive and psychomotor impairments are all caused by the world's most socially accepted drug, alcohol.[1,2] Rising incidents of motor vehicle crashes due to the consumption of ethyl alcohol, or ethanol, pose road safety concerns.[3] Forensic toxicology assists in maintaining public safety by identifying and quantifying impairing substances within the human body, such as Blood Alcohol Concentration (BAC).[4]

The purpose of this study was to investigate the stability of ethanol in samplings taken from blood draws, alcoholic beverages, and Quality Assurance samples (QAs). These samplings are taken in order to be analyzed via HS/GC/FID, which is a technique used to analyze volatile compounds (such as ethanol) within a given sample. The samples were consistently aliquoted using a semi-automated pipette-diluter. Each sample (or QA) was aliquoted at 0.5mL with the addition of 1.5mL of internal standard (0.085% nPA) into a headspace vial with a 20mL capacity. The samples consisted of five blood samples of varying BACs (0%, < 0.10%, 0.10-0.20%, 0.20-0.30%, and >0.30%) and one alcoholic beverage (ABV ~13%). The QAs consisted of four standardized ethanol values (0.01%, 0.08%, 0.10%, and 0.50%) to ensure instrument performance. Each HS/GC/FID run started with a volatile mix, an internal standard blank, and these four QAs. Two pipettings were taken from each sample, generating six "A" vials and six "B" vials per HS/GC/FID run. In addition, for every 10 samplings (5 duplicates), one 0.10% QA was run.

This study examined three alterations in the current methodology. The first tested the ethanol stability of samples/QAs when pipetted into sample vials and left undisturbed for 0, 12, 24, and 48-hour intervals, thus evaluating the impact on unpunctured headspace vials. The second tested the ethanol stability at 0, 12, 24, and 36-hour intervals after repeated injections of the same headspace vials, thus evaluating the impact on punctured headspace vials. The third tested the ethanol stability after recapping the vials 6-hours after injection (to simulate a workday run) and 24-hours after injection (to simulate an overnight run). A positive correlation was observed between the time elapsed and the degradation of QAs for most methodologies, with an increasing number of QAs falling outside of the established quality control criteria as the time increased toward 48 hours. Reinjection of the same vials, in particular without recapping, produced the greatest number of QAs to fall outside of acceptable quality control criteria. The blood and alcoholic beverage samples, however, retained a Coefficient of Variance (CV) within the same sample around 1-2% for each iteration. When the undisturbed vials were analyzed, they produced the lowest CVs (< 0.10%), while the recapped and reinjected vial analysis had generally consistent CVs. The sample with the largest inconsistencies in CV was the < 0.1% blood sample.

This study demonstrated that ethanol levels within samplings of blood and alcoholic beverages generally stay consistent for 24 hours while QAs showed some instability. This suggests that ethanol stability may be dependent on the conditions under which the samplings are handled. Additional investigation into the degradation of ethanol in blood versus a QA matrix may provide greater insight into this observation.

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Blood Alcohol Concentration; Quality Assurance; Chromatography

L8 Forensic "ToxAIcology": Is It Really Over for the Forensic Human Community?

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Learning Objectives: By participating in this presentation, attendees will be given the opportunity to understand the impact of Artificial Intelligence (AI) in the study of a clinical case involving a toxicological-forensic issue.

Impact Statement: The impact on the audience will be in terms of analyzing the strengths and weaknesses with respect to the introduction of AI tools to support forensic toxicology activities.

Abstract Text: AI has now become part of various areas of everyday life, not least forensic science, of which toxicology is a part.[1,2] There are several AI software applications that can form opinions in response to inquiries in the widest range of subject areas. Their usage has grown, moving from a straightforward comparison to a possibly vital consultation. The aim of the here-presented study is to evaluate the plausibility and appropriateness of the responses given by an AI software for the resolution of a clinical scenario. These answers were recorded through the presentation of an interrogation carried out utilizing experimental technique. In particular, the responses were assessed for compliance with the forensic and forensic-toxicological ascertainment procedures. First, starting from a hypothetical clinical scenario of intoxication from synthetic cannabinoid, AI software was asked to predict both clinical and toxicological parameters in the simulated case. In addition, it was asked to predict a strategy to manage the intoxication. The program was then asked to make an educated guess on a diagnosis based on several symptoms given as input, also asking for a progressively increasing level of detail. Every response has been recorded and assessed based on the diagnostic algorithm's technical-procedural correctness, compliance with best practices and standards for clinical management, and toxicological-forensic analysis. Compliance was specifically examined in relation to the algorithm's selected approach, which additionally supplied fictitious blood and urine concentration data of the chemical that was "analyzed."

Finally, all data provided by AI software were rearranged as a scientific paper, with minimal to no modification and submitted as a case report to journals with different aims and scopes (clinical, forensic, and emergency) provided with an algorithm to check for plagiarism and AI utilization. Results deriving from this study are presented accompanied by both technical methodological considerations from a forensic toxicological perspective and ethical considerations for collective reflection to be proposed to the forensic science community.

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Artificial Intelligence; Toxicology; Software

L9 Leveraging Surveillance Data for the Analysis of Trends, Adulteration, and Novel Substances in the District of Columbia Needle Exchange Programs: A Four-Year Review

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Learning Objectives: After attending this presentation, participants will understand the potential of a crime laboratory analyzing and reporting on needles received via a used syringe exchange program and the impact on the public health of the community.

Impact Statement: The impact of this presentation on the field of forensic sciences is the explanation of a model that can be implemented in other jurisdictions to evaluate drug use trends in a community in near real-time, allowing rapid communication of emerging health threats.

Abstract Text: The District of Columbia (DC) Department of Forensic Sciences (DFS) has conducted a comprehensive analysis of syringes collected from needle exchange programs across the District over the past four years.[1] This study aims to present key findings on the types and prevalence of controlled substances, patterns of adulteration, the emergence of Novel Psychoactive Substances (NPS), and the geospatial distribution of drug use. This program contributes to a broader effort aimed at understanding the illicit drug supply in the District of Columbia and the consumption patterns of Intravenous (IV) drug users, particularly in the context of the ongoing overdose epidemic. The study's objective is to establish a comprehensive monitoring program that delivers real-time data critical for public health organizations, while also providing preliminary insights into the drugs detected in the local supply.

The analysis of syringe residues is conducted in the Forensic Chemistry Unity (FCU), which is accredited by the American National Standards Institute (ANSI) National Accreditation Board (ANAB). The laboratory utilizes Gas Chromatography/Mass Spectrometry (GC/MS) for qualitative analysis, with methods validated for this purpose. Testing is performed using an Agilent 8890 GC paired with an Agilent 5977B MS. The validation process evaluated key performance specifications, including selectivity, specificity, stability, matrix effects, precision (repeatability and reproducibility), and robustness, ensuring reliable qualitative identification of substances present in the samples. Limitations to GC/MS analysis include an inability to determine the salt vs. base form of the substance, possibly not being able to distinguish certain chemical isomers, and an inability to distinguish thermally labile compounds from their pyrolytic products.

From July 2020 to July 2024, over 8,000 syringes were analyzed using advanced GC/MS. Analyses identified a wide array of substances, including opioids, stimulants, and their adulterants. Fentanyl, methamphetamine, cocaine, and heroin were the most commonly detected drugs, with significant increases in fentanyl-related adulteration over the four-year period. Emerging trends include a notable increase in the presence of dipentylone and medetomidine, particularly in syringes also containing fentanyl, xylazine, and heroin. This points to a significant rise in polydrug use. Novel synthetic opioids, such as fluoro valeryl fentanyl, have also been detected, posing new challenges for forensic science analysis and public health responses.

The study highlights significant adulteration patterns and geographic variations in drug use, with certain areas showing higher concentrations of specific substances and adulterants. This geospatial analysis underscores the potential of targeted public health interventions and resource allocation. Adulteration patterns revealed a high prevalence of substances like xylazine, caffeine, and diphenhydramine, which may complicate the clinical management of overdoses and the forensic analysis of drug samples. Adulterants are pharmacologically active compounds that affect the Central Nervous System (CNS). When added to the various substances present in drug street samples, they can increase the user health risks. These findings underscore the need for continuous monitoring and adaptation of forensic methodologies to keep pace with the evolving drug landscape.

In conclusion, this study provides valuable insights into the dynamics of illicit drug use through data garnered from needle exchange programs. Key advantages of this study, over data gathered from traditional forensic evidence samples, are that the data are closer to real-time updates on emerging drug trends as well as poly-drug use data. By presenting this data, we aim to contribute to the broader understanding of drug trends and promote informed strategies for improving public health and safety.

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Drug Analysis; Substance Abuse; Chromatography

L10 Mapping Mortality: A Decade's Review of Drug Usage in Maryland (2014–2024)

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Learning Objectives: This presentation will: (1) highlight the prevalence and patterned trends of illicit substance use in Maryland over a decade's time, (2) empirically highlight growing trends of concern, with possible adulterants, and (3) highlight concurrent usage between narcotics.

Impact Statement: By examining a decade's worth of toxicology, we can demonstrate growing concerns and trends in narcotic prevalence, thereby alerting other forensic examiners of the growing problems.

Abstract Text: **Introduction**: Over the past decade, synthetic opioids (and their derivatives) have increasingly infiltrated the drug market, resulting in a significant rise in overdose deaths. Often mixed with other drugs, either intentionally or unknowingly, these adulterants have dramatically enhanced the risk of fatal overdoses. In this study, we examined the epidemiological and toxicological data obtained from autopsies performed by the Maryland Office of the Chief Medical Examiner (OCME) from 2014 to 2023. Our goal was to provide a comprehensive survey surrounding the prevalence and patterns of drug usage and possible adulterants over the first ten years of the current opioid epidemic in order to highlight the critical need for targeted strategies in mitigating the devastating impacts on public health.

Results: Between 2014 and 2024, there were approximately 57,000 autopsies performed (96.22 per 100,000 population) at the OCME for the state of Maryland. Over this period, the average annual increases of documented prevalence of the following illicit substances, as reported on death certificates, were: fentanyl, 196%; cocaine, 109%; methamphetamine, 8.3%; and phencyclidine, 1.9%. An increase in polysubstance use was also revealed, with an annual average increase of 7.4% in concurrent fentanyl and cocaine usage. The data will be extended through 2024 at the conclusion of the year.

Discussion and Conclusion: As highlighted by our study, the rising trends in illicit drug use, particularly the increasing prevalence of polysubstance abuse, poses a grave threat to public health. Over recent years, there has been a troubling escalation in the concurrent use of substances (such as fentanyl and cocaine.) This surge in polysubstance use exacerbates the already severe consequences of drug use and abuse, including the heightened risks of overdose, the intensified physical health issues, and the increased strain on health care systems. The synergistic effects of combining drugs often lead to more complex and challenging health crises, making treatment and intervention more difficult. Furthermore, these trends contribute to an immense societal burden, which manifests themselves as increased health care costs, lost economic productivity, and an increasingly overwhelming demand for emergency and rehabilitation services. Addressing this issue requires a multifaceted and multidisciplinary approach, including continued evaluation and recognition of the data trends, enhanced prevention strategies, improved access to treatment, and robust public health initiatives to mitigate the devastating impacts on individuals and communities alike.

Toxicity; Drug Abuse; Epidemiology

L11 Revolutionizing Anti-Doping and Sports Forensics: Comprehensive Screening of Nutritional Supplements for WADA-Banned Substances Using High Resolution Mass Spectrometry

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Learning Objectives: The goals of this presentation are to understand the importance of identifying contaminants and World Anti-Doping Agency (WADA) -banned substances in nutritional supplements and to gain insights into advanced forensics techniques for detecting prohibited substances.

Impact Statement: This study highlights the critical role of High Resolution Mass Spectrometry (HRMS) in improving the detection and identification of WADA-prohibited substances in nutritional supplements, thereby safeguarding the integrity of competitive sports by mitigating the risk of inadvertent doping. The findings establish a methodological benchmark for future forensic investigations in sports science, while also promoting fair play and enhancing athlete safety through advanced analytical techniques and comprehensive screening protocols.

Abstract Text: HRMS has revolutionized anti-doping and sports forensic analysis, offering unparalleled sensitivity and preciseness for detecting banned substances. HRMS has the capability for both targeted and non-targeted screening, marking it essential for identifying potential and novel doping agents. Techniques such as quadrupole Time-Of-Flight/Mass Spectrometry (qTOF/MS) and GC Orbitrap can be used to detect stimulants, diuretics, beta-blockers, narcotics, anabolic steroids, and modulators in sports supplements.

In this study, we conducted a market survey in India, analyzing 100 nutritional supplement products for the presence of 200 WADA-banned substances using HRMS. The methodology involved optimized sample preparation utilizing Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction with dispersive Solid-Phase Extraction (d-SPE) for cleanup. Chromatographic separation was achieved using an HSS T3 column with a 16-minute run time. Mass spectrometric detection employed qTOF Electrospray Ionization in both positive and negative modes (ESI+/-) with MS^e data acquisition to ensure comprehensive screening and confirmation across various supplement matrices. Validation parameters adhered to WADA's technical document TD 2023 IDCR, including ion ratio analysis, mass error calculation, and isotope intensity matching, thereby enhancing the accuracy and reliability of screening for prohibited substances. Samples were processed in UNIFI software using an in-house developed database using certified reference materials.

The study demonstrated outstanding performance in market samples, ensuring the integrity of competitive sports by preventing the use of prohibited substances. 17 % of the market samples were found to be contaminated with testosterone, methyltestosterone, higenamine, selegiline, and sibutramine belonging to S1 (Anabolic steroids) and S6 (Stimulants) category as per WADA list 2024.

The comprehensive research approach adopted in the present study not only enhances the detection capabilities by HRMS but also sets a benchmark for future forensic investigations in the field of sports science, supporting fair play, enhancing athlete safety, and mitigating inadvertent doping globally.

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Anti-Doping Forensics; Nutritional Supplements; High Resolution Mass Spectrometry (HRMS)

L12 The Opioid Crisis in Pakistan and Toxicological Interpretation: A Case Report

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Learning Objectives: The presentation will cover a comprehensive analysis of opioid crisis, focusing on the global prevalence and patterns, including heroin and polydrug toxicity. Attendees will learn to recognize the pharmacological impact of adulterants like Dextromethorphan (DXM) in street heroin samples and interpret the significance of various toxicological markers in post-mortem samples. The discussion will highlight the effects of storage temperatures on the stability of these markers and identify best storage practices. Finally, attendees will formulate recommendations for proper sample storage and handling, and suggest improvements in toxicological analysis protocols to enhance the accuracy and reliability of postmortem findings in opioid-related deaths.

Impact Statement: The presentation of this case study will significantly impact the forensic science community by highlighting the complexities and challenges associated with polydrug overdoses, particularly involving opioids like heroin and adulterants such as DXM. It will emphasize the critical role of advanced toxicological analysis in accurately identifying and quantifying various substances in postmortem samples. The findings underscore the importance of proper sample storage and handling protocols, which can greatly affect the stability and detectability of toxicological markers, such as 6-Acetyl Morphine (6-AM).

Abstract Text: Opioid overdose remains a major concern in various parts of the world since the late 20th century [1], with heroin overdoses and polydrug toxicity reported in the international literature.[1-4] In some cases, Central Nervous System (CNS) depressants and heroin abuse explain the deaths due to overdoses.[2-6] DXM is a pharmaceutically active adulterant and diluent (alone or with other substances) observed in street samples of heroin.[7] The aim of this study was to report a case of heroin and DXM fatal overdose. Effect of storage temperature on toxicological markers of drug exposure is also briefly discussed.

A 27-year old male was found dead in the countryside after missing from his home far from his residence. His dead body was stored in the mortuary for 14 days before a postmortem examination that showed few antemortem injuries on his face and arms, insufficient to cause trauma. No injection marks were observed on the dead body and no seized drug samples were submitted for analysis in this case.

Postmortem blood and gastric contents were stored at refrigerator (2-8°C) and freezer (-10 to -20°C) temperatures prior to toxicological analysis. Samples were screened for poisons (cyanide and phosphine) by colorimetric tests, drugs of abuse by immunoassay and qualitative identification for drugs and poisons by Gas Chromatography/Mass Spectrometry (GC/MS) using scan mode. Confirmation and quantification of opiates and benzodiazepines were performed by validated methods using GC/MS on Selected Ion Monitoring (SIM) mode. Samples were extracted by solid phase extraction using nalorphine as internal standard. Using a validated opiate confirmation method, a GC oven was operated at 150ºC for 2 minutes then ramped at rate of 50ºC per minute to 230ºC, stayed for 1min, again ramped at rate of 1ºC per minute to 247ºC, stayed for 0.4 minutes, and finally ramped at rate of 325ºC per minute and stayed for 2.8 minutes, making a total run time of 30 minutes. Sample volume (1µL) was injected in inlet maintained at a temperature of 250ºC and DB-5MS column (30m x 250µm x0.25µm) was used. MS was operated on SIM mode having a temperature of 250ºC of MS source and 200ºC for quadrupole.

GC/MS analysis of blood confirmed tramadol (0.36mg/L), DXM (5.94mg/L), codeine (0.95mg/L), morphine (17.16mg/L), diazepam (0.1mg/L), nordiazepam (0.05mg/L), dextrorphan, the principal DXM metabolite (qualitative), and 6-AM, (greater than 0.12 mg/L) while gastric contents contained all mentioned analytes and tetrahydrocannabinol, cannabidiol, and chloroquine.

Interpretation of toxicological findings reveal the polydrug overdose (from heroin and DXM). The presence of drugs in the gastric contents and the absence of injection marks on the body suggests the oral route of absorption and subsequent toxicity. The presence of benzodiazepines, DXM, and opium alkaloids suggests that a street heroin sample of unknown purity was consumed by the deceased. Furthermore, in contrast to the blood specimen stored in the freezer, 6-AM (primary marker of heroin abuse) was not detected in blood specimens stored in the refrigerator, showing the chemical instability of this compound and the importance of storage from -10 to -20°C for postmortem samples.

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Polydrug; Opioid Crisis; Toxicology

L13 The Reliability of a Dual Basic and Acidic Liquid-Liquid Extraction and LC/TOF Method for Routine Toxicology Drug Screening at the Indiana State Department of Toxicology Between 2019 and 2022

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WITHDRAWN

L14 The Underreporting of Psychoactive Adulterants of Illicit Drugs of Abuse in Toxicological Samples

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Learning Objectives: This presentation will discuss the application of an original Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method to authentic toxicological samples for the quantitative analysis of eight psychoactive adulterants. The analysis of authentic samples by this method revealed that several adulterants, including phenacetin and quinine, may be underreported through the current workflow of toxicological analysis.

Impact Statement: This presentation will impact the forensic science community by presenting data from the analysis of authentic toxicological samples that emphasizes the underreporting of several adulterants. It will also present a comprehensive LC/MS/MS panel for psychoactive adulterants that can be implemented into the laboratory workflow to help track the current trends in adulteration of illicit drugs of abuse.

Abstract Text: 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 product.[1,2] Adulteration with psychoactive substances, such as veterinary or unscheduled prescription drugs, instead of inactive cutting agents 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] 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.

Analysis was completed using a quantitative panel for the analysis of eight psychoactive adulterants: levamisole, xylazine, lidocaine, benzocaine, phenacetin, quinine, and tramadol and O-desmethyl tramadol. These adulterants were selected for their psychoactive properties and because they are well-established in the toxicology literature as frequent adulterants of drugs of abuse. Liquid-liquid extraction was performed using 70:30 N-butyl chloride:ethyl acetate as the extraction solvent. Instrumental analysis was performed on an Agilent 6495 LC/MS/MS system. Chromatographic separation was achieved with an Agilent InfinityLab Poroshell EC-C18 (3.0 x 100 mm, 2.7µm) analytical column combined with gradient elution with 10mM ammonium formate and 0.1% formic acid in methanol with an overall run time of five minutes. Prior to the analysis of authentic samples, method validation was completed in accordance with Academy Standards Board (ASB) 036 guidelines, including calibration model evaluation, carryover evaluation, bias, precision, determination of the limit of detection and limit of quantitation, evaluation of interferences, and evaluation for ion suppression.[4] The calibration model was validated in human blood. Matrix matching to serum and urine was performed to evaluate bias and precision for each analyte. Benzocaine, lidocaine, tramadol, xylazine, phenacetin, and levamisole were matrix-matched to both serum and urine. Quinine was matrix-matched to urine and O-desmethyltramadol was matrix-matched to serum. Dilution integrity studies at dilution ratios of 1:1, 1:10, and 1:100 were performed according to the ASB 036 additional validation parameters to allow for the dilution of authentic forensic samples over the quantitative range of the method.[4]

Fifty authentic toxicological samples, including blood, serum, and urine, were obtained and analyzed by this LC/MS/MS method for the initial purpose of comparing the method results to concentrations of one adulterant in the panel that had been reported from previous toxicology testing by several different methods. Unexpectedly, 23% of the samples had at least one additional adulterant present that had not been detected by direct toxicological testing. Adulterants that had not been detected by direct testing included quinine, phenacetin, and lidocaine. These analytes were each present in 8% of the tested samples at various concentration ranges. Quinine was detected in blood at 49.7-678ng/mL and in urine at 12.2ng/mL. Phenacetin was detected in blood at 9.9-163ng/mL and in urine at 11.7-53.1ng/mL. Lidocaine was detected in blood at 15.8ng/mL, in urine at 149ng/mL, and in serum at 42-72ng/mL. These results demonstrate an apparent underreporting of psychoactive adulterants, which may be attributed to the current screening and confirmation workflow used in many toxicology laboratories. By introducing this quantitative LC/MS/MS panel into existing analytical procedures, laboratories will obtain more information from toxicological samples and gain a better understanding of trends for less common psychoactive adulterants such as quinine, phenacetin, and lidocaine. Widespread use of this method would continue to help the forensic community track the ever-changing trends in the adulteration of illicit drugs of abuse.

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Adulterants; Toxicology; LC/MS/MS

L15 A Comparison of Sample Preparation Techniques for the Analysis of Drugs of Abuse in Oral Fluids

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Learning Objectives: By attending this presentation, attendees will be informed about the advantages and disadvantages of different sample preparation techniques on a variety of Drugs Of Abuse (DOA) and Novel Psychoactive Substances (NPS) in oral fluids.

Impact Statement: This work impacts the forensic science community by presenting various sample preparation techniques for the analysis of DOA in oral fluid. This will allow laboratories to make informed choices on methodology depending on their list of analytes, resource availability, and performance acceptance criteria.

Abstract Text: Testing for drugs in biological matrices is an important part of toxicology and workplace drug testing. The "gold standard" matrices that have been used for decades are blood and urine; however, the collection of these fluids is invasive. The analysis of DOA in oral fluids is gaining popularity due to its less invasive collection technique compared to blood or urine. However, there are some unique challenges associated with oral fluid testing. It can be difficult to remove all of the surfactants and preservatives present in the oral fluid collection device's buffer solution, which can cause matrix effects and poor column lifetime. It is also difficult to get complete recovery for all of the analytes because of varying techniques to empty the sponge in the collection device. Oftentimes, solid phase extraction or lengthy extraction techniques are utilized. Finding a method that uses a simple sample preparation paired with accurate and robust quantitation of the analytes is important for laboratories running these tests.

A Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method was developed using a 50 x 2.1mm, 2.7µm Raptor Biphenyl column. Mobile phase A consisted of 0.1 % formic acid in water and mobile phase B consisted of 0.1% formic acid in methanol. The analytes were separated under gradient conditions, with a total cycle time of 10 minutes. Samples were prepared in oral fluid and combined with Quantisal buffer. Samples were tested using three different sample preparation techniques, Salt-Assisted Liquid-Liquid Extraction (SALLE), Supported Liquid Extraction (SLE), and dilute-and-shoot, and underwent a full quantitation analysis.

A total of 68 DOA and NPS were analyzed in oral fluid. These analytes included common DOA compounds from different classes, including opioids, stimulants, and benzodiazepines, as well as some NPS. The chromatographic method was able to separate all sets of isobars. Aliquots were tested using SALLE, SLE, and dilute-and-shoot. The results of these extraction techniques were investigated and compared using performance metrics such as recovery, linearity, matrix effects, and accuracy and precision. It was found that the SALLE sample preparation worked best for a broader list of analytes. All 68 analytes passed accuracy and precision experiments when using the SALLE and there were no observed matrix effects. The SLE approach worked well for most analytes, but the analyte's performance was highly dependent on the analyte's properties, such as polarity. There were a handful of analytes that did not elute fully using the elution buffer for the SLE, and therefore these analytes did not pass the performance metrics. Dilute-and-shoot is an easy and cheap technique, but it did not work as well as the other techniques to remove buffer surfactants, leading to issues with analyte performance, matrix effects, and column lifetime. It was also difficult to achieve certain limits of detection using the dilute-and-shoot method. Therefore, a majority of the analytes that underwent dilute-and-shoot did not pass the performance metrics. When comparing the Low QC (5ng/mL), the SALLE and SLE approaches showed a significant increase in sensitivity for all of the analytes, some up to four times compared to dilute-and-shoot.

Overall, this work demonstrates an accurate and robust chromatographic method, able to separate the full list of 68 analytes. This work also compares sample preparation techniques for oral fluid analysis and reviews the benefits and problems with each.

Oral Fluid; Drug Abuse; Chromatography

L16 A Gas Chromatography/Mass Spectrometry Analysis of Tetrahydrozoline in Alcoholic Beverage Residue Related to Drug-Facilitated Sexual Assault

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Learning Objectives: The presentation discusses the detection of drugs in alcoholic beverage residue in place of biological samples. Specifically, the drug tetrahydrozoline is analyzed in relation to Drug-Facilitated Sexual Assault (DFSA).

Impact Statement: The research allows for the use of alternative samples in DFSA cases when biological samples are unavailable, thereby enhancing the efficiency of forensic investigations. The study also provides further understanding of the use of tetrahydrozoline in DFSA cases and its ability to be detected and eventually quantitated.

Abstract Text: **Introduction:** DFSA is an issue that continues to plague the world. Drugs commonly found in DFSA cases in the United States include ethanol and cannabis in combination with other drugs.[1,2] However, alternative drugs are increasingly being used to incapacitate a victim and enable a sexual assault. These include Tetrahydrozoline (THZ), which is commonly found in over-the-counter eye drops.[3,4] With minimal research surrounding THZ in DFSA cases, agencies may process case samples with a general drug screen, which may only detect common drugs while THZ goes undetected. Additionally, alternative samples are of interest in the absence of relevant biological samples, such as urine and blood, due to delayed reporting.[5] Therefore, investigations of new drugs in DFSA should be accompanied by analyses of alternative sample types where available, such as beverage residues in containers, to aid in situations where the drug cannot be identified in a biological sample received for testing.

Objectives: The research objectives were to develop a method to detect tetrahydrozoline in alcoholic beverage residue using Gas Chromatography/Mass Spectrometry (GC/MS).

Methods: Fifty milliliters of High Noon Hard Seltzer Vodka Lemon, 4.5% by volume, was poured into a red solo cup and spiked with a THZ standard to give a 1µg/mL concentration. The beverage was poured out, and 500µL of residue was pipetted from the cup and diluted with 10mL of water with a pH greater than 8. The 10mL THZ solution was extracted using Solid Phase Extraction (SPE). The samples were derivatized with Perfluorinated Propyl Acrylate (PFPA) at 90°C for 30 minutes. After derivatization, samples were reconstituted with a 1µg/ml internal standard of eicosane and caffeine in ethyl acetate. Samples were analyzed using GC/MS with splitless injection with an initial temperature of 70°C, hold for 1 minute, ramp 30°C/min to 250°C, hold for 2 minutes, ramp 20°C/min to 300°C, and hold for 4 minutes with an overall runtime of 14.00 minutes. The same steps were repeated with the High Noon spiked with different volumes of eye drops. The validation parameters assessed were precision, the Limit Of Detection (LOD), autosampler stability, freeze/thaw storage stability of processed samples, interference studies, and linearity.

Results: THZ (from the reference standard) and THZ (from eye drops) were detected in the residue as assessed by peak area ratio. Repeatability and precision studies conducted at 0.25, 0.5, and 1µg/mL yielded Relative Standard Deviations (RSD) below 10% at all concentrations. Autosampler stability conducted over 24 hours with 19 time points indicated stability based on the graph of the Peak Area Ratios (PAR) versus injection time, having a positive slope that was not statistically significant from zero. The THZ residue from the solo cups using THZ standard and THZ (from eye drops) was able to be detected.

Discussion: Under the conditions of the study, THZ (from a standard and eye drops) was detectable in residue from a spiked alcoholic beverage. The calculated RSDs conveyed the method was repeatable. Derivatized THZ standard was found to be stable over a 24-hour period on the autosampler. The findings of this study have significant practical implications for using alternative samples in DFSA cases where biological samples are unavailable, ensuring ongoing forensic analyses. Further research includes increasing the method's sensitivity and linearity for quantitation of detectable concentrations of THZ in eye drops associated with the number of drops used.

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Chromatography; Drug Analysis; Sexual Crimes

L17 A Look at Nitazenes Our Lab Has Detected Across the Country From 2020 to 2024

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Learning Objectives: Attendees of this presentation will understand the history of the emergence of the nitazene class of Novel Psychoactive Substances (NPS), their similarity to other opioids, prevalence with other drugs of abuse, and where they have been detected in postmortem death investigations.

Impact Statement: Understanding where nitazenes are being detected is important for labs that may not have resources to develop the methods preemptively. This data can also help inform labs that primarily perform Driving Under the Influence of Drugs (DUID) work and drug chemistry labs working with seized material.

Abstract Text: NPS are important in the field of forensic toxicology. NPS can be defined as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat."[1] Previously, they emerged due to a lack of regulation surrounding their possession and sale and the availability of resources for their synthesis. There have been attempts to schedule these drugs at both a state and federal level with some success. However, scheduling of one drug may lead to the emergence of another drug that testing methodology does not include. Nitazenes are one such class of NPS that has emerged and, due to being structurally distinct from other opioids such as morphine and fentanyl, may not be detected using methods designed for those classes. Etonitazene was first synthesized and studied as a veterinary anesthetic in 1957 but never approved for use.[2] Isotonitazene, which was referenced in drug patents, was the first of the nitazenes to emerge in drug seizures in 2019.[2] Since then, many more have emerged, some of which were not described in drug patents like the N-pyrrolidino derivatives of nitazenes.[3] Due to the evolving nature of NPS detection, periodic evaluation of which ones, where they are being detected, and what their concomitant detections are is helpful information for public health groups, human performance and postmortem toxicology labs, and drug chemistry labs.

Postmortem blood specimens were collected in gray-top tubes containing sodium fluoride as a preservative. Specimens are extracted using a cold acetonitrile protein precipitation procedure and then analyzed using Liquid Chromatography paired with quadrupole Time Of Flight/Mass Spectrometry (LC-qTOF/MS). Confirmation testing was done using LC-qTOF/MS or Liquid Chromatography with triple quadrupole Mass Spectrometry (LC/MS/MS). Ethyleneoxynitazene, N-desthyl isotonitazene, N-pyrrolidino metonitazene, and N-pyrrolidino protonitazene are reported qualitatively with a cutoff of 1ng/mL. Butonitazene, etodesnitazene, etonitazene, flunitazene, isotodesnitazene, isotonitazene, metodesnitazene, metonitazene, N-pyrrolidino etonitazene, and protonitazene are reported quantitatively with a lower limit of quantitation of 1ng/mL. Methods are validated according to standards set forth by the American Board of Forensic Toxicology and International Organization for Standardization (ISO) 17025.

Our lab has had 293 detections of nitazenes in casework, which is approximately 0.3% of total casework. Indiana (178, 60.7%), Ohio (40, 13.6%), and Florida (40, 13.6%) are the top thrree states by detections. The most commonly detected nitazene is metonitazene at 202 (68.9%) detections. We have had at least one detection for all nitazenes we monitor except for etonitazene, isotodesnitazene, and ethyleneoxynitazene. Fentanyl was detected in 272 (92.8%) cases, methamphetamine in 93(31.7%), benzoylecgonine in 85 (29.0%), xylazine in 29 (9.8%), and bromazolam in 17 (5.8%) cases. While nitazene detections represent a small percentage of our casework, their detection is still important as it informs public health initiatives related to drug education and response. Nitazenes are most often found with fentanyl in our toxicology testing but that isn't always the case. Around 7% of cases did not have fentanyl, so looking for fentanyl alone as the primary opioid in suspected fatal overdoses may cause information to be missed. Additionally, nitazenes, and other NPS, may cycle through the illicit market multiple times so the removal of testing for a compound that has not been seen for a period of time is not recommended.

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NPS; Nitazenes; Postmortem

L18 A Ten-Year Retrospective Review of Death-in-Custody Toxicology Results From Jefferson County, Alabama

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Learning Objectives: The goal of this presentation is to identify trends in toxicology results from deaths in custody due to accidental drug overdose and other causes in Jefferson County, AL, between 2013 and 2023.

Impact Statement: The results demonstrated in this presentation will assist the forensic science community by identifying changing trends in means of death and toxicological findings for deaths in custody. This information will assist healthcare providers and prison officials to target interventions.

Abstract Text: Based on the most recent mortality data for state prisons, the total number of prisoners increased by 1% between 2001 and 2018. However, the number of deaths in state prisons rose 44% during that timeframe. Alabama deaths in custody reflect national trends, increasing 44%, from 87 in 2001 to 125 in 2018. During the same period, the mortality rate per 100,000 increased from 352 to 588 (67%).[1] Few recent studies have evaluated the relationship between cause of death in custody and forensic toxicology findings. This study reviewed the toxicology results from all deaths in custody in Jefferson County, AL, from 2013-2023 to identify trends in cause of death and toxicological results. Although background data is available only through 2018, more recent deaths in custody were also reviewed to facilitate identifying emerging trends.

Toxicology findings, age, sex, race, location of death, manner of death, and means of death were reviewed from the Jefferson County Coroner/Medical Examiner's Office (JCCMEO) case management database. Data for 271 cases between 2013 and 2023 were obtained; 78 cases did not have toxicology requested. Of the total cases, the manner of death was natural in 59.4% (162), accidental in 21% (57), suicide in 9.6% (26), and homicide in 6.6% (18). The most frequent natural means was malignancy in 48 cases (17.7%). Of suicidal deaths, 24 (92.3%) were due to hanging. The most common means for deaths due to homicide was the use of sharp or blunt instruments in 14 cases (77.8%). Only 9 cases (3.3%) were deemed undetermined manner. Most deaths in custody occurred in prison (164, 60.5%), as did most accidental deaths due to drug toxicity (31, 62%). Of cases with toxicology (193), the most frequently detected drugs were fentanyl (27, 14%), methamphetamine (27, 14%) and opiates other than fentanyl, including methadone (13, 6.7%). For non-drug related deaths, 31.4% (44) had positive toxicology findings for drugs and 2.9% (4) for alcohol.

During 2023, for all cases assumed by JCCMEO, accidental deaths due to opioid drug toxicity were 60% (411) of all accidental cases.[2] Accidental drug overdoses for deaths in custody accounted for 87.7% (50) of all accidental deaths with 29 cases (58%) in 2022 and 2023. Of all accidental drug overdose deaths, 64% (32) were individuals 30-49 years old, 54% (27) were Black individuals, and 94% (47) of decedents were male. The most common toxicology result was fentanyl in 26 cases (52%), followed by methamphetamine in 17 cases (34%), opioids other than fentanyl, excluding methadone, in 9 cases (18%), and cocaine in 5 cases (10%). Most cases had more than one drug detected. The fentanyl metabolite/precursor, 4-ANPP, was detected in 2022 and 2023, with the cause of death of both cases accidental due to opioid drugs.

Synthetic cannabinoids and/or metabolites MDMB-4en-PINACA and 5F-ADB 3-3 dimethylbutanoic acid were first detected in 2023 in two cases; one was determined an accidental drug overdose. In 2023, the Forensic Toxicology Lab at the University of Alabama at Birmingham initiated sending out cases to the Center for Forensic Science Research and Education (CFSRE) in cases where drug toxicity was suspected but toxicology findings did not substantiate the cause of death. This allows expanded testing, including for synthetic cannabinoids and other opioids like nitazenes for the first time.

Overall, deaths that occurred with opioids other than fentanyl or synthetic cannabinoids occurred less than anticipated. One surprising trend was the number of accidental drug overdose that occurred during 2022 to 2023. This data shows the need for continued refinement of testing panels, toxicology testing for all deaths in custody, and the need to identify at-risk populations to target interventions to stem overdose deaths.

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In-Custody Death; Forensic; Toxicology

L19 Accidental Acute Intoxication With Gummies Containing Delta-8-Tetrahydrocannabinol

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Learning Objectives: After attending this poster presentation, attendees will be familiarized with the harmfulness of semi-synthetic cannabinoids (e.g., Delta 8-Tetrahydrocannabinol [Δ8-THC]), which can be synthesized from licit hemp-derived Cannabidiol (CBD).

Impact Statement: This poster presentation will impact the forensic science community by addressing challenges related to recognition of diverse cannabis products on the market and their risk for public health.

Abstract Text: Seized cannabis products in Croatia are increasingly diverse and include a range of natural, semi-synthetic, and synthetic cannabinoids. Emerging on the cannabis market over the past several years, semi-synthetic cannabinoids are produced by processing naturally occurring cannabinoids in cannabis plants like non-psychoactive CBD from hemp, their licit precursor. Semi-synthetic cannabinoids often appear in the form of edibles and are sold in misleading packaging. According to mislabeling, the risk of accidental exposure is high, particularly among children and youths Furthermore, their pharmacological activity, toxicological properties, and harmful effects are not well studied yet, which presents a risk for public health.

In the case from October 2022, a request for toxicological analysis of suspicious gummy candies was submitted to the Croatian Forensic Science Center "Ivan Vučetić" due to accidental intoxication of two sailors after gummies' consumption. Post-consumption, they experienced general weakness, severe headache, nausea, dizziness, and hallucinations. According to their statement, the gummies were left by foreign guests that the sailors transported on the boat and were found by a cleaner. Following the onset of poisoning symptoms, the sailors sought medical attention and were subsequently transferred to the Clinical Hospital Center Split, where they successfully recovered from the consequences of unintentional poisoning. Results of blood analysis conducted at the hospital merely confirmed the presence of cannabinoids. An open colorful plastic bag (mislabeled as Skittles Gummies) containing 18 gummy candies of uniform square shape but various colors (orange (2 pieces); yellow (4 pieces); light green (3 pieces); dark red (5 pieces); and dark green (4 pieces)) was delivered to the toxicological laboratory. Gummies were submitted for qualitative and quantitative analysis using standardized analytical methods. Thin Layer Chromatography (TLC) and Gas Chromatography/Mass Spectrometry (GC/MS) were used for qualitative analysis of cannabinoids. Subsequently, Gas Chromatography with Flame Ionization Detector (GC/FID) and High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) were used for cannabinoids quantification. Obtained results showed that the gummies contained Δ8-THC with observed share ranging from 0.03% to 0.12% across the analyzed gummies. Other cannabinoids were not detected in the analyzed samples.

Δ8-THC, a phytocannabinoid which occurs naturally in low quantities in the cannabis plant, is a positional isomer of delta-9-Tetrahydrocannabinol (Δ9-THC). In recent years, Δ8-THC is often synthesized from CBD and addressed as semi-synthetic cannabinoid. Results of limited clinical studies suggest that the psychoactive effects of Δ8-THC are similar but less intensive than those of Δ9-THC (the main psychoactive component of cannabis). Most commonly reported adverse effects include respiratory problems, increased blood pressure and heart rate, confusion, sedation or reduced consciousness and decreased psychomotor activity. Psychotic-like symptoms include paranoia, hallucinations, and panic attacks.

Δ8-THC is listed at the Croatian List of drugs, psychotropic substances, and plants from which drugs can be derived and substances that can be used in drug production. This case suggests further studying of the influence of licit "CBD" market on public health. Moreover, wide availability of CBD on the market raises additional concerns since it can be used as a precursor for psychoactive cannabinoids.[1-3]

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Semi-Synthetic Cannabinoids; Δ8-THC; Forensic Analysis

L20 An Assessment of Cross-Reactivity for Cannabinoids in Blood and Oral Fluid Using Enzyme Immunoassay Kits

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Learning Objectives: After attending this presentation, attendees will understand the importance and benefit of utilizing cross-reactivity to expand detection methods for their scope of analysis. Attendees will be able to create their own cross-reactivity studies to support the expansion of their scope of analysis to include screening for emerging drugs.

Impact Statement: This presentation will impact the forensic science community by serving as a resource to implement cross-reactivity studies. This study shows the ease and accessibility of evaluating cross-reactivity, allowing laboratories to tailor their testing to the needs of their customers.

Abstract Text: Immunoassays (IA) are drug screening techniques commonly used in the field of forensic toxicology as presumptive tests to determine the presence or absence of drugs in a biological specimen. A small amount of sample can be analyzed for a wide range of drugs/drug classes using a principle known as Cross-Reactivity (CR), the level of response generated from a drug other than the intended target. At the Alabama Department of Forensic Sciences (ADFS), the Toxicology section uses a type of IA called Enzyme-Linked Immunosorbent Assay (ELISA) with instrumentation from two different manufacturers. CR is used to expand the scope of analysis, while decreasing unnecessary additional testing. Commercial IA materials often cite CR studies performed by the manufacturer, but these studies may not always include novel or emerging drugs.[1,2] Since different toxicology laboratories may have different drugs in their respective scopes of analysis, and add drugs to their scopes on a regular basis, it is important to know the ability of their IA methods to detect novel drugs, such as cannabinoids. As novel cannabinoids emerge, conducting CR studies allow for the expanded use of ELISA as a screening method.

Cannabinoid drug standards were purchased from Cerilliant, Cayman Chemical, or Lipomed and spiked into negative matrices of either bovine blood or synthetic Oral Fluid (OF). A single aliquot of the prepared specimen was analyzed on the Randox Evidence+ Analyzer for blood samples or on the Tecan Freedom Evo 75 with Immunalysis oral fluid reagents for OF samples. For each cannabinoid, starting concentrations were determined using the following: cutoff and target drug for the cannabinoid ELISA assay, and limit of detection in the confirmatory method. Additional concentrations were analyzed as needed to determine if a drug was cross-reactive, based on the concentration of the cannabinoid at the point of positive detection.

Cannabinoids were evaluated in blood on the Randox instrument and in OF on the Tecan instrument. Both (6aR,9R)-delta-10-Tetrahydrocannabinol (9R-Δ10-THC) and (6aR,9S)-delta-10-Tetrahydrocannabinol (9S-Δ10-THC) were positive in OF starting at 4.0ng/mL. In blood, 9S- Δ10-THC was positive starting at 150ng/mL and 9R- Δ10-THC was positive at 300ng/mL. In OF, Δ8-THC was positive starting at 2.0ng/mL. Δ8-THC was previously evaluated in blood per manufacturer CR studies.[2] More complex cannabinoids such as delta-9-Tetrahydrocannabiphorol (Δ9-THCP), Δ8-THCP, delta-9-Tetrahydrocannabinol acetate ester (Δ9-THCO), and Δ8-THCO were not cross-reactive using either process. 9R-Hexahydrocannabinol (9R-HHC) and 9S-HHC were analyzed in blood. 9R-HHC was positive at 100ng/mL. 9S-HHC had mixed results at 100ng/mL and was positive at 150ng/mL. 9R-HHC and 9S-HHC were not analyzed in OF at this time.

Different cannabinoids can cross-react with various cannabinoid ELISA assays due to the sample matrix and the target of each IA kit. For the cannabinoid assay, Randox uses 11-Nor-9-Carboxy- Δ9-Tetrahydrocannabinol (THC-COOH) at 10ng/mL as the target and Immunalysis uses Δ9-THC at 4ng/mL. During the initial validation of these methods, Δ9-THC, Δ8-THC, and THC-COOH were analyzed to determine the ability to detect these targets.[3] Confidence in the data resulting from CR studies can be increased by analyzing samples in replicates and/or on multiple batches. While drugs with similar chemical structures to the target drug may cross-react with that assay, this is not guaranteed. Knowledge that a drug will cross-react with a specific assay allows for the use of ELISA as a presumptive detection method and prevents the need for multiple screening and confirmation analyses, thus increasing laboratory efficiency. Additionally, CR studies can be used for any drug being added to the laboratory's scope of analysis, not solely cannabinoids. Future aims include performing additional CR studies for drugs in a variety of drug classes as new drugs emerge.

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Immunoassay; Cross-Reactivity; Cannabinoids

L21 An Evaluation of Enzyme-Linked Immunosorbent Assay (ELISA) Screening Kits for the Detection of Nitazene Analogs

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Learning Objectives: Following this presentation, attendees will gain insight into which commercially available ELISA kits, from three different manufacturers, exhibit cross-reactivity with seven distinct nitazene analogs, along with respective concentrations.

Impact Statement: This information will benefit the forensic community by assessing the viability of these kits for laboratories seeking to screen for nitazene analogs using ELISA.

Abstract Text: In the modern-day landscape of Novel Psychoactive Substances (NPS), laboratories may struggle with implementing screening methods capable of detecting emerging drugs. While some laboratories have turned to mass spectrometry-based screening methods, like Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS), others still use immunoassay-based methods such as ELISA due to the high capital costs of high-resolution mass spectrometers. Due to these high-resolution screening techniques being cost prohibitive, it is still important to assess techniques such as ELISA, which is still commonly used in many forensic laboratories. However, immunoassay methods may pose a challenge for detection in an evolving drug landscape since kits are developed for specific drugs or classes and cross-reactivities of new compounds are generally unknown. Since 2019, various NPS nitazene analogs have been identified in forensic casework and have continued to proliferate; however, their detection using ELISA has not been extensively described in the literature. Currently, no commercial ELISA kits exist for the screening of nitazene analogs, and it is unclear whether these compounds show sufficient cross-reactivity with other commonly used kits to produce false positivity.

This project tested nitazene cross-reactivity of commercially available ELISA kits from three forensic manufacturers (Neogen, Immunalysis, and Randox) using whole blood. Fentanyl and oxycodone/oxymorphone kits were purchased from all vendors and opiate kits were purchased from Neogen and Immunalysis. Opiate and opioid kits were chosen for evaluation due to their combination with nitazenes in casework, despite structural dissimilarities to the 2-benzylbenzamidazole class. Non-opioid kits, such as LSD, zolpidem, and tramadol, were chosen for evaluation based on structural similarities to the core nitazene molecule (e.g., diethylamine moiety). A mix of 7 nitazene analogs (4'-OH nitazene, 5-methyl etodesnitazene, isotonitazene, metodesnitazene, N-piperidinyl etonitazene, N-pyrrolidino etonitazene, and protonitazene) were tested at kit cutoff concentrations, 5X cutoff, and 10X cutoff. Manufacturer quality controls were utilized, if provided. Controls that were not provided were made in-house using certified reference materials to achieve the recommended cutoff for the kit's target analytes. Cutoff concentrations, limits of detection, and protocols varied per kit. Quality control and nitazene samples were run in duplicate and all procedures were followed in accordance with instructions provided with each kit. An Agilent BioTek Epoch microplate spectrophotometer was used to read all plates at a wavelength of 450nm.

This study expanded on current literature by testing cross-reactivity of more nitazene analogs across kits targeting different drugs from different manufacturers. All kits that were tested yielded no cross-reactivity with nitazenes at any concentration, demonstrating the importance of mass spectral-based screening methods for nitazene analogs. This affirms that nitazenes will not produce false positives using the traditionally targeted analytes from the evaluated kits and will avoid unnecessary resource allocation to downstream confirmation testing but is disadvantageous for novel opioid detection. Further, these results highlight the need for commercially available nitazene analog ELISA kits for laboratories that utilize immunoassay screening methods and have not yet transitioned to high-resolution screening.

Immunoassay; Novel Synthetic Opioids; Screening Methods

L22 An Incident of Food Poisoning Attributed to Bongkrekic Acid and a Forensic Examination of the Deceased in Taiwan

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Learning Objectives: After attending this presentation, attendees will understand the properties of bongkrekic acid, pathological findings in individuals poisoned by it, and the analytical techniques used to identify bongkrekic acid in postmortem specimens.

Impact Statement: This presentation will impact the forensic science community by conveying the importance of efficient interdisciplinary collaboration as investigation of foodborne outbreak-associated cases usually falls outside the traditional domain of forensic science.

Abstract Text: At the end of March 2024, an incident of foodborne outbreak occurred in Taiwan. Following the consumption of a particular food, numerous individuals exhibited symptoms, including varying degrees of vomiting, diarrhea, abdominal pain, fever, dizziness, weakness, palpitations, and paralysis within a few hours. Six individuals experienced a rapid deterioration in health, progressing to severe complications within a span of 3 to 7 days, ultimately leading to death due to an inability to recover. An interdisciplinary discussion involving the health and forensic departments ensued, leading to a strong suspicion of bongkrekic acid as the etiologic agent based on the food type ingested, clinical manifestations, and disease progression. The forensic department undertook the responsibility of performing autopsies and analyzing bongkrekic acid.

Bongkrekic acid is a mitochondrial toxin produced by *Burkholderia gladioli* pathovar cocovenenans (B. cocovenenans) under particular environmental conditions through utilizing specific fatty acids. This compound blocks the Adenine Nucleotide Translocase (ANT) located on the inner mitochondrial membrane, which acts as an Adenosine Triphosphate/Adenosine Diphosphate (ATP/ADP) exchanger. Consequently, ATPs generated by mitochondria are unable to exit the mitochondrial matrix and reach the cytoplasm for cellular utilization.[1] This interference results in cellular dysfunction, ultimately leading to cell damage or death.

Four out of the six deceased have undergone forensic investigations, including pathology and toxicology examinations. In the pathology section, all four deceased were found to have extensive destruction of liver cells, severe rhabdomyolysis, acute tubular necrosis, disseminated intravascular Coagulation (DIC), local or diffuse alveolar destruction, perivascular edema of the brain, and systemic edema.

In the toxicology part, Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) was employed to quantify the amount of bongkrekic acid in postmortem specimens, using bongkrekic acid-$^{13}C_{28}$ as the internal standard for quantification.[2] A 1g tissue sample was placed into a homogenization tube containing pre-filled ceramic beads, followed by the addition of 2mL of deionized water and the internal standard. Following homogenization, the resulting homogenate was transferred to a screw-capped test tube, to which 2mL of acetic acid/acetate buffer (pH 3.0) and 2mL of ethyl acetate were added. After thorough mixing and centrifugation at 3,500rpm, the upper layer was carefully transferred to a separate tube and evaporated to dryness. The residue was subsequently reconstituted with 100µL of methanol for analysis. A calibration curve was established using pig liver tissue as the matrix, incorporating five concentration points: 0.01, 0.025, 0.05, 0.1, and 0.2µg/g of bongkrekic acid, respectively. The linearity of the calibration curve demonstrated an r^2 value greater than 0.995.

Bongkrekic acid was detected in all four deceased. The concentrations of bongkrekic acid in the tissues of cases 1 to 4 are as follows: brain: 0.022, 0.058, 0.030, 0.024µg/g; lung: 0.657, 1.614, 0.079, 0.207µg/g; heart: 0.230, 0.241, 0.064, 0.171µg/g; spleen: 1.754, 0.976, 0.088, 0.089µg/g; liver: 3.288, 0.607, 0.034, 0.307µg/g; and kidney: 3.293, 2.750, 0.393, 1.698µg/g, respectively. The quantitative results indicate that bongkrekic acid is predominantly concentrated in the liver, lungs, and kidneys. The cause of death was attributed to massive hepatocytic damage and severe rhabdomyolysis. The determination of the manner of death is currently pending due to the incomplete status of the investigation.

This report will present a case study on bongkrekic acid poisoning as an example of the practical application of the technique. It is recommended that medicolegal death investigators become familiar with the principles of bongkrekic acid poisoning.

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Food Poisoning; Forensic Toxicology; Bongkrekic Acid

L23 Assessing the Stability of NPS Benzodiazepines in Blood, Urine, and Serum Using LC/QqQ/MS

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Learning Objectives: After attending this presentation, attendees will be able to understand the effects of storage conditions, matrices, and storage containers on the stability of novel benzodiazepines.

Impact Statement: This presentation will impact the forensic science community by providing stability knowledge of novel benzodiazepines which can be applied to forensic casework and testing.

Abstract Text: Novel Psychoactive Substances (NPS) continue to appear in forensically relevant biological specimens, including postmortem and antemortem specimens. NPS benzodiazepines are one of the largest subclasses observed in forensic cases, either as drugs used on their own or in combination with more common traditional drugs (e.g., fentanyl). Traditional benzodiazepines have a history of being prescribed due to their beneficial pharmacological effects on the central nervous system. NPS benzodiazepines, on the other hand, are often not approved for human use and are encountered as illicit substances masquerading as commonly prescribed drugs like alprazolam. Commonly encountered pharmaceutical benzodiazepines serve as the starting point for NPS derivatives, with structural and pharmacological differences. The evolving nature of NPS benzodiazepines in the United States necessitates increased testing for these drugs, which in turn necessitates understanding about drug stability in biological specimens.

A Liquid Chromatography/Tandem Quadrupole/Mass Spectrometry (LC/QqQ/MS) quantification method was successfully developed and validated for the quantification of six novel benzodiazepines and four metabolites from a previously existing developed method. This scope of testing included bromazolam, clonazolam, etizolam, flubromazolam, flubromazepam, flualprazolam, 8-aminoclonzolam, 3-hydroxy flubromazepam, alpha-hydroxy bromazolam, and alpha-hydroxy clonazolam. Sample preparation was performed using a basic liquid-liquid extraction using sodium bicarbonate buffer (pH 9) and a mix of MTBE and N-butyl chloride (60:40, v:v). Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in methanol. Chromatographic separation was achieved using an Agilent Infinity Lab Poroshell C-18 (2.7µm, 3.0 x 100mm) column using a gradient with standard reverse phase chromatography. The calibration range was 5-500ng/mL. The limit of quantitation was 5ng/mL. A stability study was conducted for the ten benzodiazepines and metabolites in three matrices: blood, urine, and serum. The stability of these drugs was assessed over 120 days. The parent drugs and metabolites were fortified separately, in matrix at a final concentration of 50ng/mL. Blood stability was evaluated in preserved gray top tubes, while urine and serum stability were evaluated unpreserved. Samples were stored at room temperature (~20°C), refrigerator (4°C), and freezer (-20°C). Analysis occurred on days 0, 1, 2, 7, 14, 21, 28, and 120. Twenty-two authentic samples were analyzed, and results were compared to the initial analysis.

In this study, a drug was considered unstable once the concentration decreased 20% from initial. Of the samples stored in gray top tubes, 6 analytes were stable at room temperature, 8 in the refrigerator, and 9 in the freezer. In serum, 8 analytes were stable at room temperature, 9 in the refrigerator, and 8 in the freezer. In urine, 5 analytes were stable at room temperature, 6 in the refrigerator, and 6 in the freezer. For the most accurate quantitation of NPS benzodiazepines, analytes in blood samples stored at 4ºC and -20 ºC were most stable, while analytes in urine and serum samples were most stable at -20 ºC. It is recommended for more reliable concentration data from forensic laboratories to test for the parent drugs (e.g., bromazolam, flubromazepam, etizolam, flubromazolam, and flualprazolam) due to their increased stability in gray top tubes and stored at 4ºC or -20ºC. Clonazolam showed the greatest instability in all matrices, temperatures, and storage containers; however, its metabolite 8-aminoclonazolam displayed greater stability, therefore showing the need for this metabolite in the scope of testing. This information will provide forensic laboratories the greatest opportunity to appropriately identify prevalent novel benzodiazepines present in authentic samples. Understanding the proper storage conditions and containers necessary for collection of samples that may contain NPS benzodiazepines can lead to more reliable interpretation of analytical results and assist in implementing proper protocols for laboratories.

NPS; Toxicology; Stability

L24 Drug/Substance Toxicity-Related Deaths Examined at the West Tennessee Regional Forensic Center and Their Prescription Drug History: A Comparison of 2023 to 2013

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Learning Objectives: This presentation will give attendees a comparison of drug/substance toxicity-related deaths and Controlled Substance Monitoring Database (CSMD) records of the decedents (examined) at the West Tennessee Regional Forensic Center (WTRFC) in 2013 and 2023.

Impact Statement: This study benefits the forensic community by providing an analysis of drug- and substance-related deaths and a discussion of the decedent's controlled prescription drug use in the prior three years of their death, as well as the pattern changes between last year and 10 years before.

Abstract Text: Following the United States opioid epidemic, there has been a progression from prescription drugs (1990-2010) to heroin (2010-2013) and now to fentanyl (2013-present).[1] To combat this growing epidemic, the CSMD was implemented in Tennessee in 2002 in accordance with the Controlled Substance Monitoring Act. CSMD was enhanced again in 2012 and 2016 by the Prescription Safety Acts.[2] However, the trends of the opioid epidemic have shifted away from prescription pills and toward synthetic opioids, particularly fentanyl. The presence of fentanyl has significantly increased the number of fatal overdoses, as it is frequently adulterated in counterfeit pills and illicit substances, particularly heroin, methamphetamine, and cocaine.[3] Shelby County is no exception to these national trends, as, since 2018, there has been an increase in fatal overdoses, a large majority of which can be attributed to fentanyl. Shelby County also leads Tennessee in fatal overdoses, with 577 in 2022. While the Tennessee Health Department has done studies tracking fatal overdoses across the state, there has been no study showing the CSMD prescription history of these decedents. We attempted this study by crosschecking data of drug/substance toxicity-related deaths examined at WTRFC with CSMD data in the three years leading up to deaths. This evaluation was replicated for decedents in 2013, and the data was compared between the two years.

There was an increase in substance-related deaths from 122 decedents (8% of all cases evaluated at our center) in 2013 to 495 (21% of all cases evaluated at our center) in 2023. The most common substances in 2013 were cocaine (n=33, 27%), heroin (n=29, 23%, and alprazolam (n=13, 10%). Of the 122 decedents in 2013, 66 decedents had polydrug toxicity at the time of their death. In 2023, the most common substances were fentanyl (n=405, 81%), cocaine (n=226, 45%), and methamphetamine (n=138, 28%). Of the 495 decedents in 2024, 392 of them had polydrug toxicity at the time of their death. In 2013, 19 decedents (15.5 % of all drug/substance toxicity-related deaths) had a CSMD prescription history, and only 3 of them had the prescribed substance in their system when they died. Of these 19 decedents, 16 had been prescribed an opioid within the three years leading up to their deaths. In 2023, 133 decedents (26.8 % of all drug/substance toxicity-related deaths) had a CSMD prescription history, and only 12 of them had the prescribed substance in their system when they died. Of the 133 decedents that had a CSMD prescription, 117 of them had a prescription for an opioid in the three years leading up to their death.

The number of substance-related deaths in our region has significantly increased in comparison to a decade ago. Although the presence of a correlation is equivocal, the ratio of people with a controlled substance prescription history among this population has also increased.

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Overdose; Toxicity; Fentanyl

L25 Evaluating Extraction Parameters for the Detection of Chronic and Single-Dose Drug Exposure in Forensic Hair Analysis Using Design of Experiments

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Learning Objectives: This research compares different parameters within the extraction procedure of hair analysis using a Design of Experiments (DoE) assessment. Information is provided about the possible use of hair analysis for detecting single doses of drugs in hair for cases of drug-facilitated crime.

Impact Statement: There is limited work on the detection of single doses of drugs in forensic hair samples, as well as no standard method for forensic hair analysis. This research provides a comparison of extraction methods for forensic hair analysis with the ultimate goal of developing a best method for detecting both chronic and single doses of drugs in hair samples.

Abstract Text: Hair as a matrix has proven to be a valuable source for evaluating exposures that extend past the typical detection window for common matrices. It takes an average of ten days for drugs to incorporate into the hair matrix and can be detected weeks to months after exposure. This can be especially helpful in cases of Drug-Facilitated Sexual Assault (DFSA) and Drug-Facilitated Crime (DFC), where a single dose of a drug is suspected. Detecting single doses presents a challenge as it requires very low Limits Of Detection (LOD) and Limits Of Quantitation (LOQ). Currently, there is very minimal work on detecting single doses of drugs in hair and no overall standardized method for forensic hair analysis.

Due to multiple challenging variables, there is a need for an optimized method for forensic hair analysis, which is the overall goal of this study. As the expected concentration range of drugs in hair following a single-dose exposure is in the low pg/mg range, highly sensitive and specific methods are required. DoE is a statistical technique that assesses the correlation between different independent variables and the resulting effects on a dependent variable. The DoE approach is used in this study to compare multiple variables of the hair extraction to determine the most optimal method. This was completed using authentic Hair Reference Material (HRM) with known concentrations to be able to compare the extraction efficiency with each changing variable. A Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) method was developed for 43 drugs and metabolites potentially related to DFC. The method uses an Agilent 1290 UHPLC coupled to a 6470 LC/QqQ/MS/MS) with electrospray ionization in positive and negative modes. The column used was a Zorbax Eclipse Plus C18 column 3.0 x 100mm, 1.8mm with guard column. The gradient starts at 5% B for 5min, then increases to 95% until 8min, and is held for 2min at a flow rate of 0.3mL/min. Aqueous mobile phase (A) is 5mM ammonium formate in 0.1% formic acid in water and organic phase (B) is 0.1% formic acid in methanol. The procedure evaluated was a solvent extraction technique that consisted of an optimized decontamination procedure consisting of one 30min wash with water followed by three 30min washes with dichloromethane.[1] The hair is then dried overnight, weighed out, and placed into a steel milling jar. For homogenization, it was pulverized in a Mini-Bead Beater 24 ball mill in 10s intervals for 30s at 3,200rpm, resulting in finely ground hair.[2] The DoE protocol was a 2^3 factorial assessment that evaluated different sample sizes (5mg or 20mg), extraction times (2h or 6h), and the effect of an ultrasonic bath (with or without) on the method. Ultrasonication paired with the solvent swelling has been shown to improve recovery of certain drugs from hair.[3] Matrix matched calibration curves were created by spiking blank hair extract. The majority of LOD and LOQ values were within ranges 0.01-0.26pg/mg and 0.03-0.81pg/mg, respectively, showing good sensitivity and selectivity. This provides a suitable method for detecting low concentrations of drugs in hair, as commonly seen in cases with single doses.

Future work will include a secondary DoE evaluation of different extraction techniques and the validation of the final optimized method using the American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 036 guidelines. The optimized method will also be evaluated for the detection of single doses of drugs in hair using authentic single-dose samples.

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Hair; Extraction; Method Development

L26 Evaluation of the Cross-Reactivity of 27 Parent and 26 Metabolite Tetrahydrocannabinol Analogs Using a Tetrahydrocannabinol Carboxylic Acid ELISA and HEIA Kit

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Learning Objectives: After attending this presentation, attendees will know the impact of the analogs and analog metabolites on current urine drug screening immunoassay methods, understand the relevant structural moieties contributing to cross-reactivity, and the consequential challenges and application.

Impact Statement: This presentation will inform the forensic science community on cross-reactivity for current cannabinoid analogs in urine using two commercial immunoassay kits, Enzyme-Linked Immunosorbent Assay (ELISA) and Homogenous Enzyme Immunoassay (HEIA), on two different instruments.

Abstract Text: With the federal legalization of hemp (Agriculture Improvement Act of 2018) and increasing decriminalization of Delta 9-Tetrahydrocannabinol (D9-THC) across the United States, the cannabinoid market has experienced exponential growth. This market includes D9-THC, Cannabidiol (CBD), and novel semi-synthetic cannabinoid analogs that include structural alterations in the position of the cyclohexenyl bond in the alicyclic ring, acetylation of the hydroxyl on the aromatic ring, or alteration of the alkyl chain length of D9-THC. These analogs are found in edibles, vape cartridges, and tinctures and many contain unlabeled analogs.

The primary D9-THC metabolite, 11-Nor-9-Carboxy (D9-THC-COOH), is commonly used to presumptively assess marijuana consumption. Emerging semi-synthetic cannabinoids may cross-react with commercially available cannabinoid/THC immunoassays due to structural similarities in both the parent compounds and their respective metabolite molecules.

The cross-reactivity of 27 THC analogs and 26 commercially available metabolites were evaluated in triplicate at 20ng/mL, 50ng/mL, 100ng/mL, 500ng/mL, and 1,000ng/mL utilizing the Immunalysis Cannabinoids (THC) Urine EIA kit on the Medica EasyRA Enzymatic Immunoassay (EIA) and the OraSure Technologies Cannabinoids Intercept Microplate EIA on the Dynex DSX Automated Enzyme-Linked Immunoassay (ELISA) system. Both kits target D9-THC-COOH at a 50ng/mL cutoff.

All analogs were prepared in verified cannabinoid-free urine. ELISA verification controls were prepared relative to the cutoff at 2X, 1X, 1/2X, along with a positive (200ng/mL D9-THC-COOH) and negative quality control (blank urine). The ELISA workflow was: THC-conjugate addition (horseradish peroxidase labeled D9-THC derivative), 30-minute incubation at ambient temperature, six-cycle wash, OraSure substrate (3,3,5,5-tetramethylbenzidine) addition, 30-minute incubation, stopping reagent (2.0N sulfuric acid), and absorbance measurement (450nm). For HEIA, a precision test utilizing the Medica Red Test Dye (n=10), a calibrator at 50ng/mL (n=3), and controls (±25% cutoff) were required to pass before samples were ran. HEIA uses Glucose-6-Phosphate Dehydrogenase (G6PDH) to convert NAD to NADH and an absorbance measurement is read at 340nm. If a positive result occurred at 1,000ng/mL, subsequent dilutions at 500, 100, 50, and 20ng/mL were analyzed to determine the extent of cross-reactivity. Compounds that generated a negative result at 1,000 g/mL were considered undetectable.

Six parent THC analogs at 1000ng/mL (22%) and one at 500ng/mL (4%) were positive at the 50ng/mL cutoff concentration on the ELISA platform. Seven parent analogs at 1,000ng/mL (26%) and four at 500ng/mL (15%) were positive at the 50ng/mL cutoff concentration on the HEIA platform. One THC analog metabolite at 1,000ng/mL (4%), seven at 500ng/mL (27%), seven at 100ng/mL (27%), and 2 at 50ng/mL tested positive (8%) at the 50ng/mL cutoff concentration on ELISA. Three THC analog metabolites (12%) at 500ng/mL and 14 at 100ng/mL (54%) tested positive at the 50ng/mL cutoff concentration on HEIA.

The results on both instruments indicate cross-reactivity at possible relevant concentrations for the carboxy and hydroxy metabolites. The number and position of bonds in the alicyclic ring, the complete cyclic ring compared to the broken cyclic ring as in CBD, and the carboxylic acid group on the alicyclic ring significantly contributed to cross-reactivity. The length of the alkyl sidechain impacted cross-reactivity minimally. The results indicate that high presumptively positive THC rates for delta-9-THC-COOH could be due to the cross-reactivity with cannabinoid analog metabolites and demonstrate certain moieties permit cross-reactivity, which may help laboratories strategically expand current confirmation methods.

Cannabinoids; Cross-Reactivity; ELISA/HEIA

L27 A Forensic Analysis of Environmental Fate and Biodegradation of Benzodiazepines in Synthetic Wastewater Using SPE-LC/MS

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Learning Objectives: The study aims to provide a comprehensive understanding of the environmental persistence and potential bioaccumulation of benzodiazepines in wastewater systems. It seeks to evaluate the degradation patterns of diazepam, etizolam, and their metabolites in both biotic and abiotic conditions, with a particular focus on the role of microbial activity in enhancing their breakdown. Utilizing advanced analytical techniques, such as Solid-Phase Extraction coupled with Liquid Chromatography/Mass Spectrometry (SPE-LC/MS), the research aims to demonstrate effective methods for detecting and quantifying trace levels of these compounds and their transformation products in synthetic wastewater. Additionally, the study investigates the enantiomeric transformation of α-hydroxyetizolam under various environmental conditions to understand how microbial processes influence enantiomeric balance and degradation pathways. Ultimately, the research aims to explore the forensic implications of benzodiazepine degradation patterns, contributing to more accurate interpretation of toxicological results in cases of drug-facilitated crimes and environmental contamination.

Impact Statement: This study provides critical insights into the environmental fate and degradation of benzodiazepines in wastewater systems, highlighting the significant role of microbial activity in breaking down these persistent pharmaceuticals. By utilizing advanced analytical techniques to monitor the transformation of diazepam, etizolam, and their metabolites, the research contributes valuable data for developing more effective wastewater treatment strategies. The findings have important forensic implications, aiding in the accurate interpretation of drug residues in complex legal cases involving drug-facilitated crimes and environmental contamination. Ultimately, this study enhances our understanding of benzodiazepine persistence and biodegradability, supporting the development of improved environmental management and forensic toxicology practices to better protect public health and ecosystems.

Abstract Text: **Introduction:** The widespread use of benzodiazepines has led to their persistent presence in the environment, particularly in wastewater systems, posing risks due to their potential bioaccumulation and harmful effects on ecosystems and human health. Effective forensic and environmental strategies are crucial to mitigate these impacts. Accurate detection and quantification of benzodiazepines are essential for forensic toxicology, especially in cases involving drug-related incidents and environmental contamination. Understanding their degradation behavior in both biotic and abiotic systems is fundamental for assessing their environmental fate and guiding management strategies.[1]

Methods: This study investigates the biodegradation of diazepam and etizolam, along with their main metabolites, in synthetic wastewater matrices prepared to replicate municipal wastewater composition.[2] Reactors were spiked with 1µg/mL of the target drugs and divided into biotic and abiotic systems. SPE-LC/MS was employed to evaluate the persistence and transformation of these benzodiazepines under controlled environmental conditions. The study also examined the enantiomeric transformation of α-hydroxyetizolam using chiral column.

The experiment spanned 14 days, with samples collected on days 0, 1, 2, 3, 7, and 14. Parameters such as pH, optical density in the biotic reactors, and drug concentrations were measured. The samples were analyzed using Liquid Chromatography/Mass Spectrometry-Electrospray Ionization (LC/MS-ESI) in positive ionization mode. Chromatographic separation was achieved using a gradient mobile phase of Solvent A (0.1% formic acid in H_2O) and Solvent B (acetonitrile with 0.1% formic acid) at a flow rate of 0.7mL/min, ensuring efficient separation and analysis of the target compounds.

Results and Discussion: The results revealed significant degradation of diazepam and etizolam, especially in biotic reactors, highlighting the role of microbial activity in their breakdown. Etizolam showed a reduction of 35.64% in the abiotic reactor and 44.44% in the biotic reactor, demonstrating that microbial activity enhances its degradation.

The behavior of α-hydroxyetizolam enantiomers varied between systems. Enantiomer E1 showed consistent degradation across both systems, decreasing by 65.57%, indicating similar susceptibility to non-biological and biological processes. In contrast, enantiomer E2 exhibited more variability, with a greater reduction in the abiotic reactor (70.59%) compared to the biotic reactor (64.86%), suggesting it is more prone to abiotic degradation, possibly due to hydrolysis, while still being influenced by microbial activity. The enantiomeric excess (%ee) of E1 remained stable in the abiotic reactor, while the biotic reactor showed fluctuations, indicating that microbial processes affect the enantiomeric balance and transformation pathways of α-hydroxyetizolam.[3,4]

Diazepam also underwent significant degradation, with a 45.83% reduction in the abiotic reactor and 66.67% in the biotic reactor, further underscoring the role of microbial activity in its breakdown. In contrast, oxazepam and temazepam displayed minimal to moderate degradation, indicating higher stability. Oxazepam showed reductions of 6.25% in the abiotic reactor and 12.5% in the biotic reactor, while temazepam decreased by 16.67% and 41.67%, respectively. These findings suggest that while oxazepam and temazepam are more resistant to degradation, microbial processes still influence their environmental fate.[3]

The results align with existing literature, emphasizing the persistence and limited biodegradability of benzodiazepines and the need for advanced treatment strategies to mitigate their impact. Understanding the degradation pathways and persistence of these drugs is crucial for accurate toxicological interpretations in cases of drug-facilitated crimes and environmental contamination. Incorporating microbial degradation data into forensic investigations can improve the accuracy of interpreting drug residues in complex environmental and legal contexts.[5]

Conclusion: In conclusion, the study demonstrated significant microbial degradation of diazepam and etizolam in synthetic wastewater, with reductions of 66.67% and 44.44% in biotic reactors, respectively. The limited degradation of oxazepam and temazepam suggests their higher stability and persistence. These findings are essential for understanding the environmental fate of benzodiazepines and have significant implications for forensic and environmental management strategies.

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Designer Benzodiazepines; LC/MS; Chirality

L28 Gabapentin Detection and Trends in Alabama Toxicology Cases (2017–2023)

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Learning Objectives: Attendees will acquire knowledge about the occurrence and prevalence of gabapentin in toxicology cases within Alabama.

Impact Statement: This presentation will serve the forensic science community by showcasing data on gabapentin's prevalence in Alabama. It will highlight the challenges associated with the absence of routine testing for gabapentin and explore potential methods to enhance testing protocols moving forward.

Abstract Text: **Background/Introduction:** In 1993, gabapentin was approved for use in the United States as a muscle relaxant and anti-epileptic drug.[1,2] Today it is also used to treat psychiatric disorders, alcohol withdrawal, restless leg syndrome, and neuropathic pain.[1,2] From 2004 to 2021, gabapentin prescriptions surged from 18 to 47 million.[3] Off-label pain management make up 95% of gabapentin prescriptions.[4] When combined with opioids, gabapentin can enhance analgesic effects and induce euphoria.[4] Due to its increasing prevalence, 23% of laboratories surveyed in of the "2024 Updates for Recommendations for Drug Testing in DUID & Traffic Fatality Investigations" suggested moving gabapentin from Tier II to a Tier I analyte.[5]

Objective: This study aims to investigate the prevalence of gabapentin cases analyzed by the Alabama Department of Forensic Sciences (ADFS) from 2017 to 2023 and to identify noteworthy cases. Confirmed gabapentin-positive cases will be evaluated based on case type, concentration, and the most common drugs found in combination.

Method: Gabapentin testing is primarily guided by case history or special request, as it does not cross-react with any assays in the current immunoassay analysis methods at ADFS. Specimens undergo protein precipitation and are then analyzed using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The calibration range for gabapentin is 1.0µg/mL to 40µg/mL with a limit of detection of 0.125µg/mL. Additionally, gabapentin cases can be identified with expanded toxicology testing (e.g., Quadrupole Time-Of-Flight/Liquid Chromatography/Mass Spectrometry (QTOF/LC/MS). Expanded toxicology testing is conducted in the absence of case history or specific requests to ensure comprehensive drug detection.

Results: Between 2017 and 2023, 682 instances of gabapentin were detected. Of these, 7% (n=45) were gabapentin-only cases, while 93% (n=637) involved gabapentin in combination with other drugs. Non-traffic fatalities comprised 72% (n=496) with a median concentration of 11µg/mL. Driving Under the Influence (DUI) cases accounted for 24% (n=162) with a median concentration of 7µg/mL, while traffic/non-death incidents made up 2% (n=17) with a median of 8.4µg/mL. Traffic fatalities represented 1% (n=7) of the cases, with a median concentration of 3.9µg/mL. The drugs most commonly found in combination with gabapentin were hydrocodone, amphetamine, alprazolam, oxycodone, and methamphetamine. In 2023, there were 99 gabapentin cases out of a total of 5,974 cases. Gabapentin ranked sixteenth (1.3%) in prevalence among DUI and traffic crash case types reported by ADFS in 2023.

Case Study #1: A 37-year-old White male was pulled over on suspicion of driving under the influence. The driver admitted to taking amlodipine and benazepril for blood pressure. Toxicological findings revealed 66µg/mL gabapentin, 0.18ng/mL fentanyl, mitragynine, and tianeptine. The Drug Recognition Expert (DRE) opinion indicated the suspected use of narcotic analgesics and Central Nervous System (CNS) depressants.

Case Study #2: A 37-year-old White male was pulled over for making an improper turn. The subject was prescribed suboxone and gabapentin for the treatment of opioid addiction. Toxicological findings revealed 15µg/mL gabapentin, 1.9ng/mL buprenorphine, and 1.5ng/mL norbuprenorphine. The DRE determined the subject was under the influence of narcotic analgesics and CNS depressants and thus unable to operate a vehicle safely.

Conclusion/Discussion: In 2023, gabapentin ranked sixteenth in prevalence among DUI and traffic-related cases in Alabama. This ranking is likely underestimated due to the absence of routine gabapentin testing. Considering the ongoing opioid crisis, implementing gabapentin testing in opioid-related cases would be beneficial as it has been shown that gabapentin can enhance opioid-derived effects. Currently, there are no immunoassay kits for detecting gabapentin in whole blood or oral fluid samples. Including gabapentin as a target in the general unknown screening method using QTOF/LC/MS) could bridge this gap until immunoassay screening options become available.

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Gabapentin; Toxicology; Casework

L29 Improving Liquid Chromatography/Mass Spectrometry Sensitivity for Barbiturates

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Learning Objectives: Attendees will learn about High-Performance Liquid Chromatography (HPLC) and the impact of various variables such as mobile phase, pH, column geometry, and stationary phase chemistry on retention times, peak shapes, and sensitivity when coupled with a triple quadrupole mass spectrometer. In addition to HPLC parameters, attendees will explore the impact of Mass Spectrometry (MS) parameters on sensitivity. The session will also cover the differences between positive and negative ion mode MS and the utilization of chemical derivatization.

Impact Statement: This project has significant implications to the forensic science community, specifically toxicology, by enhancing the sensitivity and reliability of barbiturate detection across a variety of toxicological specimens. Barbiturates, being weak acids, require the use of negative ion mode in MS; however, many instruments are less reliable in this mode, leading to decreased sensitivity. Additionally, many of the drugs found in toxicology panels are weak bases, necessitating a separate run in positive ion mode. Improving sensitivity in negative ion mode can result in more accurate quantitation and greater reproducibility in complex biological matrices. The optimized mass spectrometer parameters and innovative chromatographic techniques can be applied to any biological matrix when paired with a matrix-specific extraction. Enhancing sensitivity in positive ion mode would have a similar impact, while also reducing workload and run times in forensic labs. Overall, this methodology has the potential to streamline drug panel analyses, enabling toxicologists to deliver more precise and reliable results when working with this challenging drug class.

Abstract Text: Many drugs encountered by forensics labs, such as amphetamines and opioids, are weak bases that produce a positive ion, whereas barbiturates are weak acids that form negative ions, necessitating the use of both positive and negative ion modes when analyzing a large drug panel, causing a consequential expenditure of resources. Negative ion mode is less reliable on many instruments, leading to decreased signal intensity and posing challenges in detection limits and reproducibility for drug analysis. This project aims to provide the forensic science community with a more sensitive method for analyzing barbiturates in both negative and positive ion modes.

The specific drugs used were barbital, phenobarbital, butalbital, butabarbital, pentobarbital, amobarbital, and secobarbital. The first step of this project involved performing infusions on each of the drugs to determine ideal mass spectrometer parameters. Despite their structural similarities, each barbiturate had varying optimal values for vaporizer temperature, spray voltage, and capillary temperature. A median value was selected to provide satisfactory results for all drugs. Ideal values for the S-lens, collision energy, and product ion were also obtained for each drug. With optimal MS conditions in place, a gradient of 20% to 35% ACN over 8 minutes was used to achieve baseline separation. Mobile phase A was chosen to be a 2.5mM ammonium acetate buffer to enhance the sensitivity of barbiturates. Amobarbital and pentobarbital have identical masses of 226.27g/mol and very similar structures, causing them to co-elute. Since the focus of this project is sensitivity, amobarbital was excluded from the panel when generating standard curves, though it was still run on each column to assess the impact of column geometry and stationary phase on separation.

Several columns with varying stationary phases, lengths, silica particles, and internal diameters were tested. While column length did not significantly affect sensitivity, it did enhance selectivity between the co-eluting peaks. Superficially porous silica particles significantly improved sensitivity and selectivity compared to fully porous silica particles. Reducing the internal diameter from 2.1mm to 1.5mm provided a near two-fold sensitivity enhancement and near-baseline separation of the co-eluting peaks; this method delivered the best sensitivity improvement . Comparisons were primarily made using C18 reverse-phase material, but a biphenyl phase and a high pH phase were also tested. The biphenyl phase reduced sensitivity and selectivity between the co-eluting peaks compared to the C18. Adjusting the mobile phase pH from 4.22 to 9.52 using ammonium hydroxide was expected to enhance ionization and sensitivity but instead halved the sensitivity on the high pH phase column. While these tests were conducted in negative ion mode, positive ion mode might yield better results, achievable via derivatization; an experiment using tetramethylammonium hydroxide as the derivatizing agent is planned for the near future.

Drug Analysis; Liquid Chromatography; Mass Spectrometry

L30 Matrix-Matched Standards for Quantitative Elemental and Molecular Analysis of Hair in Forensic Toxicology

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Learning Objectives: This presentation will demonstrate the importance of the development of a calibration standard for chemical analysis of hair in forensic toxicology. This study explores direct sampling of hair to measure toxic exposure by laser-based analysis.

Impact Statement: There is a need for a matrix-matched reference material that emulates the chemical and physical properties of human hair. This material will allow for quantitative analysis of toxic metals by Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) and drug metabolites by Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS) in a single hair. These results can pave the way for a new set of calibration standards for chemical analysis of hair in forensic science.

Abstract Text: In forensic toxicology, the analysis of hair is an established analytical procedure for the detection of drugs of abuse.[1] In comparison to other biological matrices, hair can be useful as a source of biological evidence as it provides a minimally invasive sample collection and a temporal record of analytes in the body. Hair is a routinely analyzed biological sample; however, there is a lack of reference materials that are matrix-matched.[2]

Laser-based analysis can provide direct sampling of hair with limited sample preparation and minimal sample destruction. For quantification of potential toxins, it is necessary for a standard to be matrix matched. Analytically, it is imperative for a calibration standard to show similarity to the chemical, physical, and optical properties of its matrix to reduce sample matrix effects. MALDI-MS is a growing technology, providing identification and imaging of analytes in proteins. LA-ICP/MS is a highly sensitive technique, providing elemental analysis of exposure within hair samples. We have developed a method for a new reference material using keratin film as a matrix-matched calibration standard for laser-based analysis. A new reference standard for human hair will provide opportunity for single hair analysis to obtain a quantitative chronological record of potential exposure. For this study, keratin was extracted from human hair, purified, spiked, and cross-linked to obtain a thin film. The films were doped with metals (Zn, Cu, As, etc.) and drug metabolite (Benzoylecgonine[BE]) to use as a calibration material for LA-ICP/MS and MALDI-MS analysis. The concentration of the material was validated by ICP-MS and Liquid Chromatography/tandem Mass Spectrometry (LC/MS/MS). Analytical figures of merit including Limit Of Detection (LOD), Limit Of Quantitation (LOQ), yield, and linearity were studied. These results provide a novel approach to quantify toxic metals and drug metabolites in human hair.

This study is supported by the National Institute of Justice under award "Development of a Stable Matrix-Matched Standard for Molecular and Elemental Analysis of Hair in Forensic Toxicology" (15PNIJ-23-GG-01945-RESS).

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Hair; Laser; Spectroscopy

L31 Modification of a Liquid Chromatography/Tandem Mass Spectrometry Method Targeting Lysergic Acid Diethylamide and Its Primary Metabolite to Include Nine Analogs

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Learning Objectives: After attending this presentation, attendees will have gained knowledge of an automated extraction method to detect Lysergic Acid Diethylamide (LSD) and nine analogs in human urine. Furthermore, attendees will observe the application of the method to 325 specimens as part of an ongoing surveillance program. Attendees will additionally increase their understanding of the impact of liquid chromatography conditions such as mobile phase and column particle size in method development.

Impact Statement: This presentation will impact the forensic science community by demonstrating a procedure that can assist with routine analysis and surveillance activities involved with novel psychoactive psychedelics, while providing results compliant with best laboratory practices in forensic toxicology. In addition, the significance of detailed chromatographic method development can be applied to numerous analyses.

Abstract Text: **Hypothesis:** The low confirmation rate for specimens presumptively positive for LSD via immunoassay and Agilent Rapid-Fire (RF)-tandem Mass Spectrometry (MS/MS) screen was hypothesized to be due, in part, to the presence of LSD analogs in the urine samples due to common product ions and the potential of shared metabolic pathways.

Methods: A previously published automated sample preparation method was utilized for the urine extraction of ALD-52, AL-LAD, 1B-LSD, 1cP-LSD, 1P-LSD, 1V-LSD, LSD, LSM-775, LSZ, OH-LSD, and PRO-LAD.[1] The instrumental component employed the use of a Phenomenex Kinetex biphenyl (100mm x 2.1mm, 2.6µm) Liquid Chromatography (LC) column and gradient elution with 0.1% formic acid in deionized water and 0.1% formic acid in acetonitrile; however, the nine additional LSD analogs were not adequately separated with these conditions. To optimize the chromatographic results, the analysis was shifted to an Agilent Zorbax Eclipse Plus C18 Rapid Resolution (50mm x 2.1mm, 1.8µm) LC column. The aqueous mobile phase was modified to include a buffer, and the organic mobile phase was modified from acetonitrile to methanol to decrease elution strength and aid in selectivity. The method was validated to follow the standards set forth by American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 036. Qualitative validation experiments included limit of detection, interference, carryover, ionization suppression/enhancement, and extract stability. The validated method was then utilized for the analysis of 325 specimens: 125 specimens that had screened presumptively positive via immunoassay and RF-MS/MS adjunct screen; 100 specimens that had screened presumptively positive by immunoassay, but negative via RF-MS/MS; and 100 randomly selected urine specimens that had no prior LSD-positive screen.

Results: The newly developed method allowed for improved analyte separation and peak shape while maintaining the analytical time seen in the previous method. The qualitative method was satisfactorily validated to ANSI/ASB Standard 036. The limit of detection was administratively defined at 0.1ng/mL. No exogenous or endogenous interferences were observed, and carryover studies were acceptable at 50ng/mL. Normalized ionization suppression/enhancement ranged from -22.3% to 5.0%. Results from processed sample stability studies demonstrated that all analytes were stable at 15°C up to 96h after sample preparation. The original hypothesis of a positive LSD immunoassay signaling the presence of one or more LSD analogs was not supported, as there were no LSD analogs detected in the 325 specimens.

Conclusion: Although no LSD analogs were observed in the samples analyzed, the validation results demonstrated that this method was suitable for the analysis of LSD, OH-LSD, and nine LSD analogs. This method could likely be applied to other matrices with little optimization needed. Automated sample preparation allowed for efficient analysis of these analytically challenging compounds with minimal manual handling. There also was no increased analytical time burden when modifying the method from 2 to 11 analytes. This method demonstrates how the chromatographic column and conditions can be modified to optimize separation of analytical targets while maintaining reasonable analytical runtimes. If LSD analogs are in use in the population, detection will likely improve as reference standards are made available for metabolic products of these compounds. This confirmatory method is suitable for routine LSD and OH-LSD analysis in addition to ongoing surveillance of the LSD analogs in locations where LSD and LSD analog use is of concern.

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Liquid Chromatography; LSD; Automation

L32 Old vs. New: A Novel Approach to Blood Alcohol Analysis In the Modern Day Forensic Laboratory

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Learning Objectives: The main learning objective for this project is the development of an instrument set up that allows for the analysis of blood alcohol headspace samples along with solvent extracted whole blood drug samples on a single instrument. What sets this apart from traditional dual column, dual Flame Ionization Detector (FID) blood alcohol analysis is the utilization of a single, versatile autosampler that has the capabilities to sample both headspace samples and liquid extracted samples. We will also utilize a blood alcohol Gas Chromatography (GC) column with tolerances up to 320°C, which allows the toxicologist to also run high temperature analyses for their blood drug tests on the same instrument. We will investigate the precision, accuracy, and reproducibility differences between this new testing approach vs. the traditional dual column, dual FID set up.

Impact Statement: The work performed on this project addresses the limitations faced by forensic toxicologists when it comes to the versatility of the instruments that they can use for blood alcohol headspace testing. The main limitation is the fact that the traditional blood alcohol instrument set up can only be used for blood alcohol/volatiles analysis and no other qualitative or quantitative test in the laboratory. The goal of this project is to seek out an alternate instrument set up and testing method using a GC column not traditionally used in blood alcohol analysis and an autosampler that can perform the headspace analysis along with other toxicology related sample analysis.

Abstract Text: GC/FID analysis for the measurement of blood alcohol in forensic samples utilizing the dual column, dual FID set up in forensic samples has been the gold standard in the forensic toxicology laboratories for many years. There are a few forensic toxicology laboratories that use a different approach wherein they split a blood alcohol sample coming off of the GC column onto an FID detector and a mass spectrometer so that they could use both the retention time from the FID and the mass spectrum from the mass spectrometer to verify a blood alcohol result. Whether these labs used the dual column-dual FID method or the GC-FID/MS method, the main limitation for headspace analysis of blood alcohol samples on almost every instrument is that the analyst always had to use an instrument whose sole purpose in the forensic toxicology laboratory was for blood alcohol analysis. This limited the variety of testing that could be performed on that instrument. The work performed on this project addresses the limitations faced by forensic toxicologists when it comes to the versatility of the instruments that they can use for this testing. The main limitations for not being able to use these instruments for other toxicology laboratory testing purposes are the low temperature tolerances of the traditional blood alcohol GC columns and the limited sampling capabilities of a traditional headspace autosampler. This project seeks out an alternate testing method using a GC column not traditionally used in blood alcohol analysis and an autosampler that can perform the headspace analysis and liquid sampling analysis used in blood drug toxicology testing.

The objective in this project is to develop a robust and versatile instrument setup that allows for the analysis of blood alcohol headspace samples on an instrument that can also analyze blood drug samples. This is accomplished by utilizing a versatile autosampler and single GC column split into an FID detector and MS detector for accurate quantitation and identification of volatile components. We then compare the precision, accuracy and reproducibility of the headspace samples for this instrument set up versus the results of the same headspace samples on a traditional headspace sampler with dual GC column, dual FID detector set up.

In attaining the precision, accuracy, and reproducibility results of these methods, a volatile component Certified Reference Materials (CRM) from Cerilliant was used. An N-propanol (1%) internal standard was added to each sample for quantitative purposes. The CRM samples consist of aqueous ethanol calibrators ranging from 0.01g/dL to 0.50g/dL, a multi-component standard containing ethanol, acetone, isopropanol, and methanol as well as a separate CRM containing acetaldehyde to attain proper retention times of these other analytes. Two controls (a high and low control) will be run with the calibration standards to assess the accuracy of the calibration curve. The samples will consist of 100uL of sample and 1,000uL of the internal standard.

In comparing the differences between the traditional instrument setup and this new set up, we utilized three GC columns on two different instruments. For the traditional dual column, dual FID setup, a Triplus 500 headspace instrument, a TG-ALC 1 (30m x 0.32mm ID x 1.80um) and TG-ALC 2 (30m x 0.32mmID x 1.20um) column were used with each being tied to an individual FID detector. For the new instrument setup being used for this project, a Triplus smart autosampler and a TG-624 sil MS column (30m x 0.25mmID x 1.4um) were used for the chromatographic separation with the sample being split onto an FID detector and a single quad mass spectrometer (Thermo Fisher ISQ 7610).

Chromatography; Forensic Analysis; Blood Alcohol Concentration

L33 Profiling Heavy Metals in Kratom Powder Purchased in Southeastern Pennsylvania and Southern New Jersey

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Learning Objectives: Attending this poster, participants will learn about the heavy metal profile present in several strains of the most common commercially available kratom powders

Impact Statement: The Drug Enforcement Administration (DEA) has refrained from scheduling kratom pending further public comment but rather has identified it as a drug/chemical of concern.[1] This research contributes to the growing body of literature investigating the toxicological impact of the drug.

Abstract Text: Kratom is a tropical tree native to Southeast Asia known to induce opioid-like effects arising from the presence of mitragynine and 7-OH-mitragynine.[2] The different strains of kratom (Red, Green, and White) are named for the vein color of the plant's leaves, with each having a unique profile of effects. As with other plant-based substances such as cannabis, the potential exists for phytoaccumulation of heavy metals. In a 2019 report, researchers at the Food and Drug Administration (FDA) found significant concentrations of Ni and Pb in the kratom products studied.[3] This was expanded upon by Prozialeck et al. who analyzed kratom products purchased in the Western suburbs of Chicago for a broader panel of metals. The researchers reported high concentrations of Pb, Ni, Fe and Cr along with traces of As, Cd, and Hg.[4] A follow-up study by Fleming et al. assayed kratom products purchased in the Richmond, VA, area yet reported that only Mn was identified as a potential risk for users.[5]

The objective of this study is to add to the growing body of literature surrounding the potential for heavy metals poisoning through ingestion of kratom products which in turn could result in nervous system or kidney damage, anemia, and increased risk of certain cancers.[6] The results of this study were compared with those which have previously been reported. Additionally, correlations between kratom strain, country of origin, and mitragynine concentration were explored.

Twenty kratom powders representing the three strains (7 Red Vein, 5 Green Vein, 3 White Vein, and 5 indeterminate were purchased from smoke shops and gas stations in southeastern Pennsylvania and southern New Jersey. Four different countries of origin were represented: Indonesia, Malaysia, Brunei, and Thailand. Samples were stored in the dark and refrigerated in their original packaging until processed for analysis.

Three replicate digestions of each sample (0.25g each) to which was added 1.0mL HNO_3, 0.5mLs HCl, and 2ppb Au (to reduce Hg carryover) were prepared using microwave-assisted digestion and diluted to a final volume of 50mLs followed by Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) analysis. Each sample batch included a digestion blank and second source QC standard along with a Continuing Calibration Blank (CCB) and a Continuing Calibration Verification (CCV) standard. The elements assayed were a combined panel of those previously reported on by both Prozialeck and Fleming.

The results of this study were in general agreement with those previously reported. Elevated levels of Cr (0.9ug/g to 6.1ug/g), Pb (0.32ug/g to 1.58ug/g) and Mn (1,100ug/g to 2,250ug/g) were identified, which could result in heavy metal poisoning of the user based upon common dosing practice and frequency of administration. The reminder of the panel (As, Cd, Fe, Hg, Al, Cu and Se) were all found to be present but at levels well below the toxic threshold. The concentration of Ni (1.3 to 6.5ug/g) was also well below toxic levels in contrast to what was previously reported by both Prozialeck and the FDA.

This study has concluded that concentrations of Cr, Pb, and Mn in select kratom powders commercially available in Southeastern Pennsylvania and Southern New Jersey may exceed the regulatory guidance outlined by the CDC Agency for Toxic Substances and Disease Registry (ATSDR) ,thereby posing a hazard to users. This is true at moderate (3-6g) and heavy (6-8g) doses and for those users who typically dose 2-3 times daily. No statistically significant difference was observed when comparing the concentrations of Cr, Pb, and Mn between the three strains. The limited geographic representation of samples derived from Brunei and Thailand made it difficult to draw any conclusions regarding the impact of country of origin.

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Metals; Toxicity; Drug Analysis

L34 The Quantitation of an Oral Fluid Drug Panel, Including THC, Using High Resolution Accurate-Mass (HRAM) Orbitrap Mass Spectrometry

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Learning Objectives: The objectives of this work was to understand how to run an oral fluid drug panel to satisfy oral fluid cutoff level guidelines and learn how Orbitrap Mass Spectrometry achieves sensitive levels while being able to perform retrospective analysis.

Impact Statement: This fast and quantitative method on the Orbitrap Exploris 120 was developed around 31 drugs of abuse specified by the Substance Abuse and Mental Health Services Administration (SAMHSA) and the National Safety Council's Alcohol, Drugs and Impairment Division, satisfies cutoff levels, and provides linearity through a wide dynamic range, making this method optimal for labs that want to incorporate oral fluid drug testing into their labs.

Abstract Text: **Background:** As labs move toward oral fluid for ease of collection and roadside testing, it is important to be able to test for a wide range of analytes and achieve required sensitivity. With the SAMHSA guidelines providing Limit Of Quantitation (LOQ) levels, the extraction protocol and instrumentation need to be sensitive enough to accomplish these cut-offs. Including Tetrahydrocannabinol (THC) into the assay provides challenges in the extraction as most drugs of abuse are basic and THC is neutral. This extraction workflow, which extracts THC delta-9 alongside other drugs of abuse, coupled with the Orbitrap mass spectrometer generates high-resolution accurate mass data that offers improved sensitivity, selectivity, and accuracy for detection and quantitation of drugs of abuse in oral fluid.

Methods: Nine calibration levels (ranging from 0.5 to 200ng/mL) were made by spiking stock solution of the 31 target analytes from a wide range of drug classes (including opioids, benzodiazepines, stimulants, and THC) into human oral fluid. Samples were diluted with a preserving buffer and spiked with their corresponding internal standard. 500µL of each sample were then extracted using DPX INTip SCX/WAX SPE. The SPE tips were washed with 30% methanol following the sample binding step and eluted with 48/48/4 acetonitrile/methanol/ammonium hydroxise. Drug analytes were separated with on a Thermo Scientific Accucore Biphenyl column connected to a Thermo Scientific Vanquish Horizon UHPLC system using a fast 7-minute method. Data was acquired on the Thermo Scientific Orbitrap Exploris 120 mass spectrometer using data dependent MS2 mode (ddMS2) with an inclusion list for the 31 target drugs. Thermo Scientific TraceFinder 5.2 software was used for data acquisition and processing.

Results: LOQ determined for the 31 drugs were all below the new SAMHSA guidelines cutoffs and linearity was achieved from as low as 0.5ng/mL to an Upper Limit Of Linearity (ULOL) of 1,000ng/mL for 6-MAM, fentanyl, hydrocodone, and methadone. THC delta-9 achieved an LOQ of 1ng/mL. All drugs were confirmed with mass accuracy of less than 5ppm, retention times, and library matching. This study was also able to overcome some of the issues commonly associated with THC, including "stickiness" of the drug to consumables and its susceptibility to being suppressed by oral fluid collection device buffers.

Toxicology; High Resolution; Oral Fluid

L35 The Rapid Development of Analytical Methods and the Prediction of Retention Times for Inhalants of Abuse on GC Stationary Phases Using Web-Based Modeling Software

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WITHDRAWN

L36 The Detection and Quantitation of Alcohol in Packed Red Blood Cells

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Learning Objectives: After attending this presentation, attendees will understand the significance of including ethanol testing in blood screening to enhance transfusion safety and address potential legal concerns.

Impact Statement: This presentation will impact the forensic science community by providing information about the potential presence of ethanol in donated blood used for transfusions.

Abstract Text: Current blood testing includes ABO/Rh typing and screening for infectious diseases but does not routinely test for ethanol. As a direct indicator of alcohol consumption, ethanol can affect blood quality, interfere with medical treatments, and present legal issues, such as false conclusions or assumptions. This study focuses on the identification and quantification of ethanol in Packed Red Blood Cells (PRBCs) collected from blood donor units using Headspace/Gas Chromatography (HS/GC). The presented study focuses on the presence of ethanol, but its findings will also support the potential use of other long-term biomarkers for monitoring ethanol consumption, such as Phosphatidylethanol (PEth).

The study utilized an existing validated method for the analysis of volatiles routinely employed in forensic and clinical testing. A Shimadzu 2014 GC/FID and Tekmar HT3 Headspace system were used, with the oven temperature set at 50°C and the injection port at 200°C in split injection mode, using nitrogen as the carrier gas. The column used was Rtx-BAC1 30m x 0.32mm ID x 1.80µm. The detector operated at 225°C. Sample preparation included pipetting 200µL of calibrator, control, or sample into a headspace vial with 2.0mL of working internal standard (n-propanol, 0.0234g/dL). Headspace vials were heated and analyzed, with quantification based on a calibration curve with limits of quantitation and detection of 0.010g/dL for ethanol, methanol, acetone, and isopropanol.

Accuracy and precision were validated through quality control samples, showing less than 5% variation. Positive controls were analyzed in duplicate, with deviations limited to ±6.0% for ethanol and ±10% for other alcohols. Negative controls ensured ethanol levels stayed below 0.010g/dL.

A total of 1,057 de-identified PRBC segments were analyzed, with ethanol detected in four cases at 0.011, 0.012, 0.014, and 0.028g/dL. Assuming a 70kg individual with a volume of distribution of 0.7L/kg, a person with a total body water volume of 49 liters receiving a transfusion of one pint of blood with an ethanol concentration of 0.02g/dL would experience only a 0.00019 g/dL increase in their BAC. This amount of ethanol in a naïve individual would not be reported by a laboratory and this minimal increase in an individual with ethanol present would be within the metrological error reported by the laboratory. These results demonstrate that low concentrations of ethanol may be detected in a small percentage of donor PRBCs (0.38%), but the overall impact on transfused individuals' Blood Alcohol Concentration (BAC) is negligible. The additional volatiles analyzed (acetone, isopropanol, and methanol) were below the detection limit in all PRBC segments.

In cases involving motor vehicle accidents or other trauma requiring transfusions, the presence of ethanol in transfused blood can have legal implications but is unlikely to significantly affect forensic interpretations. Ethanol biomarkers, such as PEth, have a longer half-life and remain detectable in blood much longer than ethanol. Due to its prolonged presence, PEth is likely to be detected at a higher positivity rate compared to ethanol, which may raise concerns in vulnerable populations such as pediatric patients or those receiving multiple transfusions. Therefore, analyzing these biomarkers is essential, with implications for clinical and forensic interpretations beyond Driving Under the Influence (DUI) cases.

The presence of alcohol and its biomarkers in a recipient's system due to a blood transfusion could lead to prejudicial conclusions or incorrect assumptions in sensitive situations, such as child custody or probation cases with zero tolerance policies. This underscores the need for rigorous screening and testing of blood donations to ensure not only the safety and health of recipients but improve accuracy in forensic investigations and clinical diagnoses.

Alcohol; Headspace/Gas Chromatography; Blood Screening

L37 The Extraction, Isolation, and Analysis of Clonazepam From Blood Using the High-Performance Thin-Layer Chromatography (HPTLC) Technique in Different Solvent Systems

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WITHDRAWN

L38 The Postmortem Distribution of the Synthetic Cathinone MDPHP in a Fatal Intoxication Case

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Learning Objectives: After attending this presentation, participants will understand key aspects of fatal intoxication by a synthetic cathinone 3,4-Methylenedioxy-A-Pyrrolidinohexanophenone (MDPHP). To the best of our knowledge, this was the first quantification of this synthetic cathinone in vitreous humors and hair, and the first comparison between central and peripheric blood amounts in a postmortem case.

Impact Statement: This presentation will impact the forensic science community by stimulating debate about the use of traditional methods in unconventional intoxication and encouraging researchers to publish their results to build a reference database.

Abstract Text: Synthetic Cathinones (SCs) are a class of New Psychoactive Substances (NPS) that mimic the effects of stimulants like amphetamines and cocaine. SCs have become increasingly prevalent, making up over 52% of seized New Psychoactive Substances (NPS), with MDPHP among the most common.[1] MDPHP is a synthetic cathinone structurally correlated to 3,4-Methylenedioxypyrovalerone (MDPV). It acts as a central nervous system stimulant and its main effects are hallucinations, paranoia, tachycardia, hypertension, chest pain, and rhabdomyolysis. In recent years, the number of intoxication cases has increased even if little is known about its pharmacokinetics properties. In particular, Postmortem (PM) distribution is unknown as only two lethal intoxications, involving an adult man and a newborn, have been previously reported.[2,3] In this case report, we describe a death caused by MDPHP and a first postmortem redistribution.

A 30-year-old man with a history of cocaine use was found dead in his room. The PM interval was estimated at 3-4 hours. The autopsy revealed non-specific findings, including cerebral and pulmonary oedema, with no injection marks observed. For toxicological analysis, Central and Peripheral Blood (CB and PB), left and right Vitreous Humor (lVH and rVH), Gastric Content (GC), Urine (U), and hair (two segments: proximal 0.0-1.5 and 1.5-3.0 cm) samples were collected during the autopsy. Toxicological analyses were conducted following previously published and validated methods.[4-7] BAC was measured by headspace gas chromatography; common drugs of abused were detected by gas chromatography/mass spectrometry; benzodiazepines and NPS were analyzed by Liquid Chromatography/tandem Mass Spectrometry (LC/MS/MS). Briefly, MDPHP quantification in biological fluids was performed by a protein precipitation with acetonitrile. Hair segments were extracted by sonication (2h, at room temperature) with methanol (0.1% formic acid).

BAC was 0.02g/L, thus alcohol did not contribute to the cause of death. The only psychoactive substance detected in the biological fluids was MDPHP. It was quantified at the following concentrations: 1,640ng/mL in central blood, 1,602ng/mL in peripheral blood, 12,954ng/mL in urine, 3,029ng/mL in gastric contents, 1,846ng/mL in right vitreous humour, and 2,568ng/mL in left vitreous humor. Hair analysis revealed MDPHP concentrations of 152ng/mg (0.0-1.5 cm) and 451ng/mg (1.5-3.0cm). Additionally, hair segments were positive for 3,4-Dimethylmethcathinone (DMMC) <0.01ng/mg), α-Pyrrolidinohexiophenone (α-PHP) 0.59 and 3.07ng/mg), cocaine (6.58 and 22.82ng/mg), and benzoylecgonine (1.13 and 4.30ng/mg). The MDPHP concentrations detected were significantly higher than those reported in other fatal cases.[2,3] As a result, the cause of death was attributed to the oral intake of a lethal amount of MDPHP. The similar concentrations of MDPHP in CB and PB indicated that PM redistribution was minimal. The higher lVH level could find an explanation in its partial coagulation. Hair analysis confirmed chronic cocaine use and past consumption of MDPHP and other synthetic cathinones, including DMMC and α-PHP.

To the best of our knowledge, this is the first quantification of MDPHP in both VH and hair, as well as the first to compare CB and PB levels in a PM case. In such underreported cases, a detailed examination of the distribution and PM concentrations of these substances is crucial to improving our understanding and building a valuable reference database for the scientific community.

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Synthetic Cathinone; 3,4-Methylenedioxy-A-Pyrrolidinohexanophenone (MDPHP); Fatal Intoxication

L39 The Validation of an Analytical Method for the Quantitation of Xylazine in Human Hair

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Learning Objectives: The attendee will be able to understand how a Liquid Chromatography/Tandem Mass Spectrometer (LC/MS/MS) method for the detection of xylazine in hair was validated and understand the potential benefit of this methodology.

Impact Statement: Xylazine use and exposure is a critical issue facing the toxicology and forensic science community. This presentation allows for another opportunity for testing a person who knowingly or unknowingly used/was exposed to an adulterant that can cause significant depressant effects. The forensic science community can use this type of testing to gain better understanding of the impact xylazine is having in the population and potentially allow for better resources to combat its use.

Abstract Text: **Introduction/Objective:** Xylazine is a sedative approved for veterinary medicine but not approved for human consumption. Xylazine has commonly been found as an adulterant, unbeknownst to the user, although it can also be sought out directly. Negative side effects of xylazine include Central Nervous System (CNS) and respiratory depression, hypotension, hypothermia, high blood glucose levels, miosis, hypotension, and necrotic skin ulcerations that can lead to amputation. According to the Drug Enforcement Agency (DEA), the frequency of cases that involved xylazine were 149 reported in 2015, but that number jumped to 8,938 in 2021. This poster aims to share the validation of a method for the analysis of xylazine in a keratinized matrix, specifically hair.

Methods: The hair (25mg) is weighed, washed with acetone, dried, and then powdered in a mini ball beater. Three milliliters (mL) of 0.1 M Hydrochloric Acid (HCl) is added to the powder followed by internal standard and finally the sample was capped, vortexed and incubated at 53°C for a minimum of two hours. The sample is centrifuged while Clean Screen DAU columns are conditioned with 3mL of methanol, deionized water, and pH 6 phosphate buffer, respectively. The supernatant is then added to the column. The solid phase cartridges are then washed with 3mL of deionized water, 0.1M HCl, and finally 1mL of hexane. Samples are eluted with 3mL of 80:20 methylene chloride:isopropanol with 2% ammonium hydroxide. After evaporation at 40°C under nitrogen, samples were reconstituted with 50µL of 6% acetonitrile in water with 0.1% formic acid. Samples were then analyzed on an LC/MS/MS.

The LC/MS/MS included an Agilent 1200 LC system and a Sciex 5500 Triple quadrapole tandem mass spectrometer in positive ionization mode. Separation is achieved using a Phenomenex 50mm x 2.0mm Synergi Polar-RP column with 2µm particle size and a seven minute method. Mobile phase A is deionized water with 0.1% formic acid and mobile phase B is acetonitrile with 0.1% formic acid. The following transitions were monitored; xylazine: 221.0- >90.2, 164.0, hydroxy-xylazine: 237.0- >89.9, 137.1, and d6 xylazine: 226.8- >90.1, 170.0.

Results: The method is fully validated according to the American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*. The method was tested for calibration range and model, bias, precision, matrix effect, stability, carryover, Limit Of Quantitation (LOQ) and Limit Of Detection (LOD). The model was linear and had an analytical range of 1pg/mg to 100pg/mg with an administrative cutoff of 5pg/mg for both xylazine and hydroxy-xylazine. There is no carryover up to 10,000pg/mg. The LOD is 0.1pg/mg for hydroxy-xylazine and 0.3pg/mg for xylazine. The administrative LOD is 1pg/mg and the administrative LOQ 2pg/mg. Precision and bias were acceptable at ≤20% and extracts are stable up to seven days. Matrix effect showed ion suppression greater than 25%, therefore ten lots in total were tested at LOD and LOQ levels. Endogenous analysis using ten lots showed no interference and exogenous analysis, with over 50 analytes tested, also had no observable interferences.

Discussion/Conclusion: This is the first reported method for the analysis of xylazine in keratinized specimens. The analysis is done using solid phase extraction with LC/MS/MS and is an important test for expanding detection in population sets where abuse of drugs is common. Xylazine can be abused as a drug on its own and is therefore a concern among rehab intake centers and/or child endangerment cases. Xylazine is a growing threat in the United States and is a concern in the population and accurate identification of exposure is crucial for appropriate treatment.

Validation; Adulterants; Hair

L40 An Update on Standards Development Activities in Forensic Toxicology

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Learning Objectives: After attending this presentation, attendees will better understand the status of discipline-specific standards pertinent to the field of forensic toxicology.

Impact Statement: The presentation will raise awareness of standards development activities pertinent to forensic toxicology. It will also increase awareness of training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

Abstract Text: Standards continue to impact daily work in forensic toxicology. Over the past six years, the Organization of Scientific Area Committees (OSAC) for Forensic Science and the Academy Standards Board (ASB) have published 15 forensic toxicology standards. This presentation will provide an update on the current published standards, those nearing completion, and standards you should expect to see in the coming years.

The OSAC for Forensic Science was created to strengthen the nation's use of forensic science by promoting discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs), which further develop and publish them. The OSAC also reviews published standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

This presentation will present updates related to standards development in forensic toxicology.

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 a Quality Control Program in Forensic Toxicology Laboratories*
- ANSI/ASB 098: *Standard for Mass Spectral Analysis in Forensic Toxicology*
- ANSI/ASB 113: *Standard for Identification Criteria in Forensic Toxicology*
- 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*
- ANSI/ASB 152: *Standard for the Minimum Content Requirements of Forensic Toxicology Procedures*
- ANSI/ASB 153: *Standard Practices for Proficiency Testing for Forensic Toxicology Laboratories and Breath Alcohol Programs*
- ANSI/ASB 156: *Best Practices for Specimen Collection and Preservation in Forensic Toxicology*

ASB-published documents that have yet to go through the OSAC Registry approval process:

- ANSI/ASB 055: *Standard for Breath Alcohol Measuring Instrument Calibration*
- ANSI/ASB 122: *Best Practice Recommendation for Performing Alcohol Calculations in Forensic Toxicology*

Documents currently in development by the ASB:

- ASB 056: *Standard for Evaluation of Measurement Uncertainty in Forensic Toxicology*
- ASB 118: *Standard for Breath Alcohol Instrument Specifications*
- ASB 173 *Standard for Education, Training, Continuing Education, and Certification of Forensic Toxicology Laboratory Personnel*

Documents currently being drafted at the OSAC:

- *Quality Assurance Management Systems in Forensic Toxicology Laboratories*
- *Standard Method for Blood Ethanol Identifications and Quantitations*
- *Standard for the Identification and Quantitation of Volatile Chemicals in Biological Fluids*
- *Standard for the Analysis of Carboxyhemoglobin for Forensic Toxicology Laboratories*
- *Standard Method for Breath Alcohol Subject Testing*
- *Human Factors Considerations for Forensic Toxicology Laboratories*
- *Revisions to existing standards, new documents or work product priorities, 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; OSAC; ASB

L41 *Smith vs. Arizona* and Strategies to Support the Admissibility of Toxicology Testimony: Don't Stop Reading at Page 18

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Learning Objectives: After attending this presentation, attendees will be able to discuss the reasoning of the United States Supreme Court in their ruling in *Smith vs. Arizona* on the right to confrontation of scientific witnesses offering forensic toxicology testimony and explain why forensic toxicology testimony should be considered non-testimonial and therefore admissible when presented by a qualified witness.

Impact Statement: This presentation will impact the forensic science community by increasing the likelihood of the court's acceptance of toxicological testimony, while preserving a defendant's right to confrontation.

Abstract Text: On July 21, 2024, the United States Supreme Court (SCOTUS) issued its decision in *Smith v. Arizona*, 602 U.S. _ (2024).[1] The case involved a search warrant, seizure, and "full scientific analysis" of controlled substances by chemists in the Arizona Department of Public Safety (DPS) Crime Lab. Analyst Rast performed the original testing, memorializing her testing procedures and results in a prepared set of typed notes and a signed report; both on DPS letterhead. The typed notes included Rast's statements on description, weight, testing methods, and conclusions for each substance. At trial, Rast did not testify. Analyst Longoni testified in place of Rast, offering an "independent opinion" after review of Rast's "notes and reports." *Smith* specifically states, "And he [Longoni] did come to the same conclusion, in reliance on Rast's records. Because he had not participated in the *Smith* case, Longoni prepared for trial by reviewing Rast's report and notes."

SCOTUS held that Longoni's testimony was essentially a reading of Rast's "statements" (i.e., report and notes) into evidence when Rast did not appear and testify, thereby violating the Confrontation Clause. The Court held that when an expert conveys an absent analyst's statement in support of his/ her opinion, and the absent analyst's statements are "basis testimony" that provide foundational support for the substitute analyst's testimony only if true, then the statements are offered for "the truth of the matter asserted" and are therefore hearsay. An "independent opinion" based on foundational statements offered for the truth of those statements is therefore also hearsay and thus inadmissible.

If the SCOTUS opinion ended on page 18 with the hearsay" analysis, the "sea-change" in forensic science testimony that *Smith* is alleged to be, would be a reality. The *Smith* decision, however, must be read to conclusion as pages 19-22, which address "testimonial hearsay" contain the key information for admissibility of foundational data and substitute witness testimony of critical importance to toxicology testimony. SCOTUS remanded determinations relating to whether the underlying "statements" (data, Laboratory Information Management System [LIMS] records, notes, and report) were "testimonial" back to the Arizona trial Court to decide (assuming sufficiency of record) whether the underlying statements "focused on courts" or were prepared with the reasonable expectation that the statement would be used in future litigation, in which case those statements would be "testimonial" and thus inadmissible hearsay.

Consistent with its prior cases, SCOTUS declined to define "testimonial." It did, however, affirm that the "primary purpose test" should be used to determine whether statements of forensic witnesses are testimonial. The Court directed the *Smith* trial court to specifically identify the "statements" relied upon and intended to be introduced and to articulate the principal reason each "statement" was made. SCOTUS counseled that courts should consider the wide range of recordkeeping activities that lab analysts engage in, with examples including records created to comply with accreditation, facilitate internal review, satisfy quality control, and even as a reminder-to-self. Such records would not be testimonial as their primary purpose would not be "focused on the court."

Attorneys and courts unfamiliar with the segmented workflows, methodologies, accreditation, and quality standards used in forensic toxicology should be educated through expert toxicological testimony. It is essential to convey that the primary purpose of toxicological laboratory procedures, Standard Operating Procedures (SOPs), and quality safeguards is to generate accurate results, not to support prosecution. Clear explanations of the non-testimonial nature of data, records, SOPs, LIMS documents, and accreditation standards used to produce toxicology reports and opinions can demonstrate that the "primary purpose" of forensic toxicology workflows is to generate reliable results. Consequently, the reliance of a testifying witness on these data and records is non-testimonial and, therefore, admissible.

Reference:

1. *Smith v. Arizona*, 602 U.S. _ (2024). https://www.supremecourt.gov/opinions/23pdf/22-899_97be.pd.

Expert Testimony; Judicial; Law

L42 Beyond the Green Thumb—The Role of Technology in a Case of Suicide by Pesticide Poisoning

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Learning Objectives: After attending this presentation, attendees will recognize the importance of not underestimating any case, the need to contextualize circumstantial evidence, and the critical role of technology in forensic toxicology.

Impact Statement: This presentation will impact the forensic science community by showing how technology has been effective in solving a case initially misinterpreted as a death by natural causes.

Abstract Text: **Introduction:** Every death case, no matter how simple it may appear, can hold unexpected findings and must always be cautiously approached. In October 2023, a dead man was found in a church near Ferrara, Italy, with a fruit juice bottle near him. The CCTV videos captured the man drinking from the bottle before collapsing in what resembled a heart attack. The police identified the man as a 64-year-old farmer with no pathologies in his medical history nor ongoing pharmacological therapy.

Method and Results: The external examination showed red-purplish hypostasis and numerous bilateral conjunctival petechiae. The autopsy was performed expecting to find a myocardial infarction, however, from the first skin incision, an intense sulfurous odor was detected. The internal examination revealed an abnormal bright green color of the gastric wall, perigastric soft tissues, duodenum, and spleen; the presence of a creamy emerald-green fluid in the upper gastric tract up to 30cm from duodenojejunal flexure; several pulmonary petechiae; organ congestion and edema.

Histopathological results confirmed cerebral and pulmonary congestion and edema, acute neuronal injury in the brain, and contraction band necrosis in the myocardium. Esophageal and gastric samples showed congestion and mucosal autolysis. Suspecting self-poisoning, samples of gastric content, bile, urine, and blood were collected. A series of distinct toxicological analyses were conducted, each contributing a crucial step toward uncovering the substance responsible and ultimately solving the case.

The initial analysis involved Scanning Electron Microscopy (SEM) by using a carbon-coated stub, like the one used for gunshot residues: some drops of the emerald-green liquid found in the bottle were placed onto the adhesive stub and dried at 60°C (140°F) for 24 hours. SEM analysis revealed the presence of carbon, oxygen, sulfur, sodium, calcium, chlorine, magnesium, and nitrogen atoms.

Assuming that the substance belonged to the world of agriculture pesticides, an Open-Source AI was asked to identify a green-colored pesticide containing those atoms and it suggested carbamates or organophosphates as the most likely options. An attempt with Raman Spectroscopy was made, but failed, to determine the exact organic compound, probably because the layer above the stub was too thin, even after repeating the process with a double layer of the substance.

Further toxicological analyses were performed in another laboratory using Gas Chromatography/Mass Spectrometry (GC/MS), a semi-quantitative method that revealed that the green liquid in the bottle was oxamyl. Through an Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS), high concentrations of oxamyl were detected in blood, urine, and bile.

Discussion: Oxamyl is a systemic nematocidal pesticide belonging to the carbamate family. Carbamates are acetylcholinesterase inhibitors that kill parasites by leading to high concentrations of acetylcholine in synaptic clefts. Adverse health effects from chronic exposure are known, but sudden death from overdose or acute effects on human organs have never been observed.[1,2]

Reversible acetylcholinesterase inhibition causes cholinergic crisis (SLUDGE syndrome), which is treated with atropine. The more fearsome complication of carbamate poisoning is multifactorial respiratory failure, caused by the acetylcholine effect on the autonomic nervous system.

Conclusion: This report describes a case of an uncommon suicide method, at least in Italy, by the ingestion of a pesticide that was identified with the help of advanced technological tools. The cause of death was attributed to an acute oxamyl intoxication, leading to cardiorespiratory failure and consequent massive cerebral and pulmonary edema. What initially might have seemed like a natural death turned out to be a suicide by a chemical poison. While AI suggested the class of pesticides but failed to identify the exact substance, it was the step-by-step combination of sophisticated technological instruments that ultimately solved the case.

References:

1. Dassanayake et al. Subacute and chronic neuropsychological sequalae of acute organophosphate pesticide self-poisoning: a prospective cohort study from Sri Lanka. *Clinical Toxicology* (2021), pp. 118-130.
2. Leoci R., Ruberti M. Pesticides: an overview of the current health problems of their use. *Journal of Geoscience and Environment Protection* (2021), pp. 1-20.

Pesticide; Raman Spectroscopy; Scanning Electron Microscopy

L43 Comparisons of Blood, Vitreous Fluid, and Urine (When Available): Drug Screen Findings in Over 100 Cases

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Learning Objectives: Vitreous fluid samples are sometimes used in place of blood or urine for general drug screening in postmortem forensic toxicology case work. Based on the nature of vitreous fluid and the chemical characteristics of drugs, some drugs do not enter the vitreous fluid very well or at all. Data comparing these two matrices is limited.

Impact Statement: This presentation will look at over 100 cases in which blood and vitreous fluid drug screen findings are compared. The information will aid in the interpretation of toxicology data collected from a vitreous fluid drug screen and highlight limitations of using vitreous fluid for drug screening purposes.

Abstract Text: Vitreous fluid is commonly tested to determine the absorption state of ethanol and to determine heroin exposure or recent cocaine use.[1] It can also be tested as a second specimen to confirm drug findings in another matrix. Depending upon the circumstances of a death, vitreous fluid may be the best or only specimen available for toxicology testing. Data regarding the presence and concentration of drugs in vitreous fluid as compared to whole blood is limited. Due to the properties and aqueous nature of vitreous fluid, should it be used for general drug screening in the absence of other matrices? Could some drugs be missed? Confirmed drug findings in over 100 cases were compared between blood, vitreous fluid, and urine, when available. These data are important to help determine if a specific drug would be expected to be detected in vitreous fluid. Knowledge of the limitations of using vitreous fluid as a matrix in which to perform general drug screening is necessary for the proper interpretation of the results.

The blood, vitreous fluid, and urine were screened by Liquid Chromatography/quadrupole Time Of Flight Mass Spectrometry (LC/qTOF/MS) for approximately 350 drugs in the following drug classes: amphetamines, analgesics, anesthetics, anticholinergics, anticonvulsants, anticoagulants, antidepressants, antidiabetics, antifungals, antihistamines, antipsychotics, barbiturates, benzodiazepines, cannabinoids, cardiovascular agents, designer opioids, gastrointestinal medications, hallucinogens, muscle relaxants, novel psychoactive substances, opioid analgesics, sedatives, stimulants, synthetic cannabinoids, and urological agents. Blood, vitreous fluid, and urine were sent to confirmation for those drugs that screened positive. The screening and confirmation methods were fully validated in all three matrices per the requirements of American Board of Forensic Toxicologists (ABFT) and **International Organization for Standardization** ISO) 17025.

In some confirmation methods, the vitreous fluid or blood is only validated for qualitative confirmation. Drugs that confirmed positive in both blood and vitreous fluid were members of the following classes: anesthetics, anticoagulants, select antidepressants, select antipsychotics, anticonvulsants, antihistamines, select benzodiazepines, select designer opioids, select muscle relaxants, opioids, cardiovascular agents, stimulants, sympathomimetic amines, and select urological agents. Drugs and drug classes that confirmed in blood but were not detected in vitreous fluid were THC*, THC-OH, THC-COOH*, warfarin, select benzodiazepines, select antipsychotics, select antidepressants, select urological agents, select muscle relaxants, and analgesics. Some drugs only had one or a few occurrences; therefore, more data for these drugs are needed. There was one case with a very high concentration of THC (252ng/mL) and THC-COOH (597ng/mL) that confirmed for THC and THC-COOH in vitreous fluid just above the reporting limits of 1ng/mL and 3ng/mL, respectively. This case also confirmed for THC-OH (82.7ng/mL) in the blood but was not detected in the vitreous fluid with a reporting limit of 3ng/mL. All other cases with THC-COOH confirmed in the blood did not screen positive in vitreous fluid with a cut-off of 15ng/mL. Commonly encountered illicit drugs did confirm in vitreous fluid. In fact, a lingering opioid death may be able to be determined by comparing blood and vitreous fluid concentrations. Two cases involving fentanyl will be discussed as an example with blood fentanyl concentrations of 1 and 1.9ng/mL, and vitreous fluid fentanyl concentrations of 11.7 and 7.8ng/mL, respectively.

Vitreous fluid can be useful in the detection of some drugs but not all drugs. In an acute overdose, drugs may not have had enough time to pass into the vitreous fluid before death occurred. Also, drugs at lower concentrations in blood may not be detectable in vitreous fluid, as well as those drugs that are lipophilic like benzodiazepines. Vitreous fluid has its place in toxicology testing; however, due to its limitations, using it as a specimen for general drug screening should be avoided.

Reference:

1. Antonides, H.M., Kiely E.R., and Marinetti L.J., (2007) Vitreous fluid quantification of opiates, cocaine, and benzoylecgonine: comparison of calibration curves in both blood and vitreous matrices with corresponding concentrations in blood. *J Anal Toxicol*. Oct; 31 (8):469-76.

Drug Analysis; Postmortem; Specimen

L44 The Determination of Measurement Uncertainty of Non-Routine Measurements in Forensic Toxicological Casework

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Learning Objectives: After attending this presentation, attendees will understand how to determine measurement uncertainty of non-routine measurements in forensic toxicological casework.

Impact Statement: This presentation will impact the forensic science community by illustrating how measurement uncertainty can be determined in non-routine forensic toxicological analyses.

Abstract Text: In forensic toxicological casework, measurements are frequently performed on substances in biological materials (e.g., the concentration of amphetamine in femoral blood). With these measurements comes the concept of Measurement Uncertainty (MU). The MU is often expressed in terms of confidence intervals, which show the range within which the true concentration of a substance is believed to be, with a certain degree of confidence. The intervals are determined using the variability observed in quality control measurements using the analytical method. Determination of MU is important for a correct interpretation of the measurement results and the ISO-17025 norm even mandates the assessment of MU.

In routine forensic toxicological analyses, quality control measurements are performed on reference/control material and recorded on control charts. Numerous guidelines and literature exist on how to determine MU.[1-3] In non-routine analyses, however, control charts are not available due to the infrequent nature of measurements, for instance a certain Novel Psychoactive Substance (NPS) or an alternative biological matrix. Typically, here the measurement procedure is such that reference material is prepared in-house by adding known amounts of the substance in question to a blank matrix at several concentration levels (e.g., low, middle and high). Using standard addition on the case material itself is usually not possible because of the limited amount of case material. Since it is not very clear from the literature how MU can be determined in this case, we developed a statistical procedure.

The procedure is top-down as the analysis is non-routine with only a limited amount of reference data and includes the following steps. Four control levels of the reference material are measured in duplicate. The statistical model assumes that for each control level, the measurements have a fluctuating relative deviation from 1 (100%). This fluctuation is modeled by a normal distribution around some overall (relative) bias and with a certain (relative) standard deviation. The bias and the standard deviation are estimated using the observed deviations per control level, and a confidence interval in the casework is determined based on these estimates.

In more statistical terms: if the average over duplicate measurements at each control level with true concentration c_i is given by X_i, the relative deviations $D_i = X_i/c_i - 1$ are calculated. The mean of these four relative deviations, mean(D), is taken as the optimal estimator of the overall bias d. The sample variance of these four relative deviations, together with an estimation on the variance on the assumed true value of the reference material, is used to estimate the overall variance u^2. This overall variance is by definition the variance of the difference between a new relative measurement corrected by the optimal estimator of the overall bias. If the measurement in the case is Y and the true concentration of the material is m, the 95% confidence interval is determined by the following inequality: $|Y/m - (1 + \text{mean}(D))| \leq k \times u$, with the coverage factor k connected to a t-distribution with n-1 degrees of freedom. Here, n is the number of control levels used (in this example n is 4).

Since the model provides an interval for Y/m, transformation into an interval for m will result in reciprocal numbers in the confidence interval. The procedure will be illustrated through an example. The developed procedure is implemented at the Netherlands Forensic Institute to determine MU in circumstances where there is limited reference data.

References:

1. ASB Standard 056, First Edition 2024, draft. *Standard for evaluation of measurement uncertainty in forensic toxicology*.
2. ENFSI Guideline QCC-GDL-001, 2024. *Guideline for calculating measurement uncertainty in quantitative forensic investigations*.
3. Nordtest NT TR 537, edition 4, 2017. *Handbook for calculation of measurement uncertainty in environmental laboratories*.

Measurement Uncertainty; Non-Routine Analyses; Forensic Toxicology

L45 Evaluating the Pharmacokinetics of Xylazine in Humans: A Paired Forensic and Clinical Model

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Learning Objectives: After attending this presentation, attendees will be able to assess the forensic and clinical impacts of a human pharmacokinetic study involving xylazine and other drugs.

Impact Statement: This presentation will impact the forensic science community by expanding knowledge of forensic toxicology interpretation with respect to fentanyl, xylazine, and metabolites.

Abstract Text: Understanding the pharmacokinetics and pharmacodynamics of drugs and their metabolites is pivotal to interpretive forensic toxicology efforts. Xylazine is a Central Nervous System (CNS) depressant and alpha-2 agonist commonly found alongside fentanyl as an adulterant in the recreational opioid supply across the United States. Due to its veterinary uses, the pharmacology of xylazine is well understood in animals; however, because xylazine is not approved for human use, there is no Good Manufacturing Practices (GMP) supply to permit clinical pharmacokinetic studies. To gain this important information, a naturalistic study of xylazine pharmacokinetics in emergency department patients was established, which may be a model that can benefit future studies of emerging drugs and adulterants.

Adult patients presenting to UMass Memorial Medical Center Emergency Departments following a suspected opioid overdose are being evaluated for inclusion in an Institutional Review Board (IRB) -approved study. The goal is to recruit 12 participants with suspected xylazine ingestion to complete serial blood collections over a 4-hour period. Seven total blood specimens are collected from each participant at 30–60-minute intervals using collection tubes containing sodium fluoride and potassium oxalate. Specimens are stored in the refrigerator and shipped directly to the laboratory for analysis.

A quantitative analytical method was developed and validated to measure the concentration of drugs and their metabolites in antemortem blood specimens. The scope of the assay includes fentanyl and its metabolite norfentanyl; xylazine and its metabolites 2,6-xylidine, 1-(2,6-xylyl)-2-thiourea, 3-hydroxy xylazine, and 4-hydroxy xylazine; and medetomidine and its metabolite 3-hydroxy medetomidine. The internal standard is fentanyl-D5. The quantitative range varies, with lower limits spanning 0.1-1ng/mL and an upper limit of 100ng/mL. Samples are analyzed using a Waters Acquity UPLC coupled with a Waters Xevo TQ-S micro Liquid Chromatograph/Tandem Quadrupole/Mass Spectrometer (LC/QqQ/MS). The analytical column is an Agilent Poroshell 120 EC-C18 (3.0x150mm, 2.7µm). Mobile phases are 0.1% formic acid in water and acetonitrile. The total method run time is 7.5mins. Xylazine metabolite isomers (3- and 4-hydoxy) are resolved analytically for specific identification.

To date, serial antemortem blood specimens have been collected from three participants and analyzed using the previously described method. All participants tested positive for fentanyl and xylazine. Initial blood concentrations were 40, 110, and 2ng/mL for fentanyl, and 50, 200, and 30ng/mL for xylazine. Of the xylazine metabolites included in this study, only 3-hydroxy xylazine and 1-(2,6-xylyl)-2-thiourea have been detected, with concentrations ranging from < 1-5ng/mL and 5-20ng/mL, respectively. Prior research shows that the half-life of xylazine in large animals is approximately 30-60 minutes, but early evaluations of the data from the current study suggest the half-life of xylazine in humans may be longer. Due to the naturalistic nature of the study, parameters such as time of drug use and time elapsed between last use and blood sampling are variable, thus the data might be best explored with a sparse sample analytical technique, such as a population pharmacokinetic assessment. However, this approach will be determined once samples from all 12 participants are available.

The development and validation of a quantitative assay for drugs and their metabolites has allowed for the naturalistic evaluation of xylazine pharmacokinetics, with the anticipation of using this model to assess other emerging drugs and adulterants when no GMP-grade drug source is available. Understanding the pharmacokinetics of xylazine and its metabolites will allow for better toxicological interpretation, especially in the context of the pharmacology of parent drugs and their metabolites.

Xylazine; Pharmacokinetics; Validation

L46 Paired Toxicology Assays for the Quantitation of Medetomidine and the Differentiation of Dexmedetomidine and Levomedetomidine

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Learning Objectives: After attending this presentation, attendees will be able to describe the appearance and impact of the novel adulterant medetomidine on public health and safety.

Impact Statement: This presentation will impact the forensic science community by emphasizing the importance of quantitative testing for drugs and adulterants, as well as enantiomeric differentiation to determine species present and to establish reference concentrations.

Abstract Text: Adulterants and diluents are commonly found alongside recreational drugs in the United States, either to enhance the effects of the primary psychoactive component or add bulk to the drug product for final sale. The opioid supply has experienced this adulteration phenomenon, whether it be heroin containing diphenhydramine or, more recently, fentanyl containing xylazine. Beginning in 2023, medetomidine emerged as the latest adulterant identified with fentanyl in powder form and in biological specimens collected following opioid exposures. Medetomidine is an alpha-2 agonist and is approximately 200 times more potent than xylazine in animal models. Medetomidine exists in two enantiomeric forms: dexmedetomidine, the active and potent enantiomer, and levomedetomidine, the primarily inactive enantiomer. Dexmedetomidine is used in both human and veterinary emergency medicine, while levomedetomidine in the racemic mixture is only used in veterinary medicine. Differentiation of medetomidine's enantiomers in apparent opioid overdoses may enable distinguishing hospital administration from veterinary or clandestine sources. The identification and quantitation of medetomidine is imperative for forensic laboratories as this new adulterant can cause bradycardia and respiratory depression.

Two laboratory methods were developed for the quantitation of medetomidine and the differentiation of dexmedetomidine vs. levomedetomidine. The scope of the quantitative method included medetomidine (undifferentiated), 3-hydroxy medetomidine, fentanyl, norfentanyl, xylazine, 3-hydroxy xylazine, 4-hydroxy xylazine, 2,6-xylidine, and 1-(2,6-xylyl)-2-thiourea. The scope of the identification method included only dexmedetomidine and levomedetomidine. Fentanyl-D5 was used as the internal standard for both methods. Quantitation was performed using external calibration and the range was assessed from 0.1-100ng/mL. Blood (0.5mL) was prepared using a single-step basic liquid-liquid extraction with borate buffer (0.1M, pH 10.4) and an extraction solvent of *N*-butyl chloride/ethyl acetate (70:30 *v:v*). Method validation was conducted according to the Academy Standards Board (ASB) 036 validation standard. Authentic forensic specimens where medetomidine was confirmed present and not administered in hospital were collected and subjected to the analysis workflow.

Quantitative analysis was performed using a Waters Xevo TQ-S Micro tandem quadrupole mass spectrometer coupled with a Waters Acquity I-Class ultra performance liquid chromatograph. Chromatographic separation was achieved using an Agilent InfinityLab Poroshell 120 EC-C18 (3.0 x 150mm, 2.7µm) analytical column under gradient elution. Differential analysis was performed using an Agilent 6495 tandem quadrupole mass spectrometer coupled with an Agilent 1290 Infinity ultra-high performance liquid chromatograph. Chromatographic separation was achieved using a Phenomenex Lux Cellulose-3 Chiral (2.0 x 150mm, 3.0µm) analytical column under isocratic elution.

Eight postmortem blood specimens and 13 antemortem blood specimens collected in early 2024 were quantified and differentiated using the described workflow. Thirteen drug materials from Philadelphia, PA, were also subjected to differentiation. Toxicology cases originated from Pennsylvania (n=13) and Illinois (n=8). Ten of 21 blood samples have been quantitated thus far using the described method. Blood concentrations of medetomidine ranged from 1.2 to 16ng/mL (mean: 6.1±5.4ng/mL, median: 3.9ng/mL). Fentanyl concentrations ranged from 1.5 to 8.9ng/mL (mean: 4.3±2.3ng/mL, median: 3.0ng/mL) and xylazine concentrations ranged from 3.4 to 60ng/mL (mean: 21±19ng/mL, median: 15ng/mL). All samples contained both dexmedetomidine and levomedetomidine. In the 13 drug materials, dexmedetomidine and levomedetomidine were also both identified at approximately 1:1 ratio (i.e., racemic). Medetomidine was often detected alongside fentanyl (97%), cocaine (39%), bromazolam (15%) metonitazene (9%), and other common adulterants such as xylazine (78%), quinine (48%), and diphenhydramine (45%).

Our paired toxicological assays were successful in accurately identifying, distinguishing, and quantifying medetomidine and its enantiomers, dexmedetomidine and levomedetomidine, in biological specimens and drug materials. Blood concentrations observed were lower than those of xylazine. Our results indicate that the medetomidine detected does not originate from human pharmaceutical origins as levomedetomidine was detected in all cases.

Adulterants; MDI; Clinical

L47 Two Fatalities Involving Hydroxychloroquine

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Learning Objectives: Attendees will learn the toxicological and pathological findings in two hydroxychloroquine-related fatalities.

Impact Statement: This case study presents some of the highest postmortem concentrations of hydroxychloroquine published. Such information will assist toxicologists and pathologists when establishing cause and manner of death in cases involving hydroxychloroquine.

Abstract Text: **Introduction:** Hydroxychloroquine is a derivative of chloroquine used to treat malaria, chronic inflammatory conditions, such as lupus, and off-label for COVID-19. While relatively safe, hydroxychloroquine is associated with prolonged QT interval, torsades de pointes, and other arrhythmias. Most published case reports involve non-fatal overdoses that resolve with supportive care. Here we present two hydroxychloroquine-involved fatalities: one following a two-day hospitalization and the other found dead on scene.

Case Histories: Case 1 was a 16 year-old female with history of morbid obesity, type 2 diabetes mellitus, lupus, bipolar disorder, and recent hospitalization for a suicide attempt via ingestion of melatonin. One day after being released from the hospital, the decedent admitted ingesting 120 hydroxychloroquine pills. She was transported to the hospital and complained of abdominal pain and dizziness. She became hypotensive and bradycardic and went into cardiac arrest. She was diagnosed with multisystem organ failure, and following no improvement in her condition after two days, care was withdrawn. Organ procurement procedures were attempted. An external examination was conducted; blood samples from the hospital and the procurement agency were submitted for toxicology testing.

Case 2 was a 50 year-old female with history of bipolar disorder, anxiety, attention-deficit/hyperactivity disorder, suicidal ideation, possible past suicide attempt, heavy ethanol abuse and frequent falls. She was found dead in bed and pronounced on scene. A full autopsy was conducted and toxicology samples were submitted for testing.

Methods: If requested, toxicology testing was performed for ethanol, methanol, isopropanol, and acetone via headspace gas chromatography flame ionization detection (0.010g/100mL limit of quantitation). Drug screens for amphetamine, methamphetamine, benzoylecgonine, opiates, oxycodone, fentanyl, methadone, phencyclidine, benzodiazepines, and carisoprodolwere performed by enzyme-linked immunosorbent assay and a variety of over-the-counter and prescription drugs by gas chromatography full scan mass spectrometry (variable limits of detection). Confirmation testing was performed by chromatography/mass spectrometry methods. Hydroxychloroquine testing was performed by a commercial laboratory using liquid chromatography tandem mass spectrometry with a limit of quantitation of 100ng/mL.

Results: Toxicological findings in case 1 included diazepam, nordiazepam, midazolam, lidocaine, and hydroxychloroquine in blood drawn by the procurement agency two days after hospital admission. Hydroxychloroquine quantified at 30,000ng/mL; all other compounds were identified qualitatively. The pathologist classified the cause of death as toxic effects of hydroxychloroquine and manner of death as suicide.

Pathological findings in case 2 included hepatic steatosis and watery stomach contents with fruity odor. Toxicological findings in femoral blood were 0.192 ± 0.020g/100 mL ethanol, 48 ± 11ng/mL amphetamine, and 24,000ng/mL hydroxychloroquine; the ethanol concentration in vitreous humor was 0.246 ± 0.025g/100mL. The pathologist classified the cause of death as combined toxic effects of hydroxychloroquine, ethanol, and amphetamine and manner of death as suicide.

Discussion: The clinical manifestation in case 1 is consistent with previously reported hydroxychloroquine toxicities; unfortunately, a full autopsy was unable to be conducted to correlate clinical findings to gross and/or microscopic observations. In case 2, autopsy findings were non-specific. In both cases, the blood hydroxychloroquine concentrations far exceeded the published therapeutic range of 100–500ng/mL.[1] Few published postmortem concentrations are available for comparison; 104,000ng/mL in iliac blood was reported in a child who is suspected of ingesting 12g of hydroxychloroquine.[2]

Conclusion: Like many drugs, hydroxychloroquine toxicity is not readily apparent at autopsy; therefore, comprehensive toxicological testing is required, particularly in cases where the decedent's history is unknown.

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2. A. Kemmenoe. An Infant Fatality Due to Hydroxychloroquine Poisoning. *Journal of Analytical Toxicology* 1990; 14: 186-188.11

Hydroxychloroquine; Postmortem; Toxicity

L48 An Unexpected Fatal Methadone Ingestion in a Child

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Learning Objectives: After attending this presentation attendees will understand the interpretation of methadone toxicology across multiple postmortem toxicology specimens.

Impact Statement: This will impact the forensic science community by illustrating how extensive toxicology sampling and testing are essential components in the complete evaluation of an unexpected pediatric death.

Abstract Text: Methadone has historically been used as a pain medication and transitioned to treatment for opioid addiction in the mid-1960s and 70s.[1] Approximately 400,000 patients are prescribed methadone in the United States. After March 15, 2020, opioid treatment programs were allowed to prescribe extended take-home doses to minimize COVID-19 exposure, although the increased take-home amounts were not associated with increased harm.[2] Numerous safety mechanisms are implemented by manufacturers and suggested to patients who are prescribed methadone and live with children.[3]

Here we describe a case of a 2-year, 11-month-old girl who ingested her grandfather's methadone prescription in some unknown manner. Per report, the child was given "an inch" of liquid diphenhydramine by her mother the evening before her death. She was found unresponsive in the morning and, after lifesaving attempts, was declared dead. The autopsy examination was unremarkable. Histologic examination revealed focal lipoid pneumonia. A respiratory viral panel was positive for adenovirus and human rhinovirus/enterovirus without viral pneumonia pathology on histologic examination. Toxicology was positive for methadone in the peripheral blood (240ng/mL), cavity blood (430ng/mL), bladder wash, vitreous humor, and gastric contents (8,100ng/mL). Diphenhydramine was identified in the vitreous humor only.

Initial scene investigation did not reveal any methadone in the home. Further investigation by police found that the grandfather was prescribed methadone, which was present in the house. Both adults in the home denied giving the child methadone as well as medication accessibility, although the mother stated she gave her diphenhydramine. Liquid methadone and pediatric diphenhydramine can look similar depending on formulations and manufacturers. Given the presence of methadone across multiple samples, including gastric contents, the cause of death was determined to be acute methadone intoxication, and the mechanism was ingestion. Given the stated diphenhydramine treatment without diphenhydramine present, it is likely that methadone was mistaken for pediatric liquid diphenhydramine.

The thorough toxicology analysis across multiple specimen types was essential to conclude that methadone and not diphenhydramine was recently ingested.

References:

1. Institute of Medicine (US) Committee on Federal Regulation of Methadone Treatment. *Federal Regulation of Methadone Treatment*. Rettig RA, Yarmolinsky A, editors. Washington (DC): National Academies Press (US); 1995. PMID: 25121195.
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Methadone; Intoxication; Pediatric

L49 A Toxicological and Pathological Re-Evaluation of Media-Reported Mitragynine (Kratom) Deaths in Florida

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Learning Objectives: After attending this presentation, toxicologists and pathologists will have new data on the effects of mitragynine concentrations following 15 daily 89.2mg mitragynine isolate doses and recently reported mitragynine concentrations in impaired drivers and postmortem investigations.

Impact Statement: This presentation will impact the forensic science community by providing new data for determining cause of death in postmortem mitragynine cases.

Abstract Text: In 2023, the *Tampa Bay Times* reported that >580 Floridians died from kratom-related overdoses during the past decade, with most deaths caused by mixtures of kratom and potent opioids or other substances.[1] Toxicology results in 46 of these cases reported the presence of only mitragynine/kratom. The lethal blood mitragynine concentration is not yet determined.

National Medical Services (NMS) Labs performs most mitragynine impaired-driving and postmortem analyses in the United States and recently published data on mitragynine concentrations in these two populations.[3] The authors stated the significance of the data was difficult to ascertain because of confounding variables of a limited scope of toxicology testing and the frequent presence of abused drugs and prescription medications. In 127 mitragynine-impaired driving cases, mean and median mitragynine concentrations were 133 and 62ng/mL (8.8–1000ng/mL). In 5,413 postmortem cases, mean and median mitragynine were 360 and 120ng/mL (5.4–11,000ng/mL). In 6,860 cases, >90% had ≤1,000ng/mL mitragynine; approximately 9.5% of cases had mitragynine concentrations of >1,000ng/mL. The authors stated, "The work described herein supports the authors' initial conclusions that blood mitragynine concentrations of >1,000ng/mL are more often associated with severe adverse events, up to and including death."

Following controlled administration of 15-daily kratom leaf powder doses (4,000mg kratom) each containing up to 53.2mg mitragynine, plasma mitragynine and 7-OH-mitragynine concentrations to healthy outpatient volunteers were up to 215 and 38ng/mL, respectively.[4] Other healthy volunteers received 15-daily oral mitragynine isolate doses containing up to 89.2mg mitragynine. Plasma mitragynine and 7-OH-mitragynine concentrations were up to 753 and 63ng/mL, respectively.[5] There are no data on the blood/plasma ratios of mitragynine or 7-OH-mitragynine concentrations. This is a limitation in making a direct comparison to postmortem drug concentrations. There were no serious adverse events in the study.

We reevaluated 46 cases reported by *Tampa Bay Times* as mitragynine-only deaths, including full review of the autopsy reports. Of 46 cases, 51% of decedents had mitragynine concentrations ≤1,000 ng/mL, within concentrations found in impaired live drivers and not much higher than after controlled administration. In 49% (n=22) with concentrations ≥1,000ng/mL, 17 were men, with median (range) mitragynine concentration of 2,000 (1,200-5,696)ng/mL, and 5 women 2,300 (1,353-6,100)ng/mL. Three decedents had documented seizure disorders, 2 had sudden cardiac deaths, 1 died of food asphyxiation, 3 were chronic alcoholics, and 17 of 22 had other drugs present in the postmortem blood, and these factors were not included in the listed cause of death. In many of these cases, there was incomplete toxicological analysis and, at the time, there was a lack of controlled mitragynine administration data and the NMS data on mitragynine concentrations in impaired-driving and postmortem cases were not yet available.

Medical examiners and toxicologists need to be aware that these new data suggest that mitragynine concentrations ≤1,000ng/mL may not be the cause of death. Comprehensive toxicology testing should be performed to ensure identification of all drugs that might contribute to toxicity.

Another limitation is that only mitragynine alkaloid concentrations are provided in both sets of new data, while kratom contains multiple other alkaloids that could contribute to toxicity but are not measured in any forensic toxicology laboratory. Consumers should be aware of the consequences of taking exceedingly large kratom doses and combining kratom with other drugs due to potential drug-drug interactions.

References:

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Overdose; Cause of Death; Toxicity

L50 Endogenous Carbon Monoxide Due to Hemolytic Anemia: A Forensic Red Herring

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Learning Objectives: After attending this presentation, attendees will understand endogenous carbon monoxide (CO) and how it is produced from disease.

Impact Statement: This presentation will impact the forensic community by providing information regarding a rare disease that produces clinically measurable CO that may mislead a medicolegal investigation.

Abstract Text: CO toxicity associated with exposure to an environmental, exogenous source is routinely investigated in the field of forensics. We report two fatalities involving endogenous CO production caused by warm Autoimmune Hemolytic Anemia (wAIHA), a condition in which the body's immune system attacks and breaks down healthy red blood cells prematurely. The anemia will negatively impact the measured hemoglobin as the body can no longer compensate, while endogenous CO from heme catabolism will be generated and further impact the oxygen-carrying capacity of the remaining hemoglobin. Both patients had clinically significant measurements of CO in their biological specimens. The elevated levels of CO complicated the death investigation in case 1, making this a thought-provoking presentation for the forensic community.

Paramedics responded to the home of a 60-year-old woman who complained of persistent nausea, dizziness, and fatigue. Her initial carboxyhemoglobin (COHb) saturation was 25% as measured by paramedics in the field via pulse CO-oximetry (SpCO) and was, 2 hours later, confirmed by hospital laboratory spectrophotometric analysis to be 16% after initial treatment in the emergency department.[1] The clinical presentation of environmental CO exposure and subsequent death notification to the North Carolina Office of the Chief Medical Examiner prompted an extensive investigation into the suspected residential source of CO, which ultimately ruled out all exogenous sources. The toxicology laboratory analyzed postmortem subclavian whole blood with a result of 5% COHb saturation. After comprehensive drug testing, only caffeine was detected. The medicolegal death investigator later discovered an updated hematology consultation note, which determined the actual source of the CO to be endogenously produced from natural disease. The second case involved a 53-year-old male who had a syncopal episode and was found upon hospital admission to be jaundiced and anemic. He continued to experience intravascular red cell hemolysis and was pronounced deceased after approximately 48 hours. Analysis of the admission peripheral blood specimen was 14% COHb saturation, again demonstrating measurable CO produced from wAIHA.

These two case studies and brief review of disease-related elevation of endogenous CO will shed light on this rare disease alerting the forensic community to its potential occurrence and need for consideration when sources of environmental CO exposure have been exhausted.

Reference:

1. Hyson BE, Wehbie RS, Haikal NA Bishop-Freeman SC. Endogenous Carbon Monoxide due to Hemolytic Anemia: A Forensic Red Herring, *J Forensic Sci*. 2022; May, 67(3), 1294-99. https://doi.org/10.1111/1556-4029.15013.

Endogenous Carbon Monoxide; Warm Autoimmune Hemolytic Anemia; Postmortem Toxicology

L51 Recommendations for Drug Testing in Driving Under the Influence of Drugs (DUID) and Motor Vehicle Fatality Cases—A 2025 Update

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Learning Objectives: After attending this presentation, attendees can compare their laboratory's screening and confirmation scopes and cutoffs for impaired driving and traffic fatality cases to consensus-based recommendations for Tier I and Tier II drug scopes.

Impact Statement: This presentation will impact the forensic science community by presenting updated guideline recommendations for laboratory testing in Driving Under the Influence of Drugs (DUID) and motor vehicle fatality cases from the National Safety Council's Alcohol, Drugs and Impairment Division (NSC ADID) to improve standardization of laboratory testing practices.

Abstract Text: The purpose of this project was to re-evaluate the 2021 NSC recommendations for Tier I and Tier II scopes and cutoffs for drug screening and confirmation to determine if changes were needed.[1] Part of the re-evaluation included disseminating a survey to laboratories throughout the United States and Canada documenting their testing practices, scope of testing and cutoffs, instrument technology, the most frequently encountered drugs, as well as compliance with the 2021 recommendations. The survey was sent via SurveyMonkey to laboratories performing DUID and motor vehicle fatality casework; 80 laboratories completed the survey.

Seventeen forensic science practitioners who participated in the survey and two external collaborators involved in impaired driver testing were invited to join the authors for a virtual consensus meeting where the 2021 recommendations were re-evaluated following analysis of the survey results and practical experience with testing in DUID cases. Participants were selected based on geographic location, agency type, matrices tested, and caseload to ensure a diverse perspective.

As previously decided, urine has been removed as a recommended matrix type for impaired driving investigations since a drug's presence in urine demonstrates only historical use or exposure.

Based on discussions and polling during the consensus meeting, carisoprodol and meprobamate were downgraded from Tier I to Tier II due to their decreased casework prevalence. Gabapentin was upgraded to Tier I from Tier II due to its increased prevalence. Alpha-hydroxyalprazolam was removed from the Tier I table to coincide with the removal of urine, since that analyte had not been included in Tier I for blood and/or oral fluid. In recognition of the increased use of chromatography/mass spectrometry techniques for drug screening, specific screening cutoffs for these techniques were added to complement the existing immunoassay cutoffs. Cutoffs were differentiated for immunoassay versus chromatographic screening for both blood and oral fluid, and minor amendments were made to cross-reactivity requirements for immunoassay screening. In blood, immunoassay screening cutoffs for clonazepam and lorazepam were raised to 15ng/mL, while the cutoff for 7-aminoclonazepam was removed. The confirmation cutoffs for buprenorphine and fentanyl were raised to 1ng/mL. For oral fluid, immunoassay screening cutoffs were removed for MDA, codeine, hydrocodone, hydromorphone, and oxymorphone, which is now consistent with blood immunoassay screening. Several immunoassay screening and confirmation cutoffs were increased for oral fluid based on recent data from casework that showed higher drug concentrations in oral fluid compared to blood.

Overall, compliance with the recommendations among laboratories continues to increase for individual drugs in the Tier I scope demonstrating laboratories willing to comply, and that the recommendations and cutoffs are analytically achievable. More laboratories are testing for Tier II drugs based on regional prevalence and increased capabilities using newer instrument technology. Given the minor changes in scope, the most frequently encountered drugs in DUID casework are captured in either Tier I or Tier II, depending on prevalence. Lastly, no cutoffs for this update decreased, eliminating the need for laboratories to perform extensive revalidation to meet the recommendations.

Updates to the 2021 recommended scope and cutoffs will be distributed by NSC ADID in early 2025.

Reference:

1. D'Orazio, A.L., Mohr, A.L.A., Chan-Hosokawa, A., Harper, C., Huestis, M.A., Limoges, J.F., Miles, A., Scarneo, C.E., Kerrigan, S., Liddicoat, L.J., Scott, K.S., Logan, B.K. (2021) Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities—2021 Update. *Journal of Analytical Toxicology,* 45(6), 529-536. doi: 10.1093/jat/bkab064

DUID; Cutoffs; Guidelines

L52 New York's Drug Recognition Expert (DRE) Oral Fluid Field Certifications

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Learning Objectives: Attendees will learn how oral fluid samples can be implemented into DRE training programs. An overview of testing results from various training event locations will be provided.

Impact Statement: This presentation will impact the forensic science community by informing toxicologists and other traffic safety partners about the utility of oral fluid samples for the toxicology testing required for the DRE field certification training.

Abstract Text: **Introduction:** DRE training relies on toxicology testing for the final stage of the process. The candidate DRE must obtain a minimum 75% toxicological corroboration rate during their supervised evaluations.[1] The programs have frequently relied on urine drug testing, but more recently, oral fluid has been utilized for the toxicology requirement. Oral fluid can have several advantages over urine: it can be easier to collect, and it provides more recent drug use information. For both specimens, on-site screening devices can be used for drug/drug class preliminary results, and/or specimens can be collected and sent to a laboratory for analysis.

Methods: In June 2023, the New York State Police (NYSP) Forensic Investigation Center began testing oral fluid samples collected during field certifications for NY's DRE training program. Candidate DREs and/or instructors collected oral fluid samples using the Quantisal collection device. Trainings at the Philadelphia, PA, location only collected oral fluid samples while trainings at the Jacksonville, FL, location also collected urine for on-site screening.

Oral fluid samples from both locations were tested at the NYSP laboratory; urine samples were only screened onsite at the Jacksonville location. The testing scheme followed the recommendations for Tier 1 compounds, plus Phencyclidine (PCP) and delta-8-tetrahydrocannabinol.[2] Oral fluid samples were screened using a liquid-liquid extraction followed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis; the screen did not differentiate cannabinoid isomers.[3] Positive Tetrahydrocannabinol (THC) samples were confirmed using a Solid Phase Extraction (SPE) method followed by LC/MS/MS analysis for delta-9-THC and delta-8-THC. All other positive screening results were confirmed using SPE and LC/MS/MS analysis.

Results: A total of 349 oral fluid samples were tested from the Jacksonville training location, collected over four events in May, June, and November of 2023, plus February 2024. The oral fluid results were compared to the DRE category identified by the candidate DRE and showed a 75% agreement for at least one DRE category. The prevalence of drugs in oral fluid by DRE category from Jacksonville was Central Nervous System (CNS) Stimulant (61%), cannabis (50%), narcotic analgesic (24%), and CNS depressant (5%); 16% of oral fluid samples had no drugs detected. Comparing laboratory oral fluid results and onsite urine screening results based on DRE category showed an 85% correlation. Cannabis positivity was 50% in oral fluid and 73% in urine. The candidate DRE called cannabis in 59% of subjects.

A total of 73 oral fluid samples were tested from the Philadelphia training location, collected in December 2023. The oral fluid results were compared to the DRE category identified by the candidate DRE and showed a 96% agreement for at least one DRE category. The prevalence of drugs in oral fluid was CNS Stimulant (93%), narcotic analgesic (89%), cannabis (19%), CNS depressant (4%); all oral fluid samples were positive.

Discussion: Using oral fluid for DRE field certifications provided a viable alternative to urine for the toxicology corroboration required in the DRE training protocol. For New York, since oral fluid was being introduced in 2024 as an evidential sample in impaired driving investigations, incorporating it into the DRE training allowed the officers to become familiar with the collection process prior to their agency implementing it in their protocols.

The most common difference between the oral fluid and urine results was the detection of cannabis in the urine, but not in the oral fluid. This is likely due to the different target analytes (carboxy-THC in urine, THC in oral fluid) and the significantly different detection windows. The subjects participating in the evaluations tend to be under the influence of stimulants (cocaine or methamphetamine) and narcotic analgesics (fentanyl), so other DRE categories are not well represented in the data set.

References:

1. www.theiacp.org/drug-recognition-experts-dres.
2. "Recommendations for the Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities – 2021 Update," A. D'Orazio, A. Mohr, A. Chan-Hosokawa, C. Harper, M. Huestis. J. Limoges, A. Miles, C. Scarneo, S. Kerrigan, L. Liddicoat, K. Scott, B. Logan. *J Anal Tox*, 2021, 45:529-536. https://doi.org/10.1093/jat/bkab064.
3. "Development and validation of a simple chromatographic method to screen oral fluid samples for drugs in DUID investigations," G. Sarris, S. Abbott, T. Moreno, K. Maychack, J. Limoges. *J Anal Tox*, 2024. https://doi.org/10.1093/jat/bkae068.

Drug Recognition Expert (DRE); Oral Fluid; DRE Training

L53 How High Will It Go? The Alarming Rise of Fentanyl Tolerance

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Learning Objectives: After attending this presentation, attendees will understand the increasing tolerance to fentanyl in living individuals. Additionally, the presentation will emphasize the importance of evaluating the totality of circumstances, including case information, when interpreting results.

Impact Statement: This presentation will impact the forensic science community by highlighting the rising concentrations of fentanyl and the recent decline in its prevalence in suspected Driving Under the Influence of Drugs (DUID) cases.

Abstract Text: Drug tolerance occurs when repetitive drug use leads to a diminished response, requiring larger or more frequent doses to achieve the same effects. Fentanyl, a potent synthetic opioid, has become the most prevalent Central Nervous System (CNS) depressant reported, excluding ethanol, in both DUID and death investigations. This is largely due to a significant rise in illicit use over the past decade. The addictive nature and easy accessibility of the drug contribute to expected tolerance among individuals with opioid use disorder.

The therapeutic fentanyl concentration in blood is up to 4ng/mL for analgesia and 10-20ng/mL for anesthesia. However, concentrations more than 25 times higher have increasingly been found in living individuals. This research reviewed elevated fentanyl blood concentrations in DUID investigation casework from 2018 through 2023 and by obtaining additional case information from submitting agencies, aimed to investigate rising tolerance among opioid-dependent users.

Blood samples submitted for toxicological analysis in DUID investigations were quantitated using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) with a reporting limit of 0.2ng/mL. Fentanyl blood concentrations greater than or equal to 100ng/mL over the six-year study period were evaluated. Submitting agencies were contacted to verify the medical use of fentanyl, case history, crash details, injury severity, and observations.

Of the 12,165 cases analyzed for fentanyl, 93 submissions from 11 states across the country confirmed blood concentrations at or above 100ng/mL, representing 0.8% of all positive cases. The concentration range was 100-620ng/mL (mean ±SD 140 ±64ng/mL; median 120ng/mL). Of these cases, 84% also tested positive for at least one other drug with fentanyl-stimulant combinations being the most common. The year-to-year breakdown counts (%) are as follows: 0 in 2018, 4 (0.2%) in 2019, 9 (0.4%) in 2020, 20 (0.9%) in 2021, 25 (1.1%), and 35 (2.2%) in 2023. Fentanyl positivity in DUID cases was 9-10% in 2018 and 2019 after its inclusion in routine drug screens. Its prevalence remained over 12% in the subsequent three years before tapering to 9.5% in 2023. Males accounted for 73% of individuals with fentanyl concentrations ≥100ng/mL. The age range was 20-63 years (mean ±SD 36 ±8; median 35). The sex and age distributions were similar to those of all fentanyl-positive cases.

Forty-one responses were received from the submitting agencies, three of which had minimal information. Of the remaining 38, 33 confirmed that fentanyl was not therapeutically administered, and five had no information regarding fentanyl administration by medical personnel. All but one respondent confirmed the driver was not involved in a traumatic injury (n=37), including 12 crash cases. Despite elevated concentrations, 32 individuals did not show overdose symptoms, while six did. Impairment was observed in 37 cases, with one unknown. Among the 24 responses providing additional observations, common signs of impairment included sedation (n=11), unsteadiness (n=9), and slurred/incoherent/slow speech (n=9). Five cases showed no visible impairment associated with CNS depressant use.

The data from this study underscores a concerning trend of increasing fentanyl tolerance, with blood concentrations frequently surpassing typical therapeutic ranges despite DUID fentanyl positivity trending below 10% for the first time since 2019. The substantial proportion of males and the consistency in age distribution over the years highlight the most at-risk demographic. The unconfirmed therapeutic use reported further emphasizes the illicit nature of fentanyl consumption in these instances. Despite the extremely high fentanyl concentrations, the absence of overdose symptoms in the majority of cases signals an alarming rise in tolerance. Observed signs of impairment confirm the severe impact on driving abilities. These findings validate the importance of maintaining open communication with submitting agencies and thoroughly evaluating each case individually.

Fentanyl; Tolerance; High Blood Concentration

L54 Driving Under the Influence Snapshot: Uncovering Drug Prevalence Trends in Washington, DC

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Learning Objectives: The primary objective of this study was to assess the prevalence and patterns of drug use in Driving Under the Influence (DUI) cases in Washington, DC, spanning the years 2016 to 2023.

Impact Statement: This presentation will offer insight into the prevalence and patterns of intoxicating substances in Washington, DC, thereby aiding in the development of more robust approaches for enforcing laws about driving under the influence.

Abstract Text: DUI continues to pose a significant challenge to traffic safety in the Washington, DC, metropolitan area. Despite concerted efforts in enforcement and public awareness campaigns, the intersection of substance use and impaired driving persists as a complex issue. This study endeavors to shed light on the patterns of drug-related incidents associated with DUI offenses in Washington, DC.

DUI cases from 2016 to 2023 were sourced from various law enforcement agencies, including the Metropolitan Police Department, United States Secret Service, United States Park Police, United States Capitol Police, and Central Intelligence Agency. Samples for analysis, which could include blood, urine, and/or serum, were received and screened at the Office of the Chief Medical Examiner's Forensic Toxicology laboratory in Washington, DC. Blood and serum specimens were screened by Enzyme-Linked Immunosorbent Assay (ELISA), and analyzed for volatiles (including ethanol, methanol, isopropanol, and acetone). Urine specimens were consistently analyzed for volatiles and synthetic cannabinoids (LC/MS/MS) during the monitoring period. Urine specimens analyzed before November 2019 were screened by ELISA. Subsequently, the ELISA screen was replaced by Homogenous Enzyme Immunoassay (HEIA). Headspace/Gas Chromatography/ Flame Ionization Detector, and liquid chromatography/mass spectrometry/mass spectrometry were utilized for volatiles and drug confirmatory analysis, respectively. The final results of 3,417 DUI cases (averaging 427 cases annually) were compiled and statistically analyzed using Microsoft Excel.

Over the eight years analyzed, the most prevalent drugs were consistently: ethanol, delta-9-Tetrahydrocannabinol (THC)/11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), and Phencyclidine (PCP), followed by benzoylecgonine/cocaine/cocaethylene and fentanyl/norfentanyl. The ethanol positivity rate peaked in 2022 and 2023, reaching 70% and 74%, respectively, despite receiving fewer cases than in preceding years. During the same time period, PCP positivity peaked in 2016 at 30% and declined in 2022, finally reaching its lowest at 11% in 2023. The THC/THC-COOH positivity rate exhibited an upward trend throughout the monitoring period, peaking in 2023 at 44%. Ethanol was the most frequently encountered drug followed by PCP, and THC/THC-COOH in 2016. For the remainder of the monitoring period (2017–2023), the prevalence remained constant, with ethanol ranking highest, trailed by THC/THC-COOH, and PCP, with average positivity rates of 65%, 5%/39%, and 21%, respectively. Of the 3,417 cases, only 206, roughly 25.7 cases per year, had no drugs detected.

Washington, DC, presents a unique landscape where PCP remains a prominent substance used, distinguishing it from many other geographical areas. However, the decline in PCP detections in DUI cases in 2022 and 2023 contrasts with previous trends. The decriminalization of cannabis in Washington, DC (2015) and neighboring areas like Virginia (2021) and Maryland (2023), resulted in the highest THC/THC-COOH detection rates ever seen in the District. DUI toxicology findings are crucial tools in assisting legal and law enforcement protocols. They offer vital insights into the prevalence and patterns of intoxicating substances, thereby facilitating the development of more robust approaches for enforcing laws about driving under the influence.

Driving Under the Influence; Ethanol; THC

L55 Drug Use by Drivers in Fatal Traffic Crashes

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Learning Objectives: After attending this presentation, attendees will be able to evaluate trends related to drug positivity in fatally injured drivers over a five-year period.

Impact Statement: This presentation will impact the forensic science community by offering an assessment of current drug positivity rates and recent trends in traffic fatalities.

Abstract Text: Drug use by drivers continues to be a significant contributor to traffic-related fatalities. Traffic fatalities reached a 16-year high in 2021, with only slight reductions reported since then, according to the National Highway Traffic Safety Administration (NHTSA). In a large study focused on serious injuries or fatalities examining drug prevalence conducted by NTHSA in 2019-2021, drug positivity ranged from 25% (cannabinoids) to 9.3% (opioids) with approximately 20% of roadway users testing positive for two or more drug classes.

Traffic fatalities involving the deaths of drivers in Connecticut were used for the data set. Toxicology testing was performed by National Medical Services (NMS) Labs and included the basic postmortem toxicology panel, which tests for all Tier I drugs recommended by the National Safety Council's Alcohol, Drugs and Impairment Division (NSC ADID). The cause and manner of death along with basic demographic information were provided by Connecticut's Office of the Chief Medical Examiner.

A total of 1,291 traffic fatality cases were reviewed between 2019 and 2023. A total of 780 drivers were identified of which 593 (76%) were confirmed positive for one of more drugs. One hundred eighty-five (24%) of the drivers had no drugs detected and two (0.26%) had testing that was canceled. To evaluate drug positivity, the matrix source was limited to blood (antemortem, if available, or postmortem peripheral blood). Results for Tier I drug positivity are shown in Table 1. With respect to polysubstance use, 235 (40%) of drivers had more than one Tier I drug confirmed in blood (metabolites excluded).

Table 1. Tier I Drug Positivity Results (n=593); only drugs with greater than 5% shown.

Drug	Count	Percent Positivity
Ethanol	296	50%
Delta-9 THC	245	41%
Delta-9-Carboxy THC	198	33%
11-Hydroxy Delta-9 THC	151	26%
Fentanyl	80	14%
Benzoylecgonine	61	10%
Norfentanyl	52	8.8%
Cocaine	33	5.6%

Ethanol was the most frequently detected drug, present in 50% of cases. Ethanol blood concentrations (n=296) ranged from 10mg/dL to 394mg/dL with a mean and median of 155 (±75) and 159mg/dL, respectively. Two hundred ten cases (97%) were above the per se threshold of 80mg/dL. Due to concerns related to interpretation of postmortem THC concentrations, only cases with antemortem blood were evaluated quantitatively (n=32). THC concentrations ranged from 0.62 to 28ng/mL with the mean and median both being 4.3ng/mL. Fentanyl was the third most-frequently detected drug (n=57) with postmortem concentrations ranging from 0.36 to 490ng/mL with mean and median concentrations of 43 (±75) and 11ng/mL, respectively. Fentanyl antemortem concentrations (n=23) were lower, with mean and median concentrations of 12 (±19) and 2.5ng/mL, respectively.

Of interest, phencyclidine, a Tier II drug, was identified in 22 drivers (3.7%). There were limited instances of the detection of Novel Psychoactive Substances (NPS) in drivers with only three positive cases over the study period. All three cases were NPS benzodiazepines, (flubromazolam [n=2] and etizolam [n=1]). In all three cases, other Tier I drugs were also confirmed.

This data provides significant insights into drug involvement in traffic fatalities with 76% of drivers being positive for alcohol and/or drugs. Postmortem blood ethanol concentrations were notably high, with 97% exceeding the legal threshold of 80mg/dL. Fifty-eight percent of ethanol-positive cases were also positive for one or more Tier I drug(s), and rates of polysubstance use (40%) were high. These findings emphasize the value of consistent testing for drug use in traffic fatalities and the use of a standardized approach to track trends and compare populations.

Postmortem Toxicology; Traffic Fatalities; Drugs

L56 Impaired Driving Cases With Gabapentin

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Learning Objectives: After attending this presentation, attendees will increase their knowledge of impaired driving investigations involving gabapentin.

Impact Statement: This presentation will impact the forensic science community by outlining the increasing incidence, polysubstance use, and case reports of gabapentin in impaired driving investigations.

Abstract Text: **Introduction**: Gabapentin is an antipsychotic prescription medication in the United States commonly used for seizures, neuralgia, anxiety, and as a general sedative. As a Central Nervous System (CNS) depressant, effects similar to other depressants, including sedation and loss of coordination, have been reported.[1] The prevalence of gabapentin identified in Driving Under the Influence of Drugs (DUID) investigations is increasing. In a 2024 survey of DUID toxicology laboratories, gabapentin was in the top 15 most prevalent drugs for 30 out of 41 (73%) that included gabapentin testing in their scope.[2] In the fall of 2023, a blood Liquid Chromatography (LC) High Resolution Mass Spectrometry (HRMS) screening method was implemented by this laboratory to include gabapentin and over 200 other substances.

Methods: A volatile analysis was performed on all blood DUID cases. This was followed by an 11-panel Enzyme-Linked Immunosorbent Assay (ELISA) (Dynex DSX with amphetamines, barbiturates, benzodiazepines, buprenorphine, cannabinoids, carisoprodol, cocaine metabolite, fentanyl, methamphetamines, opioids, and oxycodone kits from Neogen) and basic drug extraction with scan Gas Chromatography (GC) Mass Spectrometry (MS) (Agilent 7890A/5975C). After implementation of the LC/HRMS (Thermo Scientific QExactive) screen a 3-panel ELISA (benzodiazepines, cannabinoids, and buprenorphine) was performed and scan GC/MS was no longer used. All positive results were confirmed with GC/MS, LC/MSMS (Sciex 3200 QTrap or Sciex 5500+), or LC/HRMS. The validated limit of detection for gabapentin by the LC/HRMS screen was 0.05µg/mL. The limit of quantitation and limit of detection for gabapentin by LC/HRMS was 1.0 and 0.5µg/mL, respectively.

Results: Gabapentin was identified in seven blood DUID cases from January 2018 to October 2023 after directed testing was performed based on case history or officer request. To evaluate the impact of the new LC/HRMS blood screen on drug prevalence, the laboratory compared 58 cases prior to implementation of the LC/HRMS screen and 58 cases after. Gabapentin went from 18th to 9th most prevalent and has been identified in four cases in eight months after implementing the LC/HRMS screen. The gabapentin mean, median, and range were 7.0, 2.1, and < 1.0 to 19µg/mL, respectively. Other psychoactive drugs were identified in all 11 cases. The most common combinations of other active drugs identified were opioids (55%) and antipsychotics (36%). Case history was obtained for 5/11 cases. Other than polysubstance detection (5/5), common observations included: traffic crash (4/5), confusion/memory issues (4/5), and extreme drowsiness (3/5).

Discussion/Conclusion: The incidence of gabapentin in DUID investigations has increased. Due to polysubstance use, interpretation of gabapentin findings can be complex. A previous study demonstrated impairing depressant effects in six cases where gabapentin was the only significant finding.[1] In five polysubstance cases presented herein involving gabapentin, impaired driving and/or CNS depressant effects were observed. Due to the CNS depressant effects of gabapentin, it is a risk to traffic safety, and its prevalence will continue to increase as more laboratories expand their scope to include it.

References:
1. Bierly, J.J., Chan-Hosokawa, A. (2024) Gabapentin in drugged driving investigations. *J Forensic Sci*, 69, 986-992.
2. D'Orazio, A.L., Mohr, A.L.A., Logan, B.K. *2024 Updates for Recommendations for Drug Testing in DUID & Traffic Fatality Investigations.* https://www.cfsre.org/images/Presentations/2024_Survey_Report.pdf. (accessed July 31, 2024).

Gabapentin; DUID; Impaired Driving

L57 2024 Novel Psychoactive Substance Trends and a Comparison Against NPS Discovery Scope Recommendations

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Learning Objectives: After attending this presentation, attendees will be able to describe the 2024 Novel Psychoactive Substance (NPS) landscape encountered in forensic toxicology casework and assess the utility of published quarterly NPS scope recommendations.

Impact Statement: This presentation will impact the forensic science by providing an up-to-date overview of emerging substances in addition to scope updates to assist laboratories in staying current.

Abstract Text: Forensic laboratories conducting seized drug analysis and toxicology testing are continually challenged to keep up with changes in NPS markets. NPS are often defined as substances that have been discovered or synthesized for the first time since the mid-2000s. Staying current can be a drain on laboratory testing resources due to rapid turnover, broad scope and variety of analytes, variable market penetration, and demographic differences. This often leads to gaps in laboratory testing and laboratories making business decisions to exclude NPS from their scope of testing. To assist laboratories in focusing on relevant drugs, NPS Discovery, an open access early warning system run by the Center for Forensic Science Research and Education (CFSRE), in conjunction with the Society of Forensic Toxicologists (SOFT) NPS Committee, publishes quarterly NPS scope recommendations.

To assess the effectiveness of the NPS Discovery scope recommendations, all forensic blood cases (postmortem and human performance) reporting synthetic stimulants/hallucinogens, Synthetic Cannabinoids (SCs), Designer Benzodiazepines (DBZDs) and/or Novel Synthetic Opioids (NSOs) for the first six months of 2024 from a large forensic toxicology reference laboratory were compared against the Tier 1 NPS scope recommendations for Q2 2024.

DBZDs continue to increase in both prevalence and variety, with the top five recommended DBZDs listed as bromazolam, desalkylgidazepam, flubromazepam, etizolam, and flualprazolam. In total, 14 different DBZD compounds (with 2 metabolite pairs) were reported in the first six months of 2024, accounting for 2,147 findings in 1,848 blood samples. Bromazolam accounted for over 76% of these findings, underscoring its top priority in the NPS Scope Recommendations. Desalkylgidazepam (n=173) accounted for an additional 8%, while flubromazepam (n=48), etizolam/alpha-hydroxyetizolam (n=63), and flualprazolam (n=66) were also reported.

For NSOs, the top five recommended compounds were metonitazene, protonitazene, carfentanil, *N*-pyrrolidino etonitazene, and fluorofentanyl. *Para*-fluorofentanyl (n=2,327) was the predominant reported compound in this category, accounting for approximately 80% of NSO findings. This is likely due to the infiltration of *para*-fluorofentanyl into the illicit opioid supply since 2020. Carfentanil (n=215) accounted for another 8% of blood reports, while additional fentanyl analogs accounted for less than 1% of findings. 'Nitazene" opioids accounted for an additional 9.3% of NSO positives, with protonitazene (n=89), metonitazene (n=43), and *N*-pyrrolidino etonitazene (n=47), encompassing the majority of that subclass, along with *N*-pyrrolidino protonitazene (n=42).

In the Stimulants & Hallucinogens category, *N*,*N*-dimethylpentylone, pentylone, alpha-PiHP/alpha-PHP, 2F-2-oxo-PCE/fluoroexetamine, and eutylone were the top recommended compounds for Q2 2024. In the first six months of 2024, *N*,*N*-dimethylpentylone and its metabolite pentylone were the dominant findings, accounting for 90% of the 156 blood synthetic stimulant/dissociative blood reports. Eutylone (n=5) and alpha-PHP/alpha-PiHP (n=8) were also reported. Confirmation testing was not available for 2F-2-oxo-PCE/fluoroexetamine during this period, so could not be assessed.

The most recent SCs in the scope recommendations include MDMB-4en-PINACA, ADB-BINACA (-BUTINACA), MDMB-BINACA(-BUTINACA), 5F-MDMB-PINACA (5F-ADB), and ADB-4en-PINACA. For SCs, MDMB-4en-PINACA accounted for 85% of the 108 positive findings reported in blood cases during the studied time frame. The 5F-ADB metabolite accounted for another approximate 7% while 5F-ADB (reported as 5-fluoro-MDMB-PINACA/5-fluoro-EMB-PINACA) was reported four times. The remaining recommended SCs were outside the scope of testing capabilities, so positivity for those is unknown.

NPS surveillance requires quick identification of emerging substances and assessing prevalence before testing development decisions. The NPS Discovery Scope Recommendations aids laboratories with these efforts. The Tier I NPS recommendations by category in this analysis were well aligned with the most frequently detected NPS in this large sample population, further supporting its utility, although limited to the submitted sample population. The NPS scope recommendations are a valuable service provided by NPS Discovery that allows laboratories to make informed decisions related to updating their analytical workflows.

Novel Psychoactive Substances; Scope Recommendations; Laboratory

L58 Metabolic Profiling of the Semi-Synthetic Tetrahydrocannabinols (THCs), Delta 10-THC, and Delta 6a,10a-THC, Using Human Liver Microsomes and LC/MS

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Learning Objectives: The metabolic profile of Delta 10-THC and Delta 6a,10a-THC, semi-synthetic isomers derived from Delta 9-THC or CBD, was previously unknown. Attendees of this presentation will learn about the observed in-vitro metabolites formed from incubation of these THC isomers with Human Liver Microsomes (HLMs).

Impact Statement: This presentation will impact the forensic science community by providing characterization of in vitro metabolic pathways of new semi-synthetic Tetrahydrocannabinols (THCs) and comparison to known metabolism of naturally occurring THCs.

Abstract Text: The pharmacology of several naturally occurring phytocannabinoid constituents of *Cannabis Sativa* L., such as Delta 9-THC, has previously been studied and well documented in the scientific literature.[1] However, since the deregulation of hemp in the 2018 USDA Farm Bill, new semi-synthetic structurally related isomers, analogs, and homologs have emerged, presenting new chemical entities with unknown metabolic profiles. The semi-synthetically derived isomers Delta 10-THC and Delta 6a,10a-THC have been reported to retain similar cannabimimetic effects in relation to the naturally occurring and structurally similar Delta 9-THC.[2,3] Therefore, understanding their metabolism would provide beneficial insights to the forensic community and enable the development of effective toxicological screening methods.

The following stereo-specific isomers were individually incubated at 37°C for 60 minutes with pooled human liver microsomes in 0.1M phosphate buffer (pH 7.4) containing NADPH regeneration system: (6a*R*,9*R*)-Delta 10-THC, (6a*R*,9*S*)-Delta 10-THC, 9(*R*)-Delta 6a,10a-THC, and 9(*S*)-Delta 6a,10a-THC.[4] Potential metabolites were screened using Liquid Chromatography with Mass Spectrometry (LC/MS). The impact of microsomal incubation time on the number and ratio of resulting metabolites was explored as well. Based on known metabolism of similar THCs, several putative authentic hydroxy and carboxy metabolite reference standards were independently synthesized and used for positive qualification.

The in vitro incubation with human liver microsomes of the semi-synthetic tetrahydrocannabinols, Delta 10-THC and Delta 6a,10a-THC, provided positive identification and characterization of several potential phase I, hydroxylated metabolites. While minor metabolites have been identified, the primary phase I metabolites of Delta 9-THC are 11-hydroxy-Delta-9-THC (11-OH-THC) and 11-nor-9-carboxy-THC (11-COOH-THC).[1] This study found that metabolism of these semi-synthetic tetrahydrocannabinols by HLMs is comparable to naturally occurring Delta 9-THC, with hydroxylation at the C-11 position being the major metabolite.

The work put forward in this presentation will be impactful because the global proliferation of semi-synthetic cannabinoids continues to pose significant challenges to seized drug chemists and forensic toxicologists who need to rapidly develop testing methods to detect and quantify emerging substances of concern.

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Metabolism; Cannabinoids; Mass Spectrometry

L59 Cytochrome P450-Mediated Metabolism of Isotonitazene, a Novel Synthetic 2-Benzylbenzimidazole Opioid

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Learning Objectives: After attending this presentation, attendees will understand the importance of potential drug-drug interactions with the nitazene analogs.

Impact Statement: This presentation will impact the forensic science community by describing the Cytochrome P450 (CYP) enzymes responsible for isotonitazene metabolism and providing context for metabolite presence in toxicological specimens.

Abstract Text: Novel synthetic opioids continue to evolve, circumventing regulatory control. The nitazenes were first synthesized in the 1950s by CIBA Pharmaceuticals but more recently re-emerged in 2019 beginning with the isotonitazene.[1-3] Nitazene analogs have been identified in conjunction with other opioids, benzodiazepines, central nervous system stimulants, and the sedative xylazine. While isotonitazene metabolites have been identified in authentic samples and produced by human hepatocytes, the CYPs responsible for isotonitazene metabolism production have not yet been isolated.[4,5]

Isotonitazene was incubated with recombinant CYP enzymes (1A1, 1A2, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 3A4, 3A5, 2E1, or 2J2) in potassium phosphate buffer with an NADPH cofactor regeneration system. Reactions were quenched with ice-cold acetonitrile and deuterated internal standard over the course of two hours. After centrifugation, sample supernatant was diluted 1:5 with mobile phase prior to Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) analysis in positive electrospray ionization mode. Chemical inhibitors were used to confirm CYP activity for 2B6 (ticlopidine), 2D6 (quinidine), and 3A4 and 3A5 (ketoconazole).

CYP 2B6, 2D6, 3A4, and 3A5 were found to produce isotonitazene metabolites. The primary metabolite, *N*-desethylisotonitazene, was produced by all four CYPs. The *O*-dealkylation, 4-hydroxynitazene, was produced by CYP 2B6 and 2D6 with minor contribution from 3A5. *N*-desethyl-*O*-desalkylnitazene was majorly produced by CYP 3A4 after the production of *N*-desethylisotonitazene. Neither the 5-nitro reduction nor hydroxylated metabolites were identified. While isotonitazene is estimated to be about 500 times the potency of morphine, *N*-desethylisotonitazene is estimated to be near the potency of etonitazene (~1,000 times).[6,7] Variation in metabolic efficiency due to the high degree of polymorphism in CYP 2D6 and the 3A family as well as induction of enzymes responsible for its production could increase the concentration of *N*-desethylisotonitazene within the body to lethal levels. *N*-Desethylisotonitazene is an essential target for toxicological interpretation given its increased pharmacological activity and abuse independent of the parent analog.

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Nitazenes; Metabolism; In Vitro

L60 Metabolite Profile Commonalities of Methoxylated Fentanyl Analogs

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Learning Objectives: Attendees will learn the impact of substitutions and additions to the fentanyl scaffold on the resulting metabolite profile via a study utilizing in vitro metabolism with pooled Human Liver Microsomes (HLMs). The focus is on the addition of methoxy groups in the ortho- and para- positions of the anilino phenyl ring of the fentanyl scaffold.

Impact Statement: The impact to the forensic science community of this research lies in the ability to discern the alterations of a potential new fentanyl analog parent drug based on the metabolites present in a clinical sample. This will aid forensic toxicological investigations when the parent drug is not present in the biological sample.

Abstract Text: The expectation of this research is that if fentanyl analogs are altered with some commonality to one another, the results of biotransformation will yield common metabolites due to the structural likeness between the parent drugs. It is well known that the primary metabolic pathway for fentanyl is oxidative N-dealkylation via the CYP450 *superfamily* of enzymes found in the human liver, specifically the isozyme CYP3A4.[1] However, additions and other alterations to the general fentanyl scaffold will impact the metabolic pathway that is favored due to the inherent changes in polarity of the overall molecule and steric hinderance of relevant atoms that result from these alterations.

This study was conducted using pooled (50 donors) HLMs for in vitro metabolism of methoxylated fentanyl analogs according to the ThermoFisher Scientific protocol for incubating HLMs.[2] The drugs of interest in this study include para-methoxy fentanyl, ortho- and para-methoxybutyryl fentanyl, as well as ortho- and para-methoxyfuranyl fentanyl, in order to assess the effect of the addition of a methoxy-group to the anilino phenyl ring of the fentanyl scaffold, as well as to determine if multiply-altered fentanyl analogs would respond similarly to biotransformation. Assays were incubated in triplicate for 60 and 90 minutes periods, with a focus on only phase I metabolism products. All assays were analyzed via Liquid Chromatography/Mass Spectrometry (LC/MS).

Preliminary results detected common metabolites within the group of methoxylated fentanyl analogs of interest, separate from the expected metabolites of fentanyl alone. Among the metabolites detected were several variations of norfentanyl, a common metabolite of fentanyl. However, these variations were adulterated based on the initial modifications from the parent fentanyl structure (i.e., a methoxylated butryryl norfentanyl metabolite was observed for para-methoxybutyryl fentanyl). A peak with a m/z of 328 was observed in the metabolite profiles of paramethoxy-furanyl fentanyl and paramethoxy butyryl entanyl and is believed to be a shared metabolite of both drugs, methoxylated and hydroxylated variant of 4-ANPP. Additional metabolites of methoxylated analogs, which have been shared between two or more drugs, were detected with an m/z of 264, 276, and 301. Although it was theorized that one metabolite could be detected for the lot of methoxylated analyzed, it was found that there might be unique metabolites to a subset od drugs in one analog class, indicating that shared metabolites can be key to determining a class of drug ingested. We are currently determining the chemical structure of these common metabolites using LC/MS/MS. The identification of these common metabolites may provide valuable insight to clinical scientists in the future when a new fentanyl analog is encountered in the field.

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Fentanyl; Metabolite; LC/MS

L61 Novel Psychoactive Substance Presence in Fentanyl/Norfentanyl-Positive Clinical Urine Specimens

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Learning Objectives: Attendees will learn about a method to analyze urine for Novel Psychoactive Substances (NPS). They will also learn about NPS presence in clinical specimens initially analyzed for Fentanyl and Norfentanyl (F/N).

Impact Statement: This presentation will impact the forensic science community by showing that even if a patient appears to be compliant when taking prescription drugs, they may have taken NPS as well. Analyzing for prescription and other common licit and illicit drugs in urine may not provide a comprehensive picture of all drugs that were ingested.

Abstract Text: NPS were initially designed as an alternative, "legal" way to take drugs. The creators of NPS are constantly changing the structures of these compounds to create new compounds that evade legislation such as the Drug Enforcement Agency scheduling measures. Individuals may take these substances to appear compliant with prescription drug use by avoiding a positive drug test for other common, more frequently tested drugs of abuse. NPS first became popular in the 2000s and since then, over 1,000 different NPS have been identified.[1-3]

Randomly selected deidentified remnant specimens that were submitted for F/N clinical drug testing at Quest Diagnostics were later analyzed for NPS between March and July of 2023. Specimens were obtained from six different geographical regions in the United States: Mid-Atlantic, Northeast, Southeast, Midwest, Southwest, and West. The F/N specimens were divided into two categories: those that had F/N quantitation values within the Analytical Measurement Range (AMR) of 0.25 to 500ng/mL and those that had F/N quantitation values above the AMR (greater than 500ng/mL). The specimens were analyzed on an NPS method containing 88 NPS in six different categories: other illicit additives, designer fentanyl analogs, designer benzodiazepines, designer opioids, designer stimulants, and synthetic cannabinoids. This method was validated following Clinical Laboratory Improvement Amendments (CLIA) and Quest Diagnostics guidelines that tested linearity, precision/accuracy, interference studies, and matrix effects. Interference studies challenged all within-panel analytes, 123 commonly prescribed and over-the-counter analytes and 90 other related analytes. Three-point calibration curves were used and all compounds had cutoff values ranging from 1.0 to 10ng/mL. The specimens were prepared using a dilute-and-shoot method, which included room temperature hydrolysis with B-One enzyme and filtration with DPX filter tips. Analysis by liquid chromatography/tandem mass spectrometry was performed and compounds were separated with a Phenomenex Kinetex F5 100A, 50x3mm, 2.6µm liquid chromatography column.

Overall, NPS positivity for the within-F/N-AMR specimens ranged from 6.1% in the West to 40.2% in the Northeast. NPS positivity for the above-F/N-AMR specimens ranged from 60.4% in the West to 100% in the Southeast. The most commonly seen NPS was xylazine (0% to 33.3% positivity seen on the within-F/N-AMR specimens and 17.0% to 90.9% positivity seen on the above-F/N-AMR specimens). Fentanyl analogs were also frequently seen, the most common of which was acetyl fentanyl, particularly in the above-F/N-AMR specimens, with 52.8% (samples from the West) to 70.4% (samples in the Mid-Atlantic) of the specimens having at least one designer fentanyl analog present. Other commonly seen NPS in these specimens included bromazolam (designer benzodiazepine), metonitazene (designer opioid), N,N-dimethylpentylone (designer stimulant), and the metabolite for MDMB-4en-PINACA (synthetic cannabinoid).

Both the within-F/N-AMR specimens and the above-F/N-AMR specimens contained numerous NPS. Presence of NPS in these urine specimens indicates that a drug may have been taken outside of prescription drug use, which could help clinicians determine that a drug was obtained illicitly. Additional within- and above-F/N-AMR specimens will be analyzed in 2024 to evaluate NPS changes.

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Fentanyl; Urine; Drug Analysis

L62 The Prevalence of P-Fluorofentanyl and Xylazine in Postmortem Fentanyl Casework: A Three-Year Retrospective Study

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Learning Objectives: The goal of this presentation is to highlight the prevalence and trend use patterns of fentanyl, xylazine, and *p*-fluorofentanyl in postmortem casework from 2021 to 2023 for five medical examiner districts in the state of Florida.

Impact Statement: This presentation will contribute to providing the toxicology community with a better understanding of polysubstance drug trends in postmortem toxicology of the studied population over the past few years.

Abstract Text: The illicit drug market in the United States has been changing rapidly. Fentanyl remains the primary drug linked to opioid-related deaths. Fentanyl is commonly found in the presence of other drugs, either as adulterants or other substances, and rarely alone. Xylazine has emerged as an adulterant in many illicit drug products. Xylazine is a veterinary drug used as a tranquilizer and sedative. The side effects include sedation, hypotension, and bradycardia, and it is not approved for human use. An increase in the trafficking of fentanyl mixed with xylazine has been reported across the US over the years, with users experiencing serious and life-threatening side effects that make it difficult to distinguish opioid overdoses from xylazine exposure.

With the rise in illicit fentanyl, several fentanyl analogs have also been reported in recent years. In 2016 during a period of fentanyl analog proliferation, *para*-fluorofentanyl (*p*-FF) started to garner attention, with a prevalence that varied across the US. After the core-structure scheduling in 2018 adopted by the US and internationally (e.g., China), *p*-FF positivity drastically dropped, but started to reappear at the end of 2020 mostly as an adulterant of fentanyl.[1,2] Fluorofentanyl is fluorinated on the aniline ring of fentanyl in the *para*-, *meta*- or *ortho*- position. Although less potent than fentanyl, *p*-FF was found to enhance the overall pharmacological effects in combination with fentanyl. The adverse effects include sedation, respiratory depression, lethargy, coma, and death.

The aim of this study was to provide data on the prevalence and trend use patterns of fentanyl, xylazine, and *p*-FF in a case series from January 2021 to December 2023 for five medical examiner districts in the state of Florida. The postmortem specimens (blood, urine, bile, liver, chest fluid, decomposition fluid, brain) or samples from hospital admission were qualitatively screened using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) and quantitated using gas chromatography-mass spectrometry (GC-MS). The method validation was described in Truver et al.[3]

A three-year retrospective analysis of data was conducted. A total of 8121 cases were submitted to the forensic toxicology laboratory for analysis over the period of study (Table 1). Among them, 1530 cases (18.8%) tested positive for fentanyl in one or more of the specimens submitted for the case. The age of the decedents ranged from 16 to 85 years (median: 41.0 years; mean: 42.6 years), with an 8-month-old outlier excluded. The majority of decedents were male (71.7%), with white being the major race (79.5%), followed by black (9.3%), hispanic (9.0%), and others (2.2%).

The concentration range for fentanyl in blood specimens (N=1356) tested was 2.5-579 ng/mL (median: 15.0 ng/mL; mean ± S.D.: 25.6 ± 44.4 ng/mL). For 69 cases, fentanyl was detected in the postmortem blood, but not quantified as the concentration was below the lower limit of quantitation (LLOQ, 2.5 ng/mL). Xylazine was identified in the blood of 78 decedents and in the urine of 391 decedents. *p*-FF was detected with fentanyl in 164 postmortem blood samples, with a concentration range of 2.5-65 ng/mL (median: 7.5 ng/mL; mean ± S.D.: 12.2 ± 12.8 ng/mL). For 66 cases, *p*-FF was positive at a concentration below the LLOQ (2.5 ng/mL). The combination of fentanyl, *p*-FF, and xylazine was detected in 70 cases.

Polysubstance use with fentanyl and xylazine was observed with other traditional stimulants (80.1%), opioids and depressants (12.4%), benzodiazepines (13.4%), ethanol (18.0%), cannabinoids (31.7%), and other drugs (27.2%). Similar trends were observed when considering polysubstance use with fentanyl and *p*-FF, or fentanyl together with both xylazine and *p*-FF, with the cocaine, methamphetamine, and amphetamine being the most common co-occurring substances.

Based on trends in the illicit drug supply, this study aimed to provide a better understanding of the fentanyl analog and adulterant epidemic in Florida and to promote increased awareness for future strategies of drug analysis and prevention.

Table 1.

Year	Total cases per year (N)	Cases tested positive for fentanyl (N)	(%) per year
2021	2523	485	19.2
2022	2669	512	19.2
2023	2929	533	18.2
Total	8121	1530	

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Fentanyl; Prevalence; Polysubstance Use

L63 The Detection of Novel Psychoactive Substances in Urine Specimens From a Large United States Prison System

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Learning Objectives: Attendees will learn about Novel Psychoactive Substances (NPS) and a high throughput method for NPS analysis in urine. They will also learn about NPS detection in clinical urine specimens from a large United States prison system.

Impact Statement: This presentation will impact the forensic science community by showing the presence of NPS in urine specimens from a prison system. These substances are making their way into the prison system, which can impact prescription compliance and behavior during incarceration.

Abstract Text: NPS are compounds designed to mimic popular drugs of abuse. These compounds were designed as alternative, "legal" ways to take drugs and started becoming popular in the 2000s. The creators of these compounds frequently change the compounds' structures, making it difficult for clinicians to know what a user may be taking. This poses challenges to regulatory agencies that are trying to schedule these compounds. It also makes finding appropriate treatment options more difficult.[1-4]

Individuals on medical therapy in a large prison system within the United States receive drug testing regularly for monitoring and compliance purposes. Clinicians started noting negative drug screen results when clinical presentations and clinical histories suggested drug use. To determine if NPS were present among individuals tested in the prison system, 187 urine specimens were obtained and tested for NPS in July 2023. These specimens were selected based on availability at the time of testing. These specimens were divided into two categories: ones that tested positive for fentanyl (87 specimens) and ones that tested negative for common licit and illicit drugs of abuse (100 specimens). In these specimens, xylazine was the initial concern for the prison system and why they asked for NPS testing.

A validated NPS method testing 88 NPS in six different categories (other illicit additives, designer fentanyl analogs, designer benzodiazepines, designer opioids, designer stimulants, and synthetic cannabinoids) was used for analysis. This method was validated using Clinical Laboratory Improvement Amendments (CLIA) and Quest Diagnostics guidelines and included linearity, precision/accuracy, matrix effects, and interference studies. Metabolites were detected whenever possible. Three-point calibration curves were employed; cutoff concentrations ranged from 1.0 to 10ng/mL. A dilute-and-shoot method with filtration, including room temperature enzymatic hydrolysis using B-One enzyme (Kura Biotech, Atlanta, GA) was used for extraction and analysis was performed using liquid chromatography/tandem mass spectrometry. Separation was achieved using a Phenomenx Kinetex F5 100A, 50x3 mm, 2.6µm liquid chromatography column.

Overall, 15 of the 87 fentanyl-positive specimens were also positive for at least one NPS (20.5%). Eight of the 100 negative specimens were positive for an NPS (8.0%). For the fentanyl-positive specimens, 8 were positive for a designer fentanyl analog, 2 were positive for an other illicit additive (xylazine), and 7 were positive for a synthetic cannabinoid. For the negative specimens, all 8 were positive for a synthetic cannabinoid. Our data demonstrates that xylazine is not the predominate concern in this population.

The NPS analysis of urine specimens from this large prison system indicates the presence of illicit drugs in the prison population. It is unknown if individuals are attempting to avoid failing a drug test by taking an NPS or if they are unsure of what may be in the drugs they are taking. Additional specimens from the large prison system will be tested in the future to determine if NPS are still present in the prison population. This method is currently being used to analyze clinical specimens submitted to Quest Diagnostics for NPS testing. NPS trends in the clinical population and how they compare to the prison population are being investigated.

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Novel Psychoactive Substances; Drug Analysis; Xylazine

L64 Two Fatal Cases Involving N-Ethylpentedrone in a Secured Forensic Psychiatric Clinic

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Learning Objectives: After attending this presentation, attendees will understand the methods of detection for N-Ethylpentedrone (NEP) in biological samples from two fatal cases, despite negative testing results using targeted and suspect screening methods (**Liquid Chromatography/Time-Of-Flight**/Mass Spectrometry [LC/TOF/MS], Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry [LC/qTOF/MS], Liquid Chromatography/Tandem Mass Spectrometry [LC/MS/MS], and Gas Chromatography/Flame Ionization Detector [GC/FID]).

Impact Statement: This presentation will impact the forensic science community by presenting the results of comprehensive pathological, toxicological and chemical investigations in two fatal cases involving NEP.

Abstract Text: NEP is a Novel Psychoactive Substance (NPS) structurally related to natural cathinone, an alkaloid present in the leaves of the *Catha edulis* (Khat) plant. It is mainly used for its euphoric and excitatory effects. Commonly reported side effects consist of hallucinations, delirium, acute psychosis, hypertension, tachycardia, and, at higher concentrations, hyperthermia and multi-organ failure, potentially leading to death.[1] Under the Dutch Opium law, NEP is currently not considered to be illegal, making it an easily accessible substance. Although first user reports of NEP already were described in the mid 2000's, postmortem toxicological data on this NPS are scarce.[2,3] Here, we describe two fatal cases involving NEP taking place in May and June 2024.

Case 1: A 46 year-old man, incarcerated in a secured forensic psychiatric clinic, was delusional, after claiming to his psychiatrist to have used a new kind of drugs. The man was transferred to a stimulus-deprived cell, where he was found unresponsive by a medical practitioner half an hour later, and died shortly after.

Case 2: Approximately two months later, a 46-year-old man claimed to have self-administered drugs in the same clinic as Case 1. About 8 hours later, he called for help with symptoms of shortness of breath and the sensation of overheating. Further medical examination resulted in a diagnosis of hyperventilation, tachycardia, and hypertension, after which the man's health deteriorated rapidly, resulting in need for resuscitation. Despite the attempted resuscitation, the man died within an hour of the initial self-reported symptoms.

For both cases, femoral blood and urine samples were initially collected at the scene by the medical examiner for toxicological analysis in a hospital pharmacy. Both of these analyses did not result in a possible toxicological cause of death, after which the cases were sent to the Netherlands Forensic Institute for additional investigation. At the Netherlands Forensic Institute, toxicological and chemical examinations were performed for both cases; Case 2 also underwent a complete forensic autopsy. Biological samples were investigated for the presence of toxic substances using headspace GC/FID, LC/qTOF/MS, and LC/MS/MS. Presumed drugs and drug paraphernalia found at both scenes were investigated using GC/MS, GC/Infrared Spectroscopy (GC/IR) and LC/TOF/MS.

For Case 1, initial toxicological investigations (LC/TOF/MS, LC/qTOF/MS, LC/MS/MS, and GC/FID) in femoral blood resulted in the detection of aripiprazole, dehydroaripiprazole, metformin, valproic acid, and zuclopenthixol, indicating low to therapeutic concentrations. Additional manual investigation of the qTOF screening data revealed the potential presence of NEP, which was analytically confirmed using a certified reference standard, indicating an active to high concentration in femoral blood.

For Case 2, initial toxicological investigations in femoral blood resulted in the detection of promethazine and aripiprazole, indicating low to therapeutic concentrations. As for case 1, NEP was detected following additional investigation of LC/qTOF/MS screening data, also indicating an active to high concentration in femoral blood. The autopsy revealed the aspecific findings of edema of the lungs and the brain, a well-filled bladder, and, throughout all organs, congestion of blood vessels (in a status post-resuscitation). There were only minor traumatic lesions and few organic alterations/diseases, which were deemed insignificant regarding the cause of death.

For both cases, chemical analysis of the presumed drugs and drug paraphernalia resulted in the detection of NEP. Based on the outcomes of the pathological, toxicological, and chemical investigations, and the obtained medical information, an NEP-related death was concluded in both cases. The presented cases emphasize the impact of a thorough toxicological examination for the detection of NPS, using a variety of targeted and untargeted screening approaches.

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N-Ethylpentedrone; NPS; Postmortem

L65 Insights Into Phosphatidylethanol (PEth) 16:0/18:1 Formation and Degradation Analysis by Supercritical Fluid Chromatography-Tandem Mass Spectrometry

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Learning Objectives: After attending this presentation, attendees will be able to identify and describe the risks of PEth formation relevant to Postmortem (PM) cases.

Impact Statement: This presentation will impact the forensic science community by increasing awareness of PEth concentrations in authentic PM samples and how PEth concentrations may change after death.

Abstract Text: **Background**: PEth consists of phospholipids formed in the presence of ethanol, as catalyzed by phospholipase D. Due to its high specificity, sensitivity, and prolonged detectability, the quantification of the predominant homolog, PEth 16:0/18:1, is presently being used for different antemortem clinical and forensic applications. Despite its potential utility in PM toxicology, there is limited research characterizing PEth formation and degradation in the early PM phase.

Objectives: The goals were to develop and validate a Supercritical Fluid Chromatography-tandem Mass Spectrometry (SFC-MS/MS) method to quantify PEth 16:0/18:1 in blood and evaluate the possibility of PEth formation and degradation following death using an in vitro model.

Methods: Liquid-liquid extraction with an 80:20 heptane: isopropanol extraction solvent was utilized. Analysis was conducted using SFC-MS/MS. Separation between PEth 16:0/18:1 and other lipids was achieved on a Waters Corp Torus DEA column (1.7µm,3mm X 100mm), with a 1mL/min flow rate of CO_2 and 0.1% ammonium acetate in 95:5 methanol:water under a 10-minute gradient at 50°C. Method validation was performed per in-house protocols, and American National Standards Institute/Academy Standards Board (ANSI/ASB) 036 recommendations. A proof-of concept study was conducted with 35 autopsy samples obtained from the Swedish National Board of Forensic Medicine.

Four heparinized blood sources were used to simulate PEth formation in vitro in the first 48 hours postmortem. Aliquots were spiked to Blood Alcohol Content (BAC) 1.5g/L and 3g/L, respectively, then incubated in a water bath at 37°C for 1 hour. The water bath was then left to cool, with duplicate measurements taken hourly for 7 hours and after 24 and 48 hours. The degradation study was conducted simultaneously with the same four blood sources (BAC 0g/L) by monitoring initial in vivo PEth concentrations over time. Aliquots were stored at 4°C and extracted within 12 hours post-sampling. Another study was later conducted with the blood fortified to BAC 3g/L, but incubating in a 37°C water bath for 24 hours.

Results: All parameters met acceptability criteria per ANSI/ASB 036 guidelines. The linear range (n=8) was 10-2,500ng/g with a 1/x weighing. The Lower Limit of Quantification (LLOQ) was 10ng/g. Bias, within-run and between-run precision for QCs (20, 200, 2000ng/g) were < -6.8%, < 17.1% and < 8.3%, respectively. Endogenous and exogenous interferences were negligible. Autopsy samples yielded PEth concentrations between 32.6-2,476.3ng/g.

Minimal degradation was noted for in vivo-formed PEth samples, with degradation rates between 0.04-0.2ng/g/hr. PEth formation occurred in an ethanol concentration-dependent manner. Higher BAC resulted in increased PEth formation rates, with statistically significant differences between BAC 1.5g/L and 3.0g/L samples using paired t-tests (α=0.05). PEth formation decreased at lower temperatures and plateaus off before reaching room temperature. Overall formation was found to be relatively low, with formation rates of BAC 1.5g/L and 3.0g/L samples in the first 7 hours ranging between 1.6-3.4ng/g/hr and 3.0-4.6ng/g/hr, respectively. PEth concentrations ranged between 18.3-44.3ng/g for BAC 1.5g/L and 26.4-58.6ng/g for BAC 3.0g/L after 24 hours.

In contrast, PEth formation exhibited a linear trend over 24 hours when BAC 3g/L blood sources were maintained at 37°C, with similar formation rates ranging between 3.6-5.4ng/g/hr and reaching final PEth concentrations between 127.0-164.4 g/g.

Conclusion: We conclude that in vivo formed PEth was stable up to 48 hours in an in vitro model mimicking the temperature decline from 37°C to ambient occurring after death. Results also showed that the in vitro formation of PEth in blood containing high BACs was relatively low and temperature dependent. Careful consideration is needed when interpreting PEth concentrations in PM cases, particularly in cases with long PM interval and when the body has been maintained at elevated temperatures.

Alcohol; Postmortem; Liquid Chromatography

L66 Alcohol Extrapolation: Contentions Within the Scientific and Legal Systems

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Learning Objectives: After attending this presentation, attendees will be able to understand the contention of the use of different types of extrapolations in the legal system, examine the application of current scientific principles to estimate Blood Alcohol Concentrations (BACs) over time, describe how to adequately calculate the amount of alcohol consumed, the strengths and weaknesses of this approach, and explain how to apply these principles to medicolegal matters and existing court decisions.

Impact Statement: This presentation will further amplify the very recently approved *Best Practice Recommendations for Performing Alcohol Calculations in Forensic Toxicology* (Academy Standards Board [ASB] 122) and impact the forensic science community by proposing methods for standardizing the use of alcohol extrapolation calculations. Our review aims to distinguish how despite academic and medical research using various forms of extrapolation, in legal testimony the use of extrapolation is often contentious, sullied by misinformation, inconsistency, improper use, and bias. In the future, we hope this knowledge will be used to adequately calculate the amount of alcohol consumed and apply these principles to medicolegal matters and existing court decisions.

Abstract Text: Alcohol is ubiquitous and the most commonly found drug in criminal investigation and civil litigation. In such cases, the legal process often requires experts to interpret alcohol test evidence through various calculations, incorporating different types of extrapolations (including "back-calculations") to answer a number of relevant legal questions about alcohol use and intoxication in a person of interest. Extrapolations are not new and are widely used in diverse disciplines such as medicine, pharmacology, epidemiology, and clinical practices. Extrapolations are used to reach conclusions from a set of known facts and scientific assumptions to predict certain consequences from data. In alcohol research, reasonable assumptions about data applicable to pharmacokinetics, including the rate(s) of absorption and elimination, drinking periods, individual anthropomorphic, and other drinker characteristics are routinely incorporated into the calculation. Careful consideration of these underlying assumptions is essential in the use of extrapolation in forensic matters.

Although clinical alcohol scientists perform these types of analyses as a regular basis course of their research, there is some lack of consensus about the use of extrapolation among forensic toxicologists whom are often called upon by the court system to estimate alcohol use and subsequent intoxication.[1] Multiple peer-reviewed scientific publications have presented mathematical models to accurately calculate alcohol use and subsequent intoxication based on known scientific principles or objective chemical tests.[2-6] For many years, alcohol researchers have recognized the need for standardization of such calculations in their field.[7-13] In April 2024, The Academy of Forensic Standards approved a "best practices" guide to further standardize the field.[14] However, not everyone may be aware of ASB 122 or the guidelines set out by the Academy. Our goal is to broaden the scope of knowledge on alcohol extrapolation that ultimately led to the development of ASB 122 and further the information to all attendees. There particularly is a growing necessity in forensic toxicology for evidence-based consensus on this topic, especially when the application of this methodology is subject to a *Frye* or *Daubert* challenge. To our knowledge, there are few, if any, scientific treatises that connect the science (extrapolation) to the law (e.g., applied to *Daubert* or *Frye*).

Specially, we will focus on four issues: (1) the use of different forms of extrapolation in biomedical and forensic applications to estimate alcohol use based on subjective or objective evidence; (2) examples of the use of anterograde and retrograde extrapolation to estimate BACs over time based on subjective or objective evidence; (3) the need to standardize and define "a drink" when extrapolation is used to estimate the consumption of different alcoholic drink formulations and subsequent intoxication; and (4) a review of the relevant case law in the United States on the use of such scientific methods.

Described in this regard are the requirements necessary to reasonably and accurately estimate alcohol use and extrapolate BACs based on objective chemical test results. The inclusion of subjective self-reporting and limitations of including them in such analyses will also be discussed in reference to legal precedents of the use of extrapolation evidence in the United States legal system.

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Alcohol; Calculations; Legal

L67 Performing Blood Alcohol Calculations According to ASB 122 Using a Simple Spreadsheet

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Learning Objectives: Attendees will learn how to access and use a simple spreadsheet tool to conduct alcohol calculations in accordance with Academy Standards Board (ASB) 122 Best Practice recommendations. The tool can easily be implemented in laboratory workflows.

Impact Statement: Implementation of validated spreadsheet tools will make it easier for toxicology laboratories and other toxicologists to adhere to the ASB best practices in their alcohol calculations. Hopefully, this will contribute to wider adoption of the guidelines and more consistent and transparent calculations across the discipline.

Abstract Text: In mid-2024, the American National Standards Institute/Academy Standards Board (ANSI/ASB) published Best Practice Recommendation 122, detailing how to perform alcohol calculations in forensic toxicology.[1] While ANSI/ASB 122 provides detailed instructions, performing these complex calculations by hand is still cumbersome and carries the risk of human error. Although advanced calculation software available to laboratories can vary considerably by resources and expertise, spreadsheets are virtually ubiquitous in the workplace as part of basic software packages provided by employers. However, not all laboratories have staff with the skillset to develop a complex and robust in-house calculation tool via spreadsheet.

To help laboratories implement ANSI/ASB 122, we developed a simple Excel spreadsheet tool that can automate the calculations. The spreadsheet covers all aspects of ANSI/ASB 122, including volume of distribution, Widmark's formula, and retrograde extrapolation. In addition, the spreadsheet allows laboratories to enter and take the measurement uncertainty of their own Blood Alcohol Concentration (BAC) methods into account in retrograde extrapolation, and allows users to increase or decrease statistical coverage factors when applicable. As a specific ratio for serum/plasma to whole blood conversion of alcohol concentrations is required by law in New York State, the spreadsheet allows the user to input and toggle between local statutory requirements for this conversion and the range provided by ANSI/ASB 122.

The user provides available case data and laboratory preferences on an input tab, and the spreadsheet provides the results and output statements, including applied assumptions. The spreadsheet is validated to ensure all example calculations included in ANSI/ASB 122 are calculated correctly.

To allow easier implementation, the spreadsheet is self-contained and does not include any macros or Visual Basic for Applications (VBA) code. In addition, calculations are separated from inputs to allow laboratories to lock and password protect the spreadsheet in order to prevent accidental changes. It is also suitable to be uploaded to laboratory quality management systems.

As part of this presentation, the spreadsheet and model instructions will be made available to attendees, and the authors are willing to assist in laboratory implementation. Attendees are free to use any parts of the spreadsheet to develop their own versions.

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Blood Alcohol Concentration; Retrograde Extrapolation; Best Practices

L68 Drug-Facilitated Crime: A Review of Findings Between 2019 and 2023

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Learning Objectives: After attending this presentation, attendees will be able to evaluate their laboratory's scope of analysis for toxicology testing in Drug-Facilitated Crimes (DFCs) and the need to supplement the recommended Academy Standards Board (ASB) scope with other drugs based on these patterns.

Impact Statement: This presentation will impact the forensic science community by presenting information on the prevalence and frequency of various drugs in victims of sexual violence. The data are based on a seven-year review of over 10,000 blood and urine samples analyzed in our laboratory.

Abstract Text: Sexual violence is sexual activity when consent is not freely provided or obtained, and hundreds of thousands of cases are believed to occur annually in the USA. Drug-facilitated crime (DFC) is when a victim is incapacitated in their physical or mental abilities through the administration of a drug, both non-consensual and self-administered, making them vulnerable to criminal acts such as robbery, sexual assault, or other forms of exploitation. Substances used in the commission of these crimes are selected for their sedating and incapacitating effects, including impairing the victim's ability to provide consent, defend themselves, to create or retain recent memories, and recall the assault occurrence or specific details.

Blood (n=2371) and urine (n=5041) samples from suspected DFC cases were submitted to NMS Labs between January 2019 and December 2023. Samples were submitted to comprehensive, validated test panels based on the ANSI/ASB Standard 121, Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in DFC Investigations including tests for ethanol, common prescription and over-the-counter drugs, recreational and misused drugs, and gamma-hydroxybutyrate (GHB). Blood and urine positivity is described below. Samples reflect different matrices were submitted in DFC investigations.
The most prevalent drug classes detected were ethanol, delta-9-THC/metabolites, and stimulants, however drug prevalence order was matrix dependent. Ethanol was more prevalent in urine versus blood (approximately 28% and 24%). Cannabinoids were more prevalent in urine than blood (approximately 35% vs 28%). The most encountered drugs within select drug classes are shown below. Within the stimulant drug class, the most prevalent analytes include methamphetamine, amphetamine, and cocaine. Benzodiazepines were present in approximately 9.1% and 14% of blood and urine cases respectively, including clonazepam, alprazolam, and lorazepam. Benzodiazepine prevalence was higher in 2019 urine samples (17%) than 2023 (13%) indicating a consistent decline in common benzodiazepines. Many designer benzodiazepines were not tested for as they are not included within the suggested ASB analysis scope. Flunitrazepam, which has a reputation as a date rape drug, was infrequently detected, with only one finding indicated in blood and urine in 2019. Opioids were detected less frequently with overall positivity of 4.5% in blood and 12% in urine, with the most frequently encountered drugs being morphine, oxycodone, and fentanyl. An increase in fentanyl prevalence was noted between 2019 (1.6% and 5.8% in blood and urine respectively), compared in 2023 (2.5% in blood and 6.9% in urine).

Drug Class	Drug	Blood Positivity	Urine Positivity
Stimulants	Amphetamine	3%	4.5%
Stimulants	Methamphetamine	13%	15%
Stimulants	Benzoylecgonine	4.4%	13%
Benzodiazepines	Clonazepam	3%	4%
Benzodiazepines	Alprazolam	1.7%	3.4%
Benzodiazepines	Lorazepam	3.2%	4.9%
Opioids	Morphine	< 1%	2.2%
Opioids	Oxycodone	< 1%	1%
Opioids	Fentanyl	2%	6%

GHB is an endogenous compound, but ASB guidelines suggest thresholds above which its presence can indicate exogenous administration. GHB detections suggesting exogenous administration were < 1% in both blood and urine, indicating it's also uncommon like flunitrazepam. As no investigative information was provided, differentiating drugs that were consumed medically, voluntarily, or were administered surreptitiously is impossible.

Given the number of sexual assault and other criminal cases involving drug use, toxicology should be routine in all investigations. Analyte detection does not necessarily mean it contributed to the alleged crime. Furthermore, if someone is under the influence of a drug, it is still considered a DFC even if the individual took the drug willingly. DFC cases must be evaluated on a case-by-case basis and findings put into the perspective of the case which includes scene investigation information, or additional testing if the case history suggests specific substances out of the laboratory's testing scope.

FC; Toxicology; Drug Trends

L69 An Investigation Into the Utility of Creatinine and the Submaxillary Gland Androgen-Regulated Protein 3B as Adulteration Biomarkers and Normalization Factors in Oral Fluid

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Learning Objectives: Attendees will hear about the quantification of creatinine and two signature peptides of the submaxillary gland androgen-regulated protein 3B (SMR3B-peptide 1 and 2) in oral fluid using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), gain insights into their concentrations in authentic oral fluid samples, and understand the application of these compounds as normalization factors for drug concentrations in this matrix.

Impact Statement: This presentation will contribute knowledge that will equip the forensic science community with an analytical strategy to improve the availability of validity tests in oral fluid and standardize drug quantification in this matrix in forensic settings.

Abstract Text: Oral fluid is a biological sample increasingly used in forensic toxicology. It is easy to obtain, shows low biohazard risk, and reflects recent drug exposure. However, the interpretation of its quantitative values is limited; drug concentrations do not correlate well with blood/plasma concentrations and show high inter- and intra-individual variability.[1] To improve the quantitative interpretation of oral fluid, it is necessary to establish markers that can detect adulteration, confirm validity, and act as normalization factors.[2] The goals of this project were: 1() to develop and validate new analytical methods for the determination of endogenous compounds, creatine, and SMR3B-peptides in oral fluid; (2) to investigate their typical concentrations and variations due to gender, age, and sample collection time; (3) to correlate blood/plasma drug concentrations with oral fluid normalized and not normalized drug concentrations.

To extract SMR3B peptides (SMR3B-1 and 2), 5µL oral fluid was digested with trypsin and filtered in the presence of an isotope-labeled synthetic peptide (SMR3B-2iso) used as internal standard (5µL 10nmol/mL). Twenty µL were injected into the LC/MS/MS and two Multiple Reaction Monitoring (MRM) transitions were monitored per compound in positive mode, following a reversed-phase chromatographic separation with 0.1% formic acid in water and acetonitrile as mobile phases in gradient mode. The method was linear 0.5-50nmol/mL (n=4) and process efficiency was 76.1% (n=10). To determine creatinine, 25µL internal standard creatinine-d_3 at 0.1µg/mL and 200µL acetonitrile were added to 25µL oral fluid. After centrifugation, 5µL of supernatant was injected into the LC/MS/MS. Chromatographic separation was performed using a Hydrophilic Interaction Liquid Chromatography (HILIC) column, with mobile phase 10mM ammonium formate and acetonitrile in gradient mode. Two MRM transitions per compound were acquired in positive mode. The method was linear 0.01-1µg/mL (n=5). Imprecision was < 11% (n=15), bias between -7.73% and 0.03% (n=15), and the overall process efficiency was 80% (n=10). Validation parameters were evaluated at 0.025 and 0. 25µg/mL.

Authentic oral fluid samples from 18 donors (n=107) were analyzed. Among the donors, 11 were females and 7 males, and 8 were < 30 years old. Fifty-three samples were self-collected in the morning and 54 in the evening. SMR3B-1 and 2 were detected in about 50% of the samples, with concentrations from 0.5 to 22.4nmol/mL. No differences were observed due to gender or age. Statistical significance ($P < 0.05$) was observed in peptide concentrations depending on the collection time (morning vs. evening). Creatinine was found in all oral fluid samples, except one participant, with concentrations from 0.02 to 0.48µg/mL. Statistically significant higher concentrations were found in male and in older participants (>30 yo). No difference depending on the collection time (morning vs. evening) was observed.

As a proof of concept, authentic oral fluid samples from participants following Tacrolimus (TAC) or Mycophenolic Acid (MPA) treatment from a previous study were analyzed.[3] For TAC, statistically significant correlations ($P < 0.05$) were observed for whole blood and oral fluid (n=48, Spearman rho=0.5255), creatinine-normalized oral fluid (n=48, Spearman rho=0.3316), and SMRB3-peptide 1 and 2-normalized oral fluid (n=24, Spearman rho=0.4192, each). However, any normalization did not strengthen the correlation between blood and oral fluid. In the case of MPA, none of the correlations between plasma and oral fluid (n=20, Spearman rho=0.1228), creatinine-normalized oral fluid (n=20, Spearman rho=0.01533) or SMRB3-peptide 1 and 2-normalized OF (n=6; Spearman rho=-0.3143 and -0.08571, respectively), were statistically significant.

Due to the scarcity of SMR3B-1 and 2 in oral fluid (50% of the samples were negative), these peptides are not good candidates for adulteration markers or normalization factors. Creatinine was present in all samples, making it a promising candidate for oral fluid validity testing. However, further studies are needed to confirm its utility as a normalization factor.

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Oral Fluid; Normalization Factor; Creatinine

L70 Postmortem Pediatric Forensic Toxicology: Children Are Not Small Adults

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Learning Objectives: After attending this presentation, attendees will gain an appreciation of the specific requirements and challenges facing forensic medicolegal scientists who investigate toxicology-related aspects of postmortem pediatric cases. The session's speakers will share their own approach style and roadmaps 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 offering guidelines and tools to medicolegal death investigators who are investigating toxicology-related deaths in the pediatric population.

Program Description: In this annual joint session of the Pathology/Biology and Toxicology sections, pediatric cases involving toxicological findings are presented and the audience is invited to interact and help bring each presentation to a conclusion utilizing expertise and commentary from both the presenter(s) and the audience. This joint session (now in its 25th year) serves as an open forum to help illuminate and clarify such issues. The session's forma typically consists of a set of short case presentations or issue-specific concerns, 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 the cause and manner of each child's death presented in the session.

Postmortem; Pediatric; Toxicology

LW1 The Doll and Its Maker: The Tale of Three Men Who Shaped Modern Forensic Practice Told Through Their Forensic Casework

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Learning Objectives: Through the lens of their forensic casework, attendees will see how some early forensic pioneers shaped modern-day forensic practice.

Impact Statement: The forensic community will learn some of the history surrounding the dawn of forensic science and will see that many of the challenges faced by early forensic practitioners persist today.

Abstract Text: In 1912, Arthur Guiterman, an American writer known for expressing his views through humorous poems, gently chided Sir Arthur Conan Doyle for disparaging other fictional detectives.[1] Conan Doyle replied in kind and ended his poem with an enigmatic couplet: "*So please grip this fact with your cerebral tentacle. The doll and its maker are never identical.*"[2]

In the couplet, Conan Doyle referred to his relationship with Sherlock Holmes, but following that thread one step further, who was Conan Doyle's "maker?" How did this underwhelming ophthalmologist generate one of the most recognizable figures in all modern literature? Also, did Conan Doyle direct his character's powers of observation on real criminals?

As it turns out, Sherlock Holmes was an amalgam of many, but one of the largest influences was that of Dr. Joseph Bell. When Conan Doyle was in medical school, Bell selected him as his outpatient clerk in his surgery clinic. This allowed Conan Doyle a ringside seat to Bell's amazing powers of both observation and deduction, something Bell called "the method." As patients entered, Bell would often identify where they came from, what they did for a living, and their malady before the patient even had the opportunity to open their mouths.

A long-time fan of detective fiction, Conan Doyle was often frustrated that readers were not given all the facts, and cases were solved by intuition or chance. After his experiences with Bell, Conan Doyle thought "*…if a scientific man like Bell was to come into the detective business, he wouldn't do these things by chance. He'd get the thing by building it up scientifically.*"[3] Bell's "*sharp piercing eyes, eagle nose, and striking features*" and how "*he would sit in his chair with his fingers together*" also strongly influenced the development of Conan Doyle's Great Detective, as did the Inverness coat and deerstalker hat that Bell often wore around Edinburgh.[4]

Conan Doyle wasn't the first to think that Bell would make a great scientific detective and, unbeknownst to Conan Doyle, Bell had already been involved in criminal casework before the two had even met. Over the course of his life, Conan Doyle would also take up the magnifying glass himself and work on many significant cases.

Conan Doyle's fictional stories also highlighted many core principles of forensic practice that were not fully understood when he wrote the stories and were sometimes not incorporated into forensic practice for years.

In addition to informing the public of the value of forensic evidence, these fictional tales inspired many (myself included) to a new calling. The most significant of those new disciples was a young orthopedic surgery intern. Edmond Locard had been fascinated by the Great Detective and in 1900, when his orthopedic surgery mentor died, Locard changed his course of study and began studying forensic medicine under Alexandre Lacassagne.

Soon, Conan Doyle's fiction became Locard's fact as the world's first police crime laboratory was born. Conan Doyle was thrilled with Locard's real-life adaptations of his work and even visited Locard at his lab in Lyon.

Locard's early casework provides many stark examples of how forensic practice transformed the criminal justice system. Yet many of the challenges faced by that first police crime laboratory are still faced by forensic practitioners today.

This presentation will focus on the unique intersection of these three men's lives and what modern forensic practitioners can still learn from Conan Doyle's fictional tales and the three men's forensic casework.

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Joseph Bell; Arthur Conan Doyle; Edmond Locard

LW2 The Lampshades and Pocket Knife Pouch From the Buchenwald Concentration Camp Are Made From Human Skin

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Learning Objectives: We show how different, unrelated techniques were integrated into one final expert witness statement over the course of three year's work. To perform conclusive tests on the last and only available remains of the Buchenwald concentration camp (the camp was destroyed after the war), we had to use a number of forensic techniques and technologies, including microscopy, hair and fiber, comparisons of old photographs, historical research, and adaptions of DNA techniques. Remarks about handling such a sensitive case will be made, including handling of press affairs and the need for independent testing techniques and stages.

Impact Statement: The uncertainty around the only remaining Buchenwald concentration camp artifacts was finally resolved. It was unknown if they were of human origin or not. The items were a matter of controversy over decades but thought to be impossible to test.

Abstract Text: A shrunken head, a heart (in liquid), a pocketknife soft pouch, and two lampshades—one fully intact, one originally given as a sample to British forces—were long thought not to be of human origin. After study of the original files relating to the production as gifts and the general use of such materials in the Buchenwald concentration camp, we decided to perform a final and conclusive check on the items using microscopy, DNA, and historical as well as criminalistic approaches. The samples are not on display any more at the memorial site due to their grim history, their political nature, and the formerly established idea of possible forgery. Another lampshade found in the United Stats coming from unclear sources had received wide public interest but was found to be made of non-human (cattle) skin. All of our samples came from the collection of the Buchenwald concentration camp memorial site's archive, including a recent acquisition from England.

Stain-wise, we performed a step-by-step approach to conserve as much material as possible. We used samples as small as possible and performed each analysis only after the previous one was fully done. This caused long delays that we decided to be preferable over a loss of stain evidence or a diagnostically less-conclusive statement.

We double-checked results in different laboratories wherever possible, including laboratories in other countries, and also including checks of negative results. All laboratories involved, except the presenting author's laboratory, did not know which samples and which case they handled to avoid possible bias.

The hair of the shrunken head was microscopically not excluded as being made of horsehair; DNA revealed that it was made of goat skin and goat hair. Some anatomical features do not match those from shrunken human heads that we usually encounter, so we reported the shrunken head skin and hair as not being of human origin.

The pieces that we cut out of two lampshades as well as the pocketknife soft pouch not only microscopically resemble the structure of human skin made into leather-like materials but also contained human DNA. Genetic fingerprinting was done with COI barcoding primers. DNA quantity was low; therefore, we applied nested Polymerase Chain Reaction (PCR) sequencing and Basic Local Alignment Search Tool (BLAST) revealed a 99% match to *Homo sapiens*.

Initially, the heart could not be identified using DNA approaches since no DNA could be extracted. Several specialized forensic and industrial testing laboratories were involved in extraction but to no avail. A clear anatomical distinction from a pig heart was not possible. By using old photographic evidence and visible features of the heart that we compared to the photographs, we found that the heart is most likely human.

Specimen; Ancient DNA Analysis; Microscopy

LW3 Weaponizing Psychiatry: Not a Thing of the Past

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Learning Objectives: The objectives of this presentation are to: (1) identify historical instances of political abuses in psychiatry and their impact on psychiatric practice; (2) evaluate the role of psychiatric associations in addressing political abuses within the field; and (3) analyze the interplay between political influences, personal agendas, and psychiatric ethics in shaping historical and contemporary psychiatric practices.

Impact Statement: This session delves into the intersection of psychiatry and politics, highlighting historical abuses and the fields resistance against such misuse. By examining past political and personal influences on psychiatric practices, attendees will gain insights into the ethical challenges and responsibilities of forensic psychiatry. Understanding these dynamics equips professionals to better uphold human rights, educate legislative and judicial bodies, and maintain the integrity of mental health care in contemporary society. This knowledge is crucial for ensuring that psychiatry remains a tool for promoting mental health and not for personal or political gain.

Abstract Text: The fundamental aim of psychiatry is to promote and protect mental health. We will begin our journey by exploring how past political abuses of psychiatry interfered with psychiatric practice, such as by using it to punish dissidents or by direct psychiatric involvement in acts which contradict accepted medical ethics. Each of these will be examined with the use of several historical examples. The ways in which the field has opposed its political abuse will be explored by a brief review of the involvement of psychiatric associations in these issues, such as the case of the World Psychiatric Association between 1983 and 1989. We will draw from the past and attempt to compare and contrast with the contemporaneous political tableau. Since its inception, the role of Forensic Psychiatry has been an informal gatekeeper of the standards of the field of psychiatry, as well as serving as an unbiased educator for legislative and judiciary bodies, the media, and the general public. As political seisms become more vigorous, it is important that we are well informed and able to discuss how our field can maintain a watchful eye and ensure the proper upholding of human rights and ethics.

In addition to political influences, it's important to acknowledge the role of personal agendas and interests in shaping the historical abuses and challenges faced by psychiatry. Beyond mere political motives, individuals with personal agendas and desires for personal gain have also played a significant role in distorting psychiatric practices.

Throughout history, we've witnessed instances where individuals exploited psychiatric power for personal gain, manipulating diagnoses or treatment plans to serve their own interests. These instances underscore the complex interplay between politics, personal motives, and psychiatric ethics.

By incorporating an understanding of personal agendas and gains alongside political influences, we gain a more comprehensive understanding of the challenges psychiatry has faced in maintaining its integrity and adhering to ethical standards.

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Forensic Psychiatry; Social Justice; Behavioral Science

LW4 (Un)solving Jack the Ripper: How Bias and Prejudice Shaped the Search for the Whitechapel Killer

Matteo Borrini, PhD, Liverpool John Moores University, Liverpool, United Kingdom*

Learning Objectives: After attending this presentation, attendees will be able to understand the historical context of the "Whitechapel murders" and understand the impact of misinformation and media sensationalism on the Jack the Ripper case. Attendees will evaluate the role of bias and prejudice in shaping the various hypotheses regarding the identity of the killer and the nature of the crimes.

Impact Statement: This presentation will impact the forensic science community by reviewing the most popular theories regarding Jack the Ripper's identity, highlighting how bias and prejudice could have influenced their development. The study will also provide an opportunity to reflect on the detrimental consequences of bias and prejudice on justice and forensic investigation.

Abstract Text: The most common misperception is the victims' identities, which could also have been used to profile the psychological characteristics of the perpetrator and their motive.[1,2] The five canonical victims have often been recognized as prostitutes in modern reconstructions of the homicides, as well as in various police reports of the time. However, only Mary Jane Kelly was known to be a professional sex worker, while Elizabeth Stride had been arrested for soliciting four years before she died in 1888. There is no evidence that Mary Ann Nichols, Annie Chapman, or Catherine Eddowes were involved in the sex trade. In all likelihood, they had been categorized as "unfortunate" because they were unmarried women living on the streets in the early morning, urgently searching for a small amount of money to acquire a bed in a common lodging house. If this major misinterpretation could have led to a misunderstanding of the real motive behind the murders, other biases fueled several hypotheses about the identity of the killer.

The Victorians probably found it challenging to accept that an individual of such an appalling nature originated from the capital of the British Empire. For this reason, the suspect was initially searched for among the foreigners and ethnic minorities that were living in London, as well as among individuals who could be considered "outsiders" due to their insanity. Alternative hypotheses targeted individuals based on their sexual orientation or professional occupation and, in other cases, implicated individuals from the artistic sphere, likely influenced by the belief that creativity and artistic aptitude make a person deviate from the norm. Over the years, various imaginary culprits have been suggested, delving into conspiracy theories and involving wealthy Victorian figures, members of royal families, and secret societies.

Noteworthy is the suggestion that the killer might have been a woman, a "Jill the Ripper," as reportedly proposed by Detective Abberline, one of the principal investigators during the murders. The idea was that a midwife would have basic anatomical knowledge to mutilate female genitalia and would be able to walk at nighttime in the streets with bloodstained clothes without raising any suspicion. Remarkably, midwives have often been viewed with suspicion and targeted during waves of witch hunt hysteria, once again demonstrating how prejudice is difficult to eradicate.

In conclusion, it is now impossible to reliably and forensically portray Jack the Ripper due to the unavailability of data collected using current scientific standards and, more importantly, the presence of biases and prejudices accumulated over the past 13 decades.

If, on the one hand, it is acknowledged that the Whitechapel murders cannot be solved anymore, they should still be seen as an essential lesson on how biases and prejudices can impact justice and forensic work and how they should be avoided from the early stage of any investigation.

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Cold Case; Serial Killer; Injustice

LW5 The Survivors of the Battle of Shiloh—A Photo Legacy

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Learning Objectives: Upon attending this presentation, attendees will learn how forensic photo analysis, used in conjunction with historical research, can be used to extract important investigative information from a photograph about which very little *a priori* information is available. Although photo-identification techniques from the forensic community were initially applied to this picture, by combining them with historical methods, it was possible to extract much more information from the photo that could then be integrated into a cohesive narrative. Attendees will learn genealogical photo-identification techniques they may not be aware of, but which can be used to enhance the investigative power of forensic analysis.

Impact Statement: This presentation will impact the forensic science community by demonstrating the synergy that exists between forensic and historical investigation. The presentation will broaden the understanding of how a seemingly irrelevant detail can be detected by one set of tools, and analyzed by the other, leading to an appreciation of the complimentary nature of the two disciplines.

Abstract Text: The Battle of Shiloh, also known as the Battle of Pittsburg Landing, was a major battle in the American Civil War fought on April 6–7, 1862. The battlefield is located between a small, undistinguished church named Shiloh and Pittsburg Landing on the Tennessee River. Two Union armies combined to defeat the Confederate Army of Mississippi. Major General Ulysses S. Grant was the Union commander, while General Albert Sidney Johnston was the Confederate commander until his battlefield death, when he was replaced by his second-in-command, General P. G. T. Beauregard. Johnston's death was a damaging blow to Confederate morale, particularly for President Jefferson Davis, who held Johnston high in personal and professional esteem. After the war, Davis wrote, "When Sidney Johnston fell, it was the turning point of our fate; for we had no other hand to take up his work in the West."

Though victorious, the Union army had more casualties than the Confederates, and Grant was heavily criticized. Critics called for him to be dismissed, but Abraham Lincoln defended his general, declaring, "I can't spare this man, he fights."

The battle was the costliest engagement of the Civil War up to that point, and its nearly 24,000 casualties made it one of the bloodiest battles in the entire war. After Shiloh, both sides realized the magnitude of the conflict, which would be longer and bloodier than they could have imagined.

Judging by the banner in the background, the group of old men in the picture must be members of the Association of the Survivors of the Battle of Shiloh. The identity of only one of the men is known: George Washington Jackson (sixth from the right, front row, small-looking face, and long white beard), of Caledonia, Jefferson County, Missouri, born on January 6, 1839. He was the great grandfather of the picture's owner. On April 6-7, 1862, Jackson fought in the Battle of Shiloh, under General Lew Wallace.

Like many photographs that we find in our personal archives, this photograph had no provenance. This talk will reveal how, using forensic and historical analysis, it has been possible to determine the exact date, general location, and occasion for the photo.

Photo Analysis; Shiloh; Civil War

Y1 Assessing the Accuracy and Precision of Crime Scene Measurements Obtained From Apple LiDAR Scans

Jason Ang, The University of Southern Mississippi, Hattiesburg, MS*

Learning Objectives: Through this presentation, the attendees will be introduced to the implementation of Apple Light Detection And Ranging (LiDAR) technology to scan a crime scene in 3D and collect measurements within the scans. The attendees will further learn how the tested applications on the device perform against a FARO Terrestrial Laser Scanner (TLS) to see their relative accuracy and precision. Finally, knowing the advantages and disadvantages of the tested applications, the attendees will be able to determine the method of choice for Apple LiDAR 3D scanning.

Impact Statement: Apple LiDAR implementation in crime scene documentation can be a cost-effective way to increase efficiency in collecting crime scene measurements while providing a visual of the scene in 3D. If deemed accurate and precise enough, this technology may potentially replace the need to collect physical measurements in crime scenes and can be an affordable alternative to TLS.

Abstract Text: Because of its ability to provide both visual and spatial information, LiDAR 3D-scanning technology is increasingly used by law enforcement agencies to document crime scenes. Although the current standard for LiDAR scanning is held by TLSs such as the FARO Focus models, newer iPhone and iPad Pro devices possess a small LiDAR sensor that can perform similar tasks. A previous study has shown that a FARO Focus scanner exhibits high accuracy against a Total Station.[1] SiteScape and Recon 3D, scanning applications that utilize LiDAR in Apple devices, have been shown in other studies separately to provide reasonable accuracy against a FARO Focus Scanner and known measurements, respectively.[2,3]

Our project attempts to assess and compare the accuracy and precision of crime scene measurements collected from SiteScape and Recon 3D on an iPhone 13 Pro Max against a FARO Focus S 350. Our experiment was conducted in a well-lit indoor mock crime scene measuring approximately 8.4m x 11.8m, in which 41 points where measurements were taken to and from were labeled with a blue arrow marker to minimize interpretation error in the point selection process. Along with the FARO, each software on the iPhone was used to scan the scene three times, each requiring multiple sub-scans that later underwent a registration process. The FARO sub-scans were registered automatically using the top view and cloud-to-cloud method within FARO Scene. The SiteScape sub-scans were registered once using SiteScape automatic registration and once manually using CloudCompare point alignment registration based on the checkerboard targets placed within the scene. While the Recon 3D scans employed the latter registration only, every sub-scan was processed with and without target detection. Therefore, in total, including the control, five datasets were collected, each containing three replicates of 820 distances ranging from 0 to 15m, computed from all possible combinations of the 41 labeled points within the scene. The random error of a particular method was measured from the standard deviations of the measurement triplicates within its dataset, while its systematic error was measured from the differences of the mean distance of the corresponding triplicates between the tested method and the control.

Among the four tested methods, Recon 3D with target detection showed the least random error with a *mean* (*SD*) of 1.53 (1.11) cm and the least systematic error at -0.72 (1.39) cm. Conversely, without target detection, Recon 3D showed a significantly greater random error at 2.19 (1.26) cm and the greatest systematic error at -9.33 (6.35) cm. In other words, for Recon 3D, target detection significantly increases both accuracy and precision and should always be used when possible. Our analysis showed that regardless of distance, the error of an individual measurement collected using Recon 3D with target detection should fall between -7.04 and 5.02cm. The automatically registered SiteScape dataset showed the greatest random error at 3.81 (2.36) cm and a reasonable systematic error at -2.32 (3.53) cm. In comparison, when manually registered, the SiteScape dataset showed a lower random error at 3.15 (1.82) cm but a greater systematic error at -4.56 (3.76) cm. In other words, manually registering the SiteScape dataset slightly increases precision but decreases accuracy. Given this minimal improvement, manual registration undermines SiteScape's practicality and time efficiency, which are its key advantages over Recon 3D. Therefore, automatic registration is recommended.

Overall, compared to SiteScape, Recon 3D can collect crime scene measurements with higher precision and accuracy and should be the method of choice when time and resources permit. Further research should include more replicates with varying scene sizes and without arrow markers.

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Crime Scene Investigation; 3D Technology; LiDAR

Y2 Utilizing Honey Bees (*Apis Mellifera*) as a Surveillance and Monitoring Technique for Bacterial Pathogens in Specific Environments

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Learning Objectives: The line between forensic microbiology, epidemiology, and biosecurity has blurred following the events of the Amerithrax investigation. This presentation will utilize preliminary data and research to demonstrate that honey bees have the potential to be used for the surveillance and monitoring of infectious diseases and pathogens within a given environment. After attending this presentation, attendees will understand the critical importance of biosecurity and biosafety implementation, recognize the current lack of effective monitoring and surveillance systems for pathogen spread, and learn how honey bees, which travel significant distances from their hives, can carry vital environmental information. Additionally, substances within the beehive can reveal microbial secrets essential for detecting threats, aiding in the identification of pathogens and transmission pathways.

Impact Statement: This presentation will have a profound impact on the forensic science community by highlighting the innovative use of honey bees for surveillance and monitoring in biosecurity and biosafety, particularly for epidemiological and forensic microbiology purposes. The goal of this research is to enhance knowledge and research in forensic microbiology, with a strong focus on biosecurity and biosafety implementation. By promoting a deeper understanding of these concepts, this presentation aims to significantly improve environmental and public safety and offer new tools for human forensic investigations to trace pathogen origins and prevent future outbreaks.

Abstract Text: **Urgent Biological Threats:** Biological threats, in the form of infectious diseases and pathogens, are increasingly attacking and harming populations worldwide, affecting various species, including humans.[1] These threats arise from both deliberate acts and naturally occurring outbreaks, posing a significant risk to public health and safety. Microbiologists, epidemiologists, and forensic scientists are working tirelessly to detect these threats and protect all populations through advanced technologies, practices, and infrastructure.[2] However, current practices are challenged, and the technology required to ensure biosecurity is limited. Effective surveillance and monitoring are crucial as the first step in detecting potentially threatening pathogens.[2-4]. Given the growing urgency of these threats, finding new and effective methods for continuous improvement in surveillance and monitoring is of utmost importance.

Innovative Surveillance Method: To address these challenges and advance the field of microbial forensics, we reviewed a novel method for the early detection of infectious diseases and pathogens.[5] This method involves testing and collecting samples from honey bees and their hives. Honey bees forage approximately one to two miles from their hive, gathering both rural and urban microbiota during their travels. Testing substrates within a hive can reveal the microbial profile of a given environment, demonstrating the potential and usefulness of this method for pathogen and epidemic surveillance and detection. This method can significantly enhance human forensic capabilities by providing critical data on environmental pathogen presence and distribution.

Study Investigation: This study will investigate this method further through three separate experiments focusing on honey bees and their hives to display microbial profiles and assist in pathogen detection. By taking samples of bee debris within each hive over time during the foraging season, this study aims to offer a deeper understanding of the environmental microbiome and further support this method for pathogen and epidemic surveillance. The results of this study could revolutionize our approach to biosecurity, providing an urgent and necessary tool in the fight against biological threats. The implications for human forensics are profound, as this method could provide crucial information for tracing the source of pathogen outbreaks and preventing future incidents, thereby protecting public health and safety.

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Honey Bees; Microbiology; Biosecurity

Y3 Human Profiling: The Application of the Oral Microbiome in the Prediction of Geographic Origin

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Learning Objectives: Oral microbiome profiles of individuals were analyzed using machine learning algorithms to predict their country of origin. By training these models using microbial composition data, this work demonstrates the potential of using oral microbiota as biomarkers for geographic and demographic differentiation.

Impact Statement: This study could significantly impact the forensic science community by providing an additional method for human profiling. By utilizing new analytical methods like machine learning, this study highlights another forensic application for the human oral microbiome in identifying or predicting an individual's geographic origins. Such a technique could enhance the accuracy and efficiency of forensic investigations and aid in cases where traditional identification methods are limited or unavailable.

Abstract Text: The human oral microbiome refers to the complex community of microorganisms residing in the oral cavity.[1] Microbial communities have been shown to play a critical role in maintaining oral and overall health, making it a target for understanding and identifying microbial biomarkers of disease.[2,3] Recent advances in microbiome research have revealed that the composition of human-related microbial communities can vary significantly based on regional, ethnic, dietary, and other cultural factors.[4-6] Thus, by analyzing the microbial signatures in the oral cavity, forensic scientists can uncover valuable information about an individual's lifestyle and even their geographic origin. This approach offers a complementary method to traditional forensic techniques, potentially increasing the accuracy and scope of human identification in forensics.

Building on these insights, this project explores the application of machine learning algorithms to predict individuals' countries of origin, based on their oral microbiome profiles. We combined oral microbiome data, obtained from other studies, which included individuals from five countries (China, Italy, South Africa, Thailand, and the United States). In addition, we collected and processed buccal swab samples from 50 individuals living in Nigeria with COPAN SMART-eNAT kit. Microbial DNA from the Nigerian swab samples was extracted, and the V4 region of the 16S rRNA gene was Polymerase Chain Reaction (PCR) amplified and DNA-sequenced. A random forest classification model was built using 70% of the dataset to assess microbial communities in each group, with the model's accuracy tested on the remaining 30% to predict the country-of-origin of those samples. After cross-validation, the model performance was shown to be approximately 86% accurate.

In addition, we assessed the effects of migration on the accuracy of the model. Considering that the microbiome is influenced by lifestyle, which could change significantly after migration, we aimed to discover if this would affect the prediction accuracy of the model. To this effect, we obtained oral swab samples from Nigerians who had recently migrated to the United Kingdom within six months of initial sample collection. For understanding the long-term effects of migration on the prediction accuracy, we sampled the same individuals twice, at three-month intervals, following the initial sampling. Upon testing data of samples taken at the final timepoint, the model accurately classified 62% of the samples as originating from a Nigerian, despite their migration to a different country.

In conclusion, our study demonstrates the potential of leveraging the human oral microbiome in forensics, particularly for human profiling. We also show that determining the country-of-origin of an individual is still possible, even after a six-month migration period. However, additional research exploring longer migration periods and expanding the number of countries built into the model would be required for greater accuracy and utility in forensic investigations.

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Oral Microbiota; Machine Learning; Geographical

Y4 Understanding Postmortem Changes in the Porcine Muscle Metabolome for Postmortem Interval Estimation

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Learning Objectives: This study aims to provide attendees with insights into the use of targeted metabolomics in forensic investigations, specifically focusing on Postmortem Interval (PMI) estimation through the analysis of porcine muscle metabolome changes after death.

Impact Statement: This research highlights the effectiveness of targeted Gas Chromatography/Mass Spectrometry (GC/MS) -based metabolomics approaches for PMI estimation. These methods enable the precise identification of relevant biomarkers, providing a robust and reproducible tool for forensic applications.

Abstract Text: Estimating the PMI remains a complex task influenced by various intrinsic and extrinsic factors affecting decomposition. Factors such as body weight, temperature, and humidity can all impact the rate at which a body decomposes, making it challenging to determine the exact time since death. However, recent advancements in molecular techniques have significantly enhanced the accuracy and reproducibility of PMI estimations. Omics technologies, such as proteomics and metabolomics, have played an increasingly significant role in forensic investigations. These technologies enable high-throughput analyses of human tissues and fluids, providing comprehensive insights into the biological data associated with decomposition. For instance, metabolomics can track the shifts in metabolites over time, offering valuable information that can be used to develop more precise models for estimating PMI. Metabolomics employs two main analytical approaches: GC-MS, which is well-suited for the identification and quantification of volatile and thermally stable compounds, and LC-MS/MS, which is suitable for detecting a broader range of metabolites, including larger and more polar molecules.

The present study aims to understand postmortem changes in the porcine muscle metabolome using an untargeted approach, through a comparative analysis of these two techniques, GC-MS and LC-MS/MS, while also evaluating the influence of employing two different instruments within the GC-MS analysis. Metabolites were extracted from 50mg sub-samples in triplicate using a methanol-water (8:2 %v/v) extraction protocol with a Precellys 24 Touch homogenizer. The extracts were then analyzed using an Agilent 6890N/5973 inert GC/MSD system and an Agilent Intuvo 9000 GC system coupled to an Agilent 5977B MSD single quadrupole mass spectrometer. LC-MS/MS analysis was conducted using a SCIEX Exion LC system coupled to a SCIEX 7600 ZenoTOF Q-TOF mass spectrometer.

Our untargeted GC-MS preliminary results were promising, revealing notable differences among samples collected at different time points, with closer intervals displaying similar metabolomic profiles. Over 60 metabolites were identified, including amino acids, oligosaccharides, and nucleobases, each showing distinct associations with PMI. Furthermore, the low Mean Absolute Error (MAE) observed in the Partial Least Squares Regression underscores its suitability for PMI predictions. We expect LC-MS/MS analyses to complement these findings by expanding the range of detectable metabolites, offering enhanced sensitivity and specificity for precise identification and quantification of biomarkers. Full results of the comparison and their implications for forensic investigations will be presented to the audience.

In conclusion, this comparative study highlights the robustness and applicability of GC-MS and LC-MS/MS based metabolomics approaches in forensic investigations, particularly in understanding postmortem changes and developing accurate PMI estimation models.

GC/MS; Metabolomics; PMI

Y5 The Rearing and Forensic Use of *Calliphora Vomitoria* Blow Fly Larvae

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Learning Objectives: In this presentation, attendees will learn about my semester long research project about the forensic application of rearing the *Calliphora vomitoria* blow fly larvae into the third instar stage and the outcomes of the rearing. I will use the data collected to talk about not only my findings but further research that can be done in the future for this to be applied to identifying unknown human remains at crime scenes using DNA extraction and sequencing. The data I will present will be on the rearing and growth patterns of the *Calliphora vomitoria* blow fly larvae.

Impact Statement: This research shows a newer side of forensic entomology and how it could possibly be used for the identification of unknown human remains with further research and data collection. Through this research, it was shown that the longer the larvae are in a habitat (or mock crime scene rearing chamber) the more they will eat/grow under my study conditions. With further research, it could be possible that if larvae are taken from a deceased person at a scene, and by following proper DNA extraction and sequencing protocols, a human DNA profile could possibly be made from the larvae gut evidence collected at the scene.

Abstract Text: For this independent research project, there were multiple different chambers set up in the back of the DNA Research lab at the University of New Haven. This setting made sure that the chambers were left undisturbed during the duration of the experiment. There were 250 larvae. There were different groups based on the day of life the larvae were in. This experiment ran for five days total so there were six individual groups (day 0 - day 5) of larvae. The purpose of this was to measure the growth patterns of each day individually.

Each larva was collected on the day of the group, based on days of life they were in, and they were measured, photographed, weighed, and examined under the microscope. It was shown that the longer the larvae were in the habitat (at the mock crime scene rearing chamber), the more they would eat and grow. Then follows the idea of extracting human DNA from larvae found at a crime scene found in the larvae stomach contents.[1-10] Further research could be done based off this study by continuing testing with human/animal blood, feeding the larvae a mixture of different blood and meats and going through the DNA extraction process and sequencing as a proof-in-principle study.[2-4,6,7] This research could be applied with further work to the identification of unknown human remains if the rearing conditions are studied as a background reference data set for comparison with future crime scenes. Creating a rearing conditions dataset would be a quick reference guide useful for studying the effects of substrate, food availability, food preference, presence of toxins and drugs, rearing time, and more. This pilot study shows that a simple rearing chamber approach can be used to train forensic scientists in basic forensic entomology techniques.

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Entomology; Blow Fly Larvae; DNA Extraction

Y6 Psychiatry Resident Compliance With the Informed Consent Process for Voluntary Psychiatric Hospitalization

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Learning Objectives: We will discuss the medical-legal topics that are recommended to be covered early in general psychiatric training, review the components necessary in consenting patients to voluntary psychiatric hospitalization, and describe the gaps that exist in resident understanding and execution of the informed consent process for voluntary hospitalization.

Impact Statement: This presentation will identify the gaps that exist in medical resident execution of the informed consent process for voluntary psychiatric hospitalization so that forensic educators in the medical setting can target these gaps and improve resident ability and competency in consenting patients. Improvement in clinical education on this matter ensures patients are informed of all constitutionally guaranteed procedural safeguards.

Abstract Text: **Introduction:** The Accreditation Council for Graduate Medical Education (ACGME) guidelines on forensic training in general psychiatry training have remained sparse and vague.[1] As a result, many psychiatric residencies have limited exposure to important medicolegal aspects of psychiatric hospitalization. The 1990 United States Supreme Court case of *Zinermon v. Burch* held that a person who is incompetent to give informed consent for voluntary admission to a psychiatric hospital is denied constitutionally guaranteed procedural safeguards when hospitalization is merely assented to.[2] Thus, a patient who is willing to sign forms, but is incapable of informed consent cannot be relied on to protest a voluntary admission and is in danger of extended confinement without procedural safeguards. After the Court's ruling in *Zinermon v Burch,* The American Psychological Association (APA) Task Force on *Consent to Voluntary Hospitalization* recommended that each patient be assessed by an admitting psychiatrist upon presentation at the hospital.[3]

Purpose: In this survey study, we aimed to identify whether any gaps existed in resident presentation of voluntary paperwork to patients that would preclude suitability for hospitalization. In particular, we sought to measure the percentage of residents who routinely informed patients of provisions governing their release and possible conversion to involuntary status.

Methods: Survey data was collected at an academic hospital in upstate New York. Thirty-five residents completed a survey on this topic across four years of general psychiatry training at the end of their respective academic years and descriptive statistics were conducted.

Results: Almost 100% (33/35) of residents evaluated patient understanding that they were signing into a unit for psychiatric treatment 100% of the time. On the other hand, intermittent compliance was noted in informing patients of provisions governing release and possible conversion to involuntary status: 58% (7/12) PGY-1s informed patients of this 50% of the time or less; 54% (6/11) PGY-2s informed patients 50% of the time or less; and 36% (3/8) PGY-3s and 0% (0/4) PGY-4s informed patients 50% of the time or less. It was noted that 54% (19/35) of all residents were never observed going through the informed consent process and had never received feedback.

Conclusions: These findings suggest that resident physicians are not fully compliant with the informed consent process for patients, particularly the criteria governing release. It was noted that over the four years of training, compliance steadily improved, suggesting residents received either the education clinically and/or didactics on the importance of the informed consent process. The ACGME recommends that residents obtain experience in evaluating potential to harm self or others, appropriateness for commitment, decisional capacity, and competency. Given the findings of this study, another recommendation would be for residents to obtain experience in evaluating appropriateness for voluntary hospitalization. Without the appropriate didactic and/or clinical education on this matter, patients are at risk of being denied constitutionally guaranteed procedural safeguards.

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Forensic Psychiatry; Education; Hospitalization

Y7 Detecting Peri- and Post-Menopausal Bone Changes Utilizing Micro-CT for Forensic Anthropology

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Learning Objectives: After attending this presentation, participants will have an improved understanding of bone changes that occur during and after menopause. In addition, they will understand how the characterization of these changes will lead to improvement in both age estimation and trauma analysis.

Impact Statement: This research impacts the forensic science community by broadening the understanding of living biological processes as they manifest at the time of death for improved forensic anthropological investigations.

Abstract Text: Defining the trajectory of bone changes during and after menopause has implications for age estimation, fracture risk, and bone functional adaptation. While these changes are well outlined in medical literature, an exploration of the topic with a forensic anthropological lens has not been conducted.

In the year prior to the final menstrual period, medical literature indicates the rate of bone loss for individuals begins to accelerate.[1] Testing has shown that N-Telopeptide (NTX), a biomarker detected in blood that suggests bone breakdown, values are significantly higher during and after menopause when compared to the period prior to menopause and can be indicative of osteoporosis.[2] Other medical literature theorizes that the increase in testosterone during menopause may lead to a temporary increase in bone deposition.[3] The purpose of this study is to see if the changes during and after menopause could be distinguished in pursuit of improved age estimation and trauma analysis for females.

In this pilot study, a sample of female individuals (n=12) was selected to be micro-Computed Tomography (micro-CT) scanned. The individuals were divided into three age cohorts based on medical literature (pre-menopausal, peri-menopausal, and post-menopausal). As a control, male individuals (n=12) were also included and had the same number of individuals per age cohort. Femora were scanned and cross-sectional properties were recorded from slices at the Sub-Trochanteric (ST) and Mid-Shaft (MS) areas for every individual. The slices were imported into the ImageJ Software for analysis. The BoneJ extension was utilized to conduct a cross-sectional analysis of each slice.

The one-way Analysis of Variance (ANOVA) test for the females in this study did not show significant results. The ANOVA compares means or averages between groups. Therefore, this test shows that there was not a significant difference between age groups. Although the statistical results of this pilot study did not support the hypotheses formed based on the known bone changes in medical literature, the trends in the means show potential. In female groups, the peri-menopausal had the highest mean for Cross Sectional Area (CSA) (MS: ~357mm, ST: ~338mm). It was expected that the second-highest group mean would be pre-menopausal. However, in this sample, the mean of the post-menopausal was higher than that of pre-menopausal females in both ST and MS slices.

There was a significant result in the ANOVA for the ST CSA values in males. This significant value (p=0.029) indicates that the cross-sectional area was significantly different between groups in the ST slices. Additionally, as remodeling rates correlate with bone loss, the average CSA values are expected to decrease with age. When plotted with a polynomial trendline fitted to the male values, a downward trend is seen in both the ST and MS slices. When compared to the female results, these are more consistently correlated with age, meaning there is likely an additional variable, aside from age, influencing the female group.

This study shows a definitive difference between male and female groups. Further investigations are needed to determine if menopause is the main driver of these differences. A larger sample is currently being examined by the authors. With this larger sample, menopausal changes could be more clearly elucidated and age estimation in females could be improved. Additionally, understanding changes in bone quality has direct implications in trauma analysis as it would affect when and how bones break.[4]

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Age Estimation; Bone; Forensic Imaging

Y8 Breaking the Silence: A Study of the Efficacy of Diverse Interrogation Methods

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Learning Objectives: Firstly, readers can understand a variety of interrogation techniques, including confrontational, general, deceptive, and sympathetic. Through this, the audience can recognize when the investigators can apply certain types of questions to the appropriate situation to do so and the potential outcomes. Readers will also gain a better understanding of how psychological profiles influence the effectiveness of these techniques. By following along with the process to come to my findings, rising scholars will learn the methodology and skills in conducting secondary evidence research, including video analysis, categorization, statistical evaluation, and discourse analysis. After the audience reads the paper, they may develop critical thinking when encountering situations involving interrogation.

Impact Statement: Potential improvement to interrogation programs could happen as it may quicken the pace of the questioning process. Practical application of the findings to real-world interrogation scenarios may improve law enforcement practices and interrogation protocols.

Abstract Text: Through active interrogations, the truth can be uncovered step by step. It is a delicate art that requires tactful and calculated maneuvers.[1] Seasoned investigators have a vast arsenal of techniques at their disposal and are able to pry out information from the most tight-lipped suspects.[2] Different approaches are often more effective, depending on unique characteristics.[3,4] This study aims to understand how different interrogation techniques vary in effectiveness across various suspects and their psychological profiles. We conducted secondary evidence research and found that there are different types of suspects involved in a case.

Interrogation questions can be tailored to extract information from suspects of different psychological profile and motivations. We closely examined 50 interrogation videos from different cases and categorized the interrogation techniques into confrontational, sympathetical, deceptive, and general. By tracking the frequency and success rate of each technique, some key insights have emerged. The confrontational approach involves direct, no-nonsense questioning that challenges the suspect's story. The sympathetic approach focuses on building rapport and emotional connection, expressing understanding and empathy.[5] The deceptive approach employs tactics like feigned evidence or false promises to manipulate suspects. The general approach uses open-ended questions and neutral methods, often as a foundational strategy.

Each technique's frequency and success rate were documented in an Excel spreadsheet, allowing us to calculate their effectiveness. Our findings indicate that confrontational and sympathetical are the most effective, with the highest success rate, while general and deceptive only seemed to work on special occasions where desperate measures were placed. Out of the 50 videos, only 5 of them used a sympathetic approach, but 4 out of 5 instances were deemed successful (as progress was made during the interrogation). However, over 20 of the videos used a general approach but only 9 of them were successful. These findings suggest that emotionally connecting with the suspect and relating to them may be the most viable method. This will increase the pace of interrogations and be a huge step in unveiling the deadliest, cruelest criminals out there.

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Interrogation; Video Analysis; Psychological Profiles

Y9 Visualizing Risk Factors of Missingness: How Spatial Software Can Illuminate Structural Violence

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Learning Objectives: After attending this presentation, attendees will learn how spatial statistics and geospatial visualization can be utilized to illuminate structural inequalities that may contribute to higher rates of missing persons.

Impact Statement: This presentation will impact the forensic science community by demonstrating how geospatial technology can be used to highlight the connection between disparate resource allocation and rates of missing persons, examining systemic issues at a "macro" level, rather than solely focusing on individual profiling. Dual usage of ArcGIS and CrimeStat to investigate patterns of missing persons exemplifies the potential for technology to be used in innovative ways, maximizing forensic anthropology's contributions to public safety and the advancement of missingness research.

Abstract Text: The identification of "hotspots," areas where crime is concentrated, is a crucial component of criminology and forensics.[1] In the United States, geospatial forensic investigation into missing persons or mortality rates has recently been done in Louisiana, Texas, and Idaho.[2-5] Before the Mississippi Repository for Missing and Unidentified Persons (MS Repository), there was no centralized resource with the ability to quantify or accurately track rates of missing people in the state of Mississippi. The MS Repository has become a clearinghouse for missing persons information throughout the state and aims to illuminate the racialized experience of going missing in Mississippi, as well as socioeconomic risk factors associated with higher rates of missing persons. The MS Repository, Geographic Information Systems (ArcGIS v 10.8.2), and CrimeStat (v 4.02)6 were used to conduct a geospatial investigation into missing persons in Mississippi and the potential correlation of socioeconomic factors with going missing by employing spatial statistics and visualization of the geographic dispersal and rates of missing persons.

The unequal allocation of resources has been theorized to result in poor health outcomes for marginalized groups, a concept known as structural violence.[7,8] Going missing can be seen as an ultimate form of poor health, seeing as it results in an individual living in a state of bare life for an indefinite amount of time and can often result in death.[9] For this project, health care access and law enforcement budgets were analyzed as proxies for resource allocation. Using GIS, the predominant demographic of missing people from each county in Mississippi was visualized. Whether the predominant demographic of missing people was representative of the county's racial makeup was tested using Chi-square tests. CrimeStat was used to analyze the spatial distribution of missing persons visually and statistically. Data was collected on law enforcement budgets and health care access by county, specifically the availability and cost of primary care visits and distribution of hospitals throughout the state. CrimeStat was also used to run spatial statistics to understand whether there is a correlation between health care access, law enforcement budgets, and missing persons.

The data on resource allocation was overlaid with data from the MS Repository to visually demonstrate the risk these two factors seem to pose to populations, unequally exposing certain groups to going missing. The utility of implementing both programs allowed for a visual and statistical geospatial analysis into the distribution of missing persons and inequalities in relation to socioeconomic factors. The novel usage of software in this project enabled the visualization of structures that enact violence unto specific populations. Preliminary results suggest that unequal allocation of accessible health care and law enforcement assets has a significant impact on who goes missing, subjecting Black, Indigenous, and People Of Color (BIPOC) groups to unnecessary risk, resulting in high rates of missing people in predominantly Black and low socioeconomic areas in the state of Mississippi. This project, in its alignment with the mission of the MS Repository, contributes to the larger goal of not only cataloging missing persons but also aiding in their recovery and identification of missing and unidentified persons throughout the state. It also attempts to contribute to a growing body of literature in forensic anthropology, moving toward conducting forensic investigation by examining systemic issues at a "macro" level.

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Missing Persons; Social Justice; Geospatial Analysis

Y10 Gendered Language Discourse in Forensic Sciences Proceedings

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Learning Objectives: Attendees will learn about: (1) the urgency of Transgender, Gender, and Sex Diverse (TGSD) inclusive language; (2) temporal and contextual trends in the use of TGSD terms in forensic sciences discourse; and (3) approaches that forensic practitioners can follow to institute TGSD-inclusive language. This presentation will also introduce the application of ATLAS.ti software and AI technology in examining trends in scientific discourse.

Impact Statement: This presentation will benefit the forensic sciences community by quantifying and contextualizing changes in TGSD language. Language use can impact the interpretation of evidence, thereby influencing case resolution and harm to TGSD individuals. This presentation demonstrates why and proposes how TGSD-inclusive language can shape theory and practice in the forensic sciences.

Abstract Text: Traditionally, the focus on gender within the forensic sciences has centered on the legal impact of language choice and the cisgender violence. Some work regarding the inclusion of gender identity in investigations has been done within the domain of death investigation, encompassing shifts in medical and death care of TGSD individuals.[1] Forensic anthropology particularly has expanded out from a methodological focus on Assigned-Sex-At-Birth (ASAB) estimation.[2] Today, forensic anthropologists agree that gender cannot be estimated from the skeleton alone, ASAB estimation methods designed for binary results exclude biocultural diversity, and recognize a need to broaden their methods to include TGSD decedents.[2-5] This has resulted in an increase in forensic anthropology scholarship incorporating contextual evidence of gender expression and non-binary ASAB estimation when constructing decedent profiles.[2] As the rates of fatal violence against TGSD people rise, forensic practitioners are more likely to encounter casework involving these individuals. Therefore, a need persists for the forensic sciences community to evaluate gendered language discourse in their fields and use inclusive approaches that reduce linguistic harm toward TGSD individuals.[6,7]

This project aims to identify patterns of gendered language within the AAFS conference *Proceedings* through time. Linguistic discourse analysis is conducted using 20 searchable sex and gendered terms within the AAFS *Proceedings* from 2004-2024. Trends in the occurrence of these terms through time are quantified within and among each of the AAFS sections. ATLAS.ti is then used to contextualize the ideological conceptions/perceptions of gendered language among forensic scientists through their operationalization of these terms. Feminist, queer, and necroviolence/biopower theories are then applied, providing critical lenses of language use in the forensic sciences.[7,8] Finally, the impact of gendered terminology on case resolution is demonstrated through a forensic anthropology case study with examples for implementing TGSD-inclusive language. Results show that gendered language in forensic sciences abstracts has focused primarily on cisgender ideology, emphasized ASAB methodologies, and yet TGSD-inclusive language and research is slowly increasing within most of the AAFS sections, mirroring cultural shifts.

When forensic scientists do not use inclusive language, this potentially permeates throughout the justice system and public eye. Language use in case reports, databases, court testimony, scholarship, and public outreach impacts the interpretation of evidence and witness credibility, alters accuracy and objectivity of analyses, policies, and may therefore harm case resolution for individuals who identify outside the normalized binary. Reflecting on the scientific discourse of gender can address biases in our analyses and align the field with recent cultural shifts. This practice is also fundamental to obtaining the goals toward diversity and inclusion within the AAFS values and vision. Along with implementing non-binary ASAB methods, intentional implementation of gender- and sex-inclusive language can include neutral pronouns and avoid binary categorizations. Such practices recognize the non-binary biocultural nature of sex and gender. They can reduce the probability of unidentified individuals being mis-gendered and/or mis-sexed and maintain more open investigative directions (e.g., not narrowing identification by 50% to one sex), which can help resolve cases.[2,7,9] Finally, by leveraging ATLAS.ti and its AI components, this study highlights the significance of integrating advanced qualitative analysis techniques into the forensic sciences. Medicolegal practitioners across the forensic sciences can apply AI technology to legal documents, witness testimonies, academic scholarship, and other textual evidence to uncover and reform discrepancies in language use.

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Gender; Language; ATLAS.ti

Y11 A Quantitative Analysis of Explosive Taggants in Forensic Samples Using Total Vaporization-Headspace/Gas Chromatography/Mass Spectrometry (TV-HS/GC/MS)

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Learning Objectives: Attendees will gain a comprehensive understanding of the role and importance of explosive taggants like 2,3-Dimethyl-2,3-Dinitrobutane (DMNB) in forensic science, particularly in aiding the identification of explosives. They will learn about the limitations of Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) for quantitative analysis due to fiber interactions and extraction efficiency issues, and how HS/GC/MS overcomes these challenges by analyzing the gas phase directly. The presentation will cover total vaporization calibration techniques to eliminate matrix effects and ensure accurate quantitation. Attendees will also learn the methodology for quantifying DMNB in the headspace of solid C4 samples using TV-HS/GC/MS, including the establishment of calibration curves and the interpretation of results. Additionally, the effects of sample properties such as compactness, age, and air exposure on DMNB detection will be evaluated.

Impact Statement: This presentation will significantly impact the forensic science community by providing a validated and robust quantitation technique for analyzing explosive taggants using HS/GC/MS. By addressing the limitations of SPME-GC/MS and presenting a more accurate and reproducible method, this research will enhance the capability of forensic investigators to detect and quantify explosive taggants like DMNB. Accurate quantitation of DMNB in the headspace is crucial for improving the training protocols and effectiveness of detection canines, ultimately leading to more reliable and efficient forensic investigations involving explosives. This advancement will contribute to the overall knowledge base and operational effectiveness of forensic professionals dealing with explosive materials.

Abstract Text: Explosive taggants are unique chemical markers added to explosives during manufacturing to aid in their identification. Headspace/Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry (HS/SPME-GC/MS) is a technique known for its high sensitivity, selectivity, and ease of use and is commonly employed for qualitative analysis. Despite its advantages, SPME can complicate quantitative analysis due to fiber interactions that can affect the reproducibility and accuracy of quantitation. These complications arise from variability in fiber coating and extraction efficiency, which can introduce errors in measuring analyte concentrations. In contrast, HS/GC/MS provides a more straightforward approach to quantitation by analyzing the gas phase directly, thereby avoiding these issues. Employing total vaporization calibration helps eliminate matrix effects that can interfere with accurate measurement, ensuring that analyte concentrations are assessed solely from the vapor phase. The total vaporization calibrants are utilized to calculate the concentration of DMNB in the headspace of solid Composition C4 samples. This study investigates the application of TV-HS/GC/MS for both qualitative and quantitative analysis of explosive taggants, emphasizing its advantages as a non-destructive method that requires small sample volumes, making it particularly suited for forensic evidence analysis.

In this research, we analyze the headspace of Composition C4 using TV-HS/GC/MS to quantify the taggant, DMNB. Calibrants of DMNB in acetone were used to establish calibration curves, relating the analyte's signal response to its known concentrations. The concentration of DMNB in the headspace of solid C4 was found to be 125.2 ± 4ppm. The study also examined the effects of the compactness of the solid sample, sample age, and air exposure. Results indicated that compactness did not affect DMNB's ability to partition into the headspace. However, less DMNB was detected in the headspace with newer C4 (most recent production year), and over weeks, surface depletion of DMNB was observed for both sealed and air-exposed C4 samples.

Our research aims to develop and validate a robust quantitation technique for forensic applications, advancing the quantitative analysis of explosive taggants. This study's primary impact lies in accurately determining the concentration of DMNB in the headspace, which is essential for assessing the levels that detection canines encounter. Providing precise measurements of DMNB in the air would enhance the training protocols and overall effectiveness of canines used in explosive detection.

Chromatography; Mass Spectrometry; Quantitative Evidence

Y12 Fingerprint Age Determination Using Spectrometry Imaging by Analyzing and Comparing the Degradation of Specific Compounds Within the Fingerprint Residue in Crime Scene and Surrounding Areas

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Learning Objectives: Attendees will learn the latest methodologies and techniques for determining the age of fingerprints in forensic science. The presentation will cover scientific principles, advancements in chemical analysis, and case studies illustrating practical applications. Participants will gain insights into the challenges and limitations of current methods and explore emerging technologies that promise to enhance accuracy and reliability in fingerprint age estimation.

Impact Statement: This presentation will significantly impact the forensic science community by introducing innovative approaches to fingerprint age determination. By enhancing the accuracy and reliability of age estimation, these methods can improve crime scene investigations, strengthen the evidential value of fingerprints, and potentially expedite case resolutions. Additionally, the presentation will encourage further research and collaboration within the community, fostering the development of advanced forensic techniques and standards.

Abstract Text: After attending this presentation, attendees will understand principles of fingerprint age determination using (one of) spectrometry imaging technology, by comparing the aging patterns between fingerprints found at crime scenes and surrounding areas, and how this method enhances forensic investigations. This presentation will impact the forensic science community by providing a new approach for determining fingerprint age, which can offer a more comprehensive understanding of the temporal dynamics at a crime scene.

Current methodologies for determining the age of fingerprints rely on analyzing the degradation of specific compounds within the fingerprint residue. The study "Determining Fingerprint Age with Mass Spectrometry Imaging via Ozonolysis of Triacylglycerols" highlights the use of Mass Spectrometry Imaging (MSI) to track the ambient aging of Triacylglycerols (TGs) and other lipids. While this approach has been effective within the crime scene, its application to the surrounding area has not been thoroughly explored.

This research focuses on comparing the aging process of fingerprints found directly at the crime scene with those collected from the surrounding areas. By employing (one of) spectrometry imaging like MSI to analyze the degradation patterns of TGs and other lipids, this study aims to establish a relative comparison that can provide better insights into the chronological framework of fingerprint deposition. Initial results indicate that fingerprints in the surrounding area show different aging patterns due to varied environmental exposures, which can be crucial for accurate age estimation.

This presentation will detail a case study demonstrating the practical application of this comparative method, illustrating how forensic experts can utilize this information to augment traditional investigation techniques. The findings suggest that by incorporating data from both the crime scene and its surroundings, forensic investigators can achieve a more accurate and reliable determination of fingerprint age, thereby improving the overall efficacy of crime scene analysis.

Forensic Science; Forensic Analysis; Crime Scene Investigation

Y13 Non-Human Proxies in Human Skeletal Research: Some Sampling and Treatment Considerations

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Learning Objectives: After attending this presentation, the attendees will gain a better understanding of the microstructural and biochemical differences between human and *Sus scrofa* (pig) bones from the perspective of gravimetry, histology, and DNA yield. This presentation will cover the sampling and treatment considerations that should be met when conducting research using non-human proxies in skeletal research.

Impact Statement: The results of this study will impact the forensic science community by demonstrating that there are many factors to consider when using non-human proxies in human skeletal research. Among them, finding a proxy of equivalent age is a necessary element to conduct appropriate research in these animals and be able to translate the results to human specimens.

Abstract Text: Non-human proxies are often used in human decomposition, skeletal, and medical research. With known structural and biochemical properties of different animal proxies, finding the "right" proxy for any type of research is possible.[1] Pigs have been used as human proxies in several studies. From the forensic anthropology point of view, in terms of bones, a deeper understanding of the differences between the non-human proxies and their human counterparts would lead to a more thorough understanding and interpretation of the results obtained using a proxy.

Bones have an organic and inorganic matrix which are comprised primarily of collagen and crystalline hydroxyapatite, respectively.[2] As any species age, the bone matrix will continue to mineralize, showing an increase in the inorganic matrix. Human bones have a circumferential lamellar bone with dense haversian canals and osteons that are rounder than other mammals and overlapping.[3,4] Pigs are said to have primarily plexiform bone with similar lamellar structure to human bone. The matrix components of pig bones are also similar to humans.[1] These matrix and structural components vary between all species and can affect the biomechanics of the skeletal structure as well as proxy research results. Previous research has compared the structural morphology of different types of common animal proxies' bones while other research has focused on the matrix components and trauma while using pigs as proxies for humans in burn studies.[1,5].

The purpose of this study was to compare the structural and biochemical properties of human and pig bones using gravimetry, histology, and DNA yield of altered (burnt) and unaltered bones.

Window cuts from a single humeri, radii, femora, and tibiae and cross sections from a single rib and metacarpal or metatarsal from each human and pig specimen were altered in a muffler oven for one hour at 60°C and then for two hours at 500°C to gain wet (water + organic matrix + inorganic matrix), dry (organic matrix + inorganic matrix), and ash weights (inorganic matrix) for the gravimetry analysis of the skeletal elements. One human and hog metacarpal or metatarsal from each specimen were set up for histology using Sirius Red and Fast Green stain to view the collagen to non-collagen protein content within the elements. And lastly, one metacarpal or metatarsal from each human and hog specimen were altered in the muffler oven at an average of 440°C for one hour for DNA yield comparison with an unaltered metacarpal or metatarsal from each specimen.

The results showed that the pig bones had a larger concentration of organic matrix than humans with similar levels of inorganic matrix. This shows that the pigs have a larger organic-to-inorganic matrix ratio, making them less dense, the opposite of what literature says about human and pig bone density. This could be the result of having human specimens that are above 50 years old and juvenile pig specimens that do not have fully mineralized bones. However, based on structural morphology applying histology, the pig bones were denser with less frequent and smaller haversian canals than human bones. The pig bones also showed higher DNA yields but similar purity as well as better preservation after exposure to heat, probably due to the higher density of organic matrix in pig bones providing more resistance than human bones to external insults.

This study leads to suggestions that factors such as age, treatment, and definitions need to be considered when conducting animal proxy research. Also, previous views on the subject were consistent and new perspectives on what to control for when using animal proxies are suggested.

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Bone; DNA; Histological

Y14 To Scale or Not to Scale: The Accuracy and Impact Behind Scaling Three-Dimensional (3D) Models

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Learning Objectives: Attendees will understand the accuracy of scaling 3D printed models for metric assessment in forensic anthropology and learn about an affordable, efficient solution when considering demonstrative aids for court testimony.

Impact Statement: This presentation impacts the forensic science community by demonstrating the accuracy and scaling of 3D printing and the ability to act as a demonstrative aid in court.

Abstract Text: 3D printing is used across disciplines from architecture to public safety and forensic analysis. In a forensic context, the importance of high-resolution 3D reproductions of skeletal remains and the ability to produce negative space with accuracy is generally well-understood.[1,2] Existing literature has explored the reliability of 3D imaging and printing to supplement toolmark evidence when using Computed Tomography (CT) and micro-Computed Tomography (micro-CT) interfaces.[3,4] The accuracy of 3D structured light scans and 3D prints is listed in recent research that further supports the use of scanning and printing to assist traditional methods of skeletal documentation.[5] With emerging scholarly dialog regarding the use of 3D models as demonstrative aids in court, this study sought to investigate the efficacy of downscaling a skull as a means of providing valid and affordable options for using these instruments.

In contrast to existing research that used CT and micro-CTs, this pilot study incorporated the use of Coordinate Measuring Machine (CMM) technology, including the FARO Design 3D ScanArm with RevEng software, along with Formlabs 3L printer with White V4 resin, Form Wash L with 99% isopropyl alcohol, Form Cure L, and Preform 3D printing software. Notably, the White V4 resin was selected for this study as it is designed for precision in comparison to other Formlabs resin options. These instruments were used to scan the plaster cast of Genocidaire Francisco Pizarro that was donated to Florida Gulf Coast University by late forensic anthropologist Dr. William Maples.

In 1984, the Peruvian government asked Dr. Maples to identify the remains of Francisco Pizarro with the cast being created at that time.[6] Then, using the Preform 3D printing technology, the original Maples' cast of Pizzaro was scanned and scaled from a 1.0 (original size) to 0.2 (scaled), and 3D printed in resin. Metric analysis using FORDISC measurements was conducted on the original and scaled casts. The measurements for both casts were compared and their correlation to the expected 20% scale were evaluated. The scaled measurements were strongly positively correlated to those of the original ($r = 0.997$, $p > 0.001$), thereby suggesting that the 3D rendering was a suitable metric comparison. Further, consistent with existing literature, the average accuracy of the scaled measurements was ±1mm of the original cast and within an acceptable level of error for use as a demonstrative aid.[5,7] Though future research is needed to validate these methodologies, the findings of this study highlighted potential positive outcomes for the utilization of scaled 3D models in forensic anthropology.

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3D Technology; Forensic Anthropology; Francisco Pizarro

Y15 A Composition of Decomposition in Three Varying Conditions Involving Body Coverage Using Pig Carcasses

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Learning Objectives: After attending this presentation, attendees will have a better understanding of how different conditions involving body coverage affect the decomposition rate in Northern Virginia.

Impact Statement: This presentation will impact the forensic science community by continuing to build upon previous studies on decomposition and establish the baseline of how different conditions affect decomposition in Northern Virginia.

Abstract Text: The purpose of this study is to document the decomposition of pigs (*Sus scrofa)* in the Forensic Research and Science Training Laboratory (FRSTL) at George Mason University in Northern Virginia under three varying conditions involving body coverage. Pigs are often used in place of human cadavers in forensic research, even though the difference in how pigs decompose compared to humans has not been widely researched. This study wants to look at how pigs decompose under different conditions involving body coverage so we can continue to build upon and look more into these decomposition differences. A previous study found that the earlier stages of decomposition in pigs differ greatly compared to humans.[1] In contrast, advanced decomposition in pigs was more similar to the previously made human scale.[2] In another study, the researchers found that decomposition occurred faster in the pigs in both the winter and summer when compared to humans.[3] This study hopes to build off of these past works and see what differences occur between the three pig carcasses and eventually compare these results to human donors.

On June 20, 2024, three pigs were delivered and placed in the designated area in the FRSTL about two hours after being put to rest. Pig number 1 was placed directly on the soil with no other added conditions. Pig 2 had a cotton T-shirt and a lightweight pair of polyester shorts put on and then placed onto the soil. Pig 3 was placed on top of a carpet, which was then wrapped. All three pigs were covered in cages in a wooded area that received similar sunlight and shade. Data collection includes observation, photographic documentation, and decomposition scoring using the total body Score (TBS) developed by Megyesi et al. at regular intervals.[2]

Preliminary results showed that after just two hours on the initial day, heavy adult blow fly activity began occurring on all three of the pigs on the face/mouth, where the gunshot trauma had been inflicted. Fly eggs were observed on all three of the pigs on Day 2. Insect activity increased more rapidly on Pig 3, which was 25-50% covered by Day 3, while Pigs 1 and 2 were less than 25%. By Day 4, insect coverage increased for Pigs 1 and 3 but not Pig 2. However, all of the pigs reached 50-100% insect activity by Day 5. Based on qualitative observations, Pig 3 reached the stages of early decomposition the fastest (3 days), while Pig 1 reached the advanced stages of decomposition the fastest (5 days). However, based on quantitative results, there is no significant difference between each of the pigs (-p = 0.952, Analysis of Variance [ANOVA]). The differences between qualitative and quantitative results highlight the need for more nuanced approaches when conducting decomposition research.

The findings of this project suggest that different conditions involving body coverage may only affect decomposition in minute ways and that they may not show up later when doing quantitative statistical tests. These results have broader implications for postmortem interval estimation. Future directions include increasing the sample size for a more robust study as well as comparing them with human donors.

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Pig Decomposition; Forensic Anthropology; Taphonomy

Y16 Designing Cerium-Doped Copper Oxide-Based Nanozymes for Microfluidic Sensing of Pesticides

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Learning Objectives: Attendees will understand the importance of pesticide detection in forensic science and public health. Attendees will gain insights into the critical role of rapid and accurate pesticide detection in addressing pesticide poisoning and its public health implications. This objective will help attendees appreciate the necessity of advanced detection methods in forensic investigations.

Explore the Synthesis and Characterization of Ce-Cu_2O Nanozymes: Attendees will learn about the synthesis process and characterization techniques used for cerium-doped Cu_2O nanoparticles. This includes understanding the doping mechanism, optimization of reaction conditions, and the use of X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Transmission Electron Microscope (TEM) for structural and morphological analysis.

Examine the Catalytic Properties and Enzyme Kinetics of Nanozymes: Attendees will explore the unique catalytic properties of Ce-Cu_2O nanozymes, including their peroxidase and laccase-like activities. They will also learn how to analyze enzyme kinetics, determine the Michaelis-Menten constant (Km), and evaluate maximum reaction velocity (Vmax).

Learn about Microfluidic Device Fabrication and Integration: Attendees will gain knowledge on the fabrication of microfluidic devices using photolithography and soft lithography techniques. This includes understanding the design optimization for efficient fluid flow and reagent interaction with nanozymes.

Evaluate the Performance and Practical Applications of the Sensor: Attendees will assess the sensor's performance in detecting pesticides like malathion, deltamethrin, and chlorpyrifos. They will also learn about the sensor's application in real-world scenarios, including field conditions, and its robustness and reliability for on-site pesticide detection.

Discuss Future Directions and Enhancements in Pesticide Detection Technology: Attendees will explore potential advancements in nanozyme-based technologies for pesticide detection. This includes discussing future research directions aimed at expanding the range of detectable pesticides and improving sensor sensitivity and specificity.

Impact Statement: This presentation will significantly impact the forensic science community by introducing Ce-Cu_2O nanozymes integrated into a microfluidic sensing platform for pesticide detection. This novel approach offers enhanced sensitivity, selectivity, and real-time, on-site analysis, improving the accuracy and efficiency of forensic investigations. The cost-effective, portable device facilitates rapid detection at crime scenes, reducing laboratory turnaround times. By showcasing an innovative combination of nanotechnology and microfluidics, this presentation will inspire further research and development in forensic methodologies. Ultimately, it enhances public health safety by enabling timely identification of pesticide residues in food and environmental samples.

Abstract Text: The pervasive use of pesticides in agriculture has led to significant concerns about pesticide poisoning and public health risks. Rapid and accurate detection of pesticide residues is critical for forensic investigations and food safety. This report explores the development of cerium-doped Cu_2O (Ce-Cu_2O) -based nanozymes for use in a microfluidic sensing platform to detect pesticides with high sensitivity and selectivity.

The research focuses on synthesizing and characterizing Ce-Cu_2O nanoparticles, leveraging their unique catalytic properties to mimic natural peroxidase and laccase enzymes. These nanozymes exhibit multienzyme-like activities, enabling the colorimetric detection of pesticides such as malathion through visible color changes. Integrating these nanozymes into a microfluidic device offers a promising solution for real-time, on-site pesticide detection, addressing the limitations of traditional analytical methods used in forensic science.

The synthesis involved a detailed analysis of the doping mechanism and the optimization of reaction conditions to achieve nanoparticles with enhanced catalytic efficiency. The characterization techniques employed included XRD, SEM, and X-ray Photoelectron Spectroscopy (XPS), providing insights into the structural and morphological attributes of the synthesized nanoparticles. The catalytic activity of the Ce-Cu_2O nanozymes was assessed through enzyme kinetics, where the Michaelis-Menten constant (Km) and Vmax for the peroxidase-like activity were determined. Notably, the laccase-like activity with the doped cerium was drastically reduced compared to the undoped Cu2O particles, highlighting the selective enhancement of peroxidase activity due to cerium doping.

The microfluidic device was fabricated using photolithography and soft lithography techniques, allowing precise control over fluid flow and reaction conditions. The design of the microchannels was optimized to ensure efficient mixing of reagents and interaction with the nanozymes. The device's performance was validated using standard pesticide solutions and real sample analyses. Results indicate that the Ce-Cu_2O nanozymes effectively detect malathion, deltamethrin, and chlorpyrifos with a low detection limit and high selectivity, even in the presence of interfering substances.

In addition to laboratory-based evaluations, the portability and practicality of the microfluidic device were tested in field conditions. The results demonstrated the device's robustness and reliability in detecting pesticide residues in various environmental samples, including water and soil extracts. The rapid response time and ease of use make this sensor highly suitable for on-site applications, providing immediate results that are crucial for timely forensic investigations and public health interventions. This work presents a novel strategy for developing a cost-effective, portable, and efficient sensor for pesticide detection, contributing to enhanced forensic analysis and public health safety. The successful integration of Ce-Cu_2O nanozymes into a microfluidic platform underscores the potential of nanozyme-based technologies in advancing analytical methodologies in forensic science and environmental monitoring. Future research will focus on expanding the range of detectable pesticides and further improving the sensitivity and specificity of the sensor, aiming to establish a comprehensive detection system for widespread forensic and agricultural applications.

Microfluidics; Nanozymes; On-Site Detection

Y17 An Evaluation of Optimal Pretreatment and Quantitative Method Pairings for the Analysis of GC/MS Data From Weathered Gasoline

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Learning Objectives: Attendees will learn about the relationship between three data structures (data matrix, total ion chromatogram, and total ion spectrum) that result from Gas Chromatography/Mass Spectrometry (GC/MS) analysis. This presentation will introduce attendees to five quantitative methods, operating on different forms of the data structure, for making pairwise comparisons between GC/MS data sets, and different methods of data pretreatments that may be considered prior to statistical analysis.

Impact Statement: GC/MS is frequently employed in forensic science applications. This presentation will illustrate to the forensic science community how different quantitative methods and data pretreatments choices may impact analytical results and conclusions drawn from GC/MS data.

Abstract Text: Research in forensic fire debris methodologies often requires quantitative assessment of data obtained by GC/MS). GC/MS analysis results in a retention time-ion abundance matrix, with rows of the matrix corresponding to single mass scans, columns corresponding to *m/z* ratios, and elements containing ion abundances. This data matrix may be reduced to a single dimension through the Total Ion Chromatogram (TIC), by summing the matrix elements across the columns. Alternatively, the matrix may instead be reduced to the opposite dimension through the Total Ion Spectra (TIS), by averaging the mass spectrum elements across time. When making quantitative comparison of GC/MS data, researchers have a choice of using the full matrix, TIC, or TIS data structure. Additionally, various pretreatments may be applied to the data prior to analysis. These include normalization, centering, scaling, and transformation.[1] The optimal choice of pretreatment may depend on both the desired analysis and quantitative method.

Recently, an experiment was conducted to assess factor effects on the recovery of Ignitable Liquid (IL) from simulated fire debris using Dynamic Vapor Microextraction (DVME).[2] DVME extracts vapor from debris headspace and the resulting sample is analyzed by GC/MS. In this experiment, factor effects were assessed by quantifying the similarity between extracted headspace captured under experimental conditions to references of neat IL. Different quantitative methods and pretreatments can be considered for this analysis.

The goal of this work was to determine the optimal combination of quantitative method and pretreatment for analysis of the collected experimental data. Five data pretreatments were considered, including constant sum normalization, mean-centering, autoscaling, fourth-root transformation, and no pretreatment. Five quantitative methods—three operating on the data matrix, one on the TIC, and one on the TIS—were considered. Quantitative methods for matrix comparisons were based on covariance mapping, eigenanalysis, and principal angle analysis.[3-5] TIC data were evaluated based on Pearson product moment correlation, and TIS data were compared through a simple distance metric.[6,7]

Data from 128 replicate samples of 50% and 80% weathered gasoline analyzed by GC/MS at two injection volumes were used to determine the optimal quantitative method/pretreatment combination. For each of the 25 quantitative method/pretreatment combinations, pairwise comparisons were made between samples of the same type injected in equal volume, samples of the same type injected at different volumes, and samples of differing weathering states injected at the same volume. Comparisons were restricted to samples analyzed on the GC/MS on the same day. The optimal method/pretreatment combinations were determined to be those that maximized discrimination between samples of differing weathering states and minimized discrimination between samples of the same weathering state analyzed at different volumes. Covariance mapping with autoscaling pretreatment and TIS comparisons with fourth-root transformation were found to have equivalent discrimination. Both methods were applied to the collected experimental data to estimate factor effects. Conclusions on factor effects were found to differ based on which method was used. Subsequent analyses indicated these discrepancies were attributable to individual chemical classes and suggested that covariance mapping paired with autoscaling provided the optimal comprehensive analysis of the experimental data.

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Fire Debris Analysis; GC/MS; Chemometrics

Y18 Integrating Machine Learning and ATR/FTIR Spectroscopy for Geographical Origin Identification to Combat Illegal Wood Trafficking

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Learning Objectives: The integration of machine learning algorithms with the spectral data from Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) spectroscopy enables the study of molecular structure of wood with more accurate identification of its geographical origins. Accurate wood source identification plays a vital role in combating illegal deforestation, which poses severe threats to biodiversity and natural ecosystems. This study not only contributes to academic knowledge but also provides practical tools for law enforcement and conservation efforts, making a meaningful impact on global sustainability initiatives.

Impact Statement: This research significantly advances the field of forensic wood identification by integrating ATR/FTIR spectroscopy with machine learning techniques, providing a powerful tool for identifying wood origins with greater accuracy. The impact of this study extends to enhancing efforts in wildlife crime prevention and environmental conservation, particularly in regions like India where illegal wood trafficking poses a severe threat to biodiversity.

Abstract Text: Wildlife crimes, including illegal deforestation, timber acquisition, and trade driven by the lumber and furniture industries, significantly threaten animal and plant diversity and deplete natural resources on the planet Earth. The ability to accurately identify the geographical origins of wood in illegal trade is crucial. However, traditional laboratory techniques like visual, chemical, and biological methods often fall short in precisely determining the origin of wood samples, leading to challenges in on-site identification for enforcement agencies.

This study introduces a novel approach by applying ATR/FTIR spectroscopy in combination with Machine Learning (ML) algorithms to develop a robust model for geographical identification of wood samples from India based on their chemical composition. The spectral peaks showed chemical constituents present in wood samples from various species and geographical locations, and this data is then subjected to an ML model in the Python framework for more accurate identification. Further, by systematically comparing various ML classifiers, this research addresses the limitations of subjective visual interpretation and aims to enhance the accuracy of wood identification.

The outcomes of the study indicate that the Logistic Regression (LR) classifier is the most effective one among others, achieving 75% accuracy for *Eucalyptus*, 68% for *Dalbergia*, and 81.5% for *Populus* wood species from four different geographical origins in India. The research methodology encompasses comprehensive data pre-processing, careful selection of classifiers, and rigorous performance evaluation, offering a promising solution for combating illegal wood trafficking as there are various portable spectroscopic instruments available today. The outcomes of this study have significant implications for wildlife crime prevention efforts. By enabling on-site tracing of illicit timber sources, apprehension of offenders, and implementation of preventive measures, this research contributes to the broader fight against deforestation and biodiversity loss. The findings provide law enforcement and conservation agencies with advanced tools to protect endangered species and preserve natural ecosystems.[1]

Reference:

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Geographical Location; Wood Identification; Machine Learning

Y19 Optimizing OdoPlex Primers for Use in Pennsylvania Elk

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Learning Objectives: The objective of this study is to show the possibility for versatility of Short Tandem Repeat (STR) primers across wildlife species. This study looks at primer optimization and capillary electrophoresis conditions.

Impact Statement: This study addresses the Organization of Scientific Area Committees (OSAC) research need for the development and validation of standardized STR panels and associated allelic ladders for North American species frequently found in wildlife forensic casework. In the success of this study, there will be a method to identify elk individuals to assist in poaching cases as well as conservation efforts. If this study should show that this multiplex does not sufficiently amplify in Pennsylvania elk, it will provide robust evidence against cross-species amplification applications.

Abstract Text: This study addresses the Organization of Scientific Area Committees (OSAC) research need for the development and validation of standardized STR panels and associated allelic ladders for North American species frequently found in wildlife forensic casework. OdoPlex is a 5-dye STR multiplex panel designed for forensic identification of North American mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*).[1] The use of this panel was investigated for use in Pennsylvania elk (*Cervus canadensis*). Due to the close relation of these species, there may be an opportunity for cross-species amplification and use in wildlife forensic casework. The study extracts DNA from both elk and White-Tailed Deer (WTD) samples using the DNeasy Blood and Tissue kit. Whole blood, ear notches, and DNA extracts were donated and utilized for this project. The WTD samples serve as a control standard when optimizing future steps in the analysis. Quantification indicated that DNA was successfully extracted from both WTD and elk samples. The Qubit dsDNA HS Quantification Assay Kit was used with the Qubit Fluorometer 4.0. Amplification was conducted using the Qiagen Multiplex PCR kit.

After observing amplification status in elk versus WTD samples, optimizing Polymerase Chain Reaction (PCR) conditions for elk samples will begin. Since this is not a commercially available kit, a custom matrix file will be created to conduct STR analysis using capillary electrophoresis. There is currently no allelic ladder for elk using this multiplex, so analysis from capillary electrophoresis data will be performed using base pair sizing. An allelic ladder will be constructed after the successful optimization of the assay. Pennsylvania has a wild elk population as well as agricultural farms. Once the assay is optimized for use in Pennsylvania elk, the end goal of this research is to conduct a population assignment of alleles to distinguish the difference between captive elk and elk from the wild herd.

Reference:

1. Brian C. Hamlin, Erin P. Meredith, Jeff Rodzen, Jolene M. Strand, OdoPlex: An STR multiplex panel optimized and validated for forensic identification and sex determination of North American mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*), *Forensic Science International: Animals and Environments*, Volume 1, 2021, 100026, ISSN 2666-9374, https://doi.org/10.1016/j.fsiae.2021.100026. (https://www.sciencedirect.com/science/article/pii/S2666937421000251).

Casework; DNA; Wildlife Forensics

Y20 An Investigation Into the Feasibility of ICP/OES With Discriminant Function Analysis for the Forensic Characterization of Red Lipstick Samples

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Learning Objectives: Attendees will learn of the usefulness of Inductively Coupled Plasma/**Optical Emission Spectrometer** (ICP/OES) in measuring elemental content in lipsticks, the significance of analytical figures of merit to this analysis and particularly in the choice of digestion method and elements chosen for the analysis, the elements that are significant in helping to differentiate different shades and brands of red lipsticks and the classification results from testing the discriminant function models and models that produced the highest accuracy for predicting the source of the unknown lipstick.

Impact Statement: This study shows the potential for the use of ICP/OES and Discriminant Function Analysis (DFA) to discriminate red lipstick samples by both brand and shade, which previously were not successful when using other methods, such as Infrared (IR) spectroscopy, which are commonly used in forensic laboratories. For brand differentiation of our limited sample set, one brand was significantly better separated compared to the other four brands studied. For shade differentiation, there was one brand that was significantly separated from the other shade, and there were two other shades separated from the other brands, which in the absence of one another would also indicate a high likelihood of being correctly attributed to the correct shade.

Abstract Text: Lipstick is used by many individuals daily, which results in the presence of lipstick on a variety of crime scenes. It is commonly found on cups, napkins, and bedding, is relatively persistent, and can remain unnoticed, making it an ideal piece of evidence. Lipstick evidence could be used to connect a victim, suspect, object, or location to each other. Previous research that has attempted to differentiate lipstick samples has primarily focused on organic data, obtained from IR and Raman spectroscopy. Investigators have used ATR/FTIR, Raman, and Principle Component Analysis (PCA) to characterize different pink lipsticks of the same brand with 95.8% accuracy.[1] Chophi et al. were able to successfully classify 38 red lipstick samples by brand using ATR/FTIR, PCA, and Linear Discriminant Analysis (LDA) with an 81.48% accuracy; however, they did not attempt to differentiate the similar shades of red.[2] These results are in line with those obtained by other investigators who were also unable to discriminate among red lipstick shades.[3]

Previous studies have reported on the elemental composition of lipstick and other cosmetic products.[4,5] However, at this time, we believe we are the first study to apply chemometric methods to characterize the lipstick with some success using ICP/OES. In our study, we attempted to differentiate 20 shades of red lipstick from 5 brands, using elemental data using ICP/OES) and Discriminant Function Analysis (DFA). We report on the elements that were most significant in the discrimination by brand and shade. We show that one lipstick brand in particular was most distinguished from the other brands when testing our method using leave-one-out and the hold out methods. A forensic scientist could report with the highest degree of certainly the results from this lipstick. Likewise, we report on the brands that hold the least evidentiary value based on the significant overlap of their elemental signatures in the discriminant function plots. Unlike the previous research studied, elemental data was more successful in shade prediction compared to the brand prediction. The shade classification was 88.3% accurate in the original model with our limited sample set. In addition, we report on the concentrations of the elements we found, including arsenic, iron, and nickel. Forensic scientists will further learn of the need for lipstick-certified standard reference materials. Our study was limited by the standard in the industry for standard reference materials used for method validation. Potentially there is an element that we did not include in our study that could prove to be significant in the differentiation of lipstick samples; however, because we only had the standard reference materials we had access to there were elements excluded from this study. Previous studies had either used standard reference materials of matrixes significantly different from lipstick, or no standard reference materials at all, which was a challenge in our method development. These reference materials are quite expensive and could be a challenge for labs, especially if they choose to include many elements in their analysis.

In conclusion, this study shows the potential for the use of ICP/OES and DFA to discriminate samples that previously were not successful when using other methods, such as IR spectroscopy, which are commonly used in forensic laboratories.

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1. Alblooshi RA, Alremeithi RH, Aljannahi AH, Nahlé A. Comparative forensic discrimination of pink lipsticks using fourier transform infra-red and Raman spectroscopy. *Vib Spectrosc* 2024;130.
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Forensic Analysis; Method Development; Statistics

Y21 Transforming Bioarchaeology: Computer Vision for Sex Identification in Skeletal Remains

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Learning Objectives: This presentation will provide: an introduction to concepts, including artificial intelligence, machine learning and computer vision; applications for computer vision in forensic science and bioarchaeology; project methodology, data collection techniques, and image preprocessing and augmentation techniques; and the preliminary results of our project.

Impact Statement: Artificial intelligence has precipitated one of the biggest revolutions in science and technology since the invention of the personal computer. By leveraging advanced mathematics with increasingly high-performance computers, forensic scientists can apply machine learning techniques to aid in nearly every step of the process from discovery to identification of human remains. This project aims to contribute to the emerging body of research around applied Computer Vision (CV) in the analysis of human skeletal remains from archaeological contexts.

Abstract Text: This project explores the use of CV for the identification of sex in human skeletal remains from archaeological contexts. The research presented here was conducted as part of an in-progress master's thesis in Anthropology at Northern Arizona University under the advisement of Dr. Kayeleigh Sharp. Training images were collected from skeletal remains housed at the Sicán National Museum in Ferreñafe, Peru, during a two-week period the summer before the start of the master's program. This preliminary work was done to determine the feasibility of the project and refine data collection techniques and methodology. The preliminary results presented here today are not comprehensive nor reflective of the final state of the project, but the work done to-date will illuminate initial findings and explore the implications of this type of research in the discipline of forensic anthropology.

The data collected for this project includes images of skeletal remains from both prehistoric and colonial-era contexts comprising a sample of 50 individuals. Relative completeness and state of preservation varied dramatically between the individuals due to varying taphonomic processes in the sampled populations. Images of the crania and innominate bone were taken when possible and depending on completeness, capturing features notable for sexual dimorphism as outlined by Buikstra and Ubelaker.[1] Various preprocessing and image augmentation techniques were applied to the images to decrease noise and increase variability, an important process to prevent the CV algorithm from "memorizing" the images rather than learning patterns of sexual dimorphism.

The research is currently in the training phase whereby labeled images are passed through a Convolutional Neural Network (CNN) in multiple epochs that recursively determine weights that the model refines as it attempts to minimize loss between the predicted and expected estimation. It is important to note that the model will be trained using professional sex estimations from bioarcheologists who worked with these collections, based on traditional sex estimation techniques. Therefore, the goal of this research is not to accurately guess sex, but rather to accurately guess what the professional sex estimation would be (a subtle but important distinction). Future work on this project will focus on expanding the training data set and validating the model using images of individuals the model has not seen before. This study underscores the transformative potential of machine learning in the field of bioarchaeology, paving the way for more innovative and interdisciplinary approaches in forensic anthropology.

Reference:

1. Buikstra, Jane; Ubelaker, Douglas (1994). Standards for data collection from human skeletal remains. *Arkansas Archaeological Survey*

Artificial Intelligence; Machine Learning; Archaeology

Y22 Investigating the Limits of Low-Input DNA Libraries for Genome Sequencing of Cryptosporidium

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Learning Objectives: This presentation will discuss research on testing the input limits of parasite DNA in three library preparation kits for Next Generation Sequencing (NGS). After attending this presentation, attendees will understand the performance potential of these kits for sequencing low-input DNA and how this research can be applied to benefit the forensic community.

Impact Statement: This presentation will impact the forensic science community by bringing awareness to the utilization of forensic parasitology in the realm of bioterrorism and other criminal investigations that may utilize protozoan parasites. Additionally, this research can bring awareness to the challenges associated with working with samples of low DNA content and offer potential solutions to those working with it in forensic science.

Abstract Text: Biological attacks are a security threat that impact human health through the dissemination of disease-causing agents.[1] According to the Centers for Disease Control and Prevention (CDC), the protozoan parasite *Cryptosporidium parvum* is classified as a Category B agent for bioterrorism of water systems. *Cryptosporidium parvum* can be spread through contaminated waterways, and infections in humans can result in severe diarrheal illness that can be fatal in immunocompromised hosts.[2] Because infection can be initiated with as few as one to ten oocysts, detection methods that are sensitive, specific, and rapid are needed to protect human health.

Genomic data is of great value for developing tools for fast, accurate, and highly sensitive parasite detection and differentiation. However, genomes for *Cryptosporidium* are lacking due in part to DNA availability. NGS library prep kits continue to lower their minimum input limit for DNA, creating new opportunities for parasite genome generation. In this study, three NGS library preparation kits were tested for their ability to generate whole genomes from low-input DNA samples.

To perform this research, we used a commercially available strain of *Cryptosporidium muris*. *Cryptosporidium* oocysts stocks were prepared in sterile water and counted in triplicate using a hemocytometer. Oocysts were prepared for DNA extraction using ten-fold dilutions ranging from 1 million (n=1) to 1 oocyst (n=8 for all oocyst concentrations below 1 million). After extracting the DNA, each sample was prepared for sequencing using the Illumina DNA Prep kit, NEBNext Ultra II FS DNA kit, and QIAseq FX DNA kit. Because DNA extractions from less than 1 million oocysts had concentrations below the limit of detection, modifications for low DNA input were made according to each kit's specific protocol. Prior to sequencing, DNA samples were quantified via Qubit and library quality was assessed via Agilent Tapestation. Whole-genome sequencing of the libraries was performed on the Illumina NextSeq. The resulting sequences were mapped to a *C. muris* reference genome, and genome coverage between kits was compared.

The NEB prep kit had the best performance and demonstrated that coverage of the reference genome exceeding 99% could be achieved with as few as 1,000 oocysts. This coverage is comparable to what was achieved from 1 million oocysts. The Illumina prep kit performed less favorably, with 10,000 oocysts needed to achieve reference genome coverage of >99%. Preliminary analyses indicate that the Qiagen kit was unable to produce enough DNA from 10,000 cells for sequencing. The findings of this study indicate that nearly complete genomes can be produced from low levels of DNA. This work can be applied to furthering the knowledge of parasite genomes and improving the current collection of publicly available genomes of parasites. Our findings are valuable for improving detection of *Cryptosporidium* and other parasites, which can have implications for parasite surveillance as part of bioterrorism or other public health programs.

References:

1. Kwak, M. L., Wallman, J. F., Yeo, D., Archer, M. S., & Nakao, R. (2024). Forensic parasitology: A new frontier in criminalistics. *Forensic Sciences Research*, 9(2), owae005. https://doi.org/10.1093/fsr/owae005.
2. Dąbrowska, J., Sroka, J., & Cencek, T. (2023). Investigating Cryptosporidium spp. Using Genomic, Proteomic and Transcriptomic Techniques: Current Progress and Future Directions. *International Journal of Molecular Sciences*, 24(16), Article 16. https://doi.org/10.3390/ijms241612867.

Next Generation Sequencing; Low DNA; Parasites

Y23 A Civil War Field Hospital: STR Analysis of Samples Collected From a Bloody Surgical Table

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Learning Objectives: This presentation will explain the effectiveness of typical forensic DNA analysis procedures when applied to approximately 160-year-old bloodstains. From sample collection to Short Tandem Repeat (STR) profiling, attendees will learn of the victories and losses throughout the research process along with ideas for potential future research.

Impact Statement: The current procedure for DNA analysis in forensic science laboratories is validated for use when the collected blood sample is relatively fresh. This research attempts to generate partial or complete STR profiles that would corroborate the findings of a study done by Jones & McClintock and add to the little research available in the forensic science community related to old bloodstains in wood.[1]

Abstract Text: The goal of this study is to generate partial or complete DNA profiles from the Hillsman House surgical table as well as determine the best methods for collection and analysis of these bloodstains. The Hillsman House was a residence that was used as a field hospital to treat hundreds of Union and Confederate soldiers during the last battle of the Civil War. In a study published in 2018, Jones and McClintock traveled to the Hillsman House and generated DNA profiles from blood found in the floorboards of the house.[1] The table was not on site at the time they were there, but there were stains on the floor where it had been.

This surgical table was recently acquired, and what were presumed to be bloodstains were located on all sides using an Alternate Light Source (ALS). The visualized stains were collected by swabbing and scraping and subjected to Phenolphthalein and Leucomalachite Green (LCG) presumptive tests to determine if blood was present in these samples. Next, the Qiagen DNeasy Blood & Tissue Kit and Qiagen Investigator Lyse & Spin Basket Kit were used as DNA extraction methods and later compared by quantification using the NanoDrop 2000 spectrophotometer, and the QuantStudio 5 Real-Time PCR system. The amplified samples are subjected to both gel and capillary electrophoresis using a 1.5% agarose gel and the AB 3500 Genetic Analyzer, respectively. Finally, electropherograms are interpreted using the GeneMapper ID-X software.

All presumptive tests yielded negative results, but after extraction, each sample yielded a quantity of DNA. No correlation between the quantity of DNA and the quality of the DNA profile is observed. Samples provided both partial and complete profiles with varying qualities between samples. By increasing the injection time from 15s to 24s it was noted that the profile quality increased. However, in many of the samples, there was a lack of characteristics that are common among highly degraded and mixture samples, including a downward slope, concentration of alleles in the lower molecular weight loci, and mixture profiles. These characteristics were present in the loci of certain samples but were absent in others. Additionally, this research shows the ineffectiveness of the Investigator Lyse & Spin Kit for DNA extraction from a wooden substrate. Lastly, this study indicates the absence of a correlation between the quality of the DNA profile and the extraction or collection technique.

Reference:

1. Jones, K. N., & McClintock, J. T. (2018). Latent Blood Detection and STR Analysis of Samples Collected from an American Civil War Field Hospital. *Journal of Forensic Identification*, 68(4) https://go.openathens.net/redirector/liberty.edu?url=https://www.proquest.com/scholarly-journals/latent-blood-detection-str-analysis-samples/docview/2158114714/se-2.

Forensic Science; DNA Analysis; Historical

Y24 Cartridge Casings in Forensic Science: An Analyzation and Comparison of Cartridge Casings Fired From Unknown and Known Firearms

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Learning Objectives: After attending this presentation, attendees will understand basic concepts behind cartridge casings and their use in the forensic science field. The concepts of class and individual characteristics will be introduced and discussed.

Impact Statement: Firearms play a crucial role in law enforcement and criminal investigations. This study was meant to develop a database in Excel to document class and individual characteristics on hundreds of donated spent cartridge casings fired from unknown firearms for later comparison and potential identification. Determining the potential origin of the donated cartridge casings can be used for the forensic science program's course curriculum involving toolmark and firearm analysis.

Abstract Text: Used cartridge casings are valuable in criminal investigations to trace firearms back to the spent casing found at a scene. Using class characteristic toolmarks found on these casings, including headstamp, firing pin impressions, breechface marks, case design, head type, color, and measurements of the neck, body, and head, can be useful in eliminating unrelated firearms and narrowing it down to a group of possible firearms. Individual characteristic toolmarks are more exclusive by narrowing them down to one specific firearm by matching striation marks found on the body of the cartridge casing to the grooves on the inside of a firearm's barrel.

Hundreds of donated spent cartridge casings fired from unknown firearms were received as well as spent casings from the Liberty Gun Range that were fired from known firearms for further comparison research to be continued. A database was developed in Excel to document class and individual characteristics on each casing for later comparison. Casings were assigned and labeled with a cartridge number. Measurements were taken of casing neck, body, rim, and head using a Mitutoyo caliper. Headstamps, striation marks, breechface marks, firing pin marks, and firing pin shapes were analyzed using a Unitron 16200 Series comparison microscope. Ejector/extractor marks were analyzed using a Accu-Scope stereo microscope. Samples were then sorted according to headstamp and placed in a chest for storage. Unknown firearm samples were compared to known firearm samples for comparison purposes within criminal investigations. It was concluded that class characteristics found on these cartridge casings generate a narrower search for matched firearms. Inconsistencies in breechface marks, firing pin shapes, firing pin marks, and extractor/ejector marks among two casings of interest propose that they came from different firearms. If both casings were found at the same crime scene, this suggests there was more than one shooter at the scene.

Individual characteristics generate even narrower searches, as unique striation marks trace back to an individual firearm. Each cartridge casing that comes from the same firearm will have similar if not identical striation marks. Certain striation marks will present themselves in the same way in each casing that comes from that firearm, building a strong case for the origins of that cartridge. Determining the potential origin of the donated unknown cartridge casings can be used for the forensic science program's course curriculum involving toolmark and firearm analysis as well as future research projects.[1-5]

References:

1. *Association of Firearm and Toolmark Examiners Glossary* 4th Ed.
2. Dutton, Gerard, "Firearms Identification, Comparison Microscopes, & the Spencer Lens Co., *AFTE Journal*, 2002, 34(2), pp. 186-198.
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Forensic Science; Firearms; Cartridge Casings

Y25 Pen Ink Analysis Via Raman Spectroscopy

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Learning Objectives: Attendees will understand the need for ink analysis research in the field of forensic trace; the current limitations of Thin-Layer Chromatography (TLC) for ink analysis, the capabilities of portable Raman Spectroscopy and Surface-Enhanced Raman Spectroscopy (SERS), how data processing improves the Raman signal, and recognize the difficulties in black ink differentiation.

Impact Statement: The research being proposed will impact the forensic science community as it will bring more attention to the capabilities of Raman Spectroscopy and SERS when differentiating pen ink. Analysts will be better informed about the suitability of this instrumentation for their laboratories. Ink analysis is an important aspect of forensics, and this research aims to update current techniques being used.

Abstract Text: Pen INK Analysis has been a very important category of forensic trace analysis since the 19th century.[1] This type of work incorporates cases dealing with questionable documents, forgery, and even fraud. The United States Secret Service investigates over 500 new ink cases each year. Their current standard method for ink analysis uses TLC.2 This technique is destructive and time consuming. Portable Raman spectroscopy is a non-destructive technique that has been used for analysis of various trace evidence including pen inks.

The aim of this study is to bridge the gaps between differentiating black colored inks with Raman spectroscopy. This is relevant as there has been research done on ink; however, the majority features blue ink as black is hard to differentiate.[3] Raman offers a non-destructive way to analyze, benefiting those who utilize this instrument to investigate important matrices.[4] We report on the usefulness of the portable Raman spectrometer in addition to SERS using gold and silver nanoparticles to enhance the signal.

After attending this presentation, attendees will understand the use of portable Raman spectroscopy to determine whether evidence writing could have originated from the source ink. Attendees will further understand how the presence of gold and silver nanoparticles affect the Raman signal to produce the SERS effect. SERS is particularly significant in the case of older and faded documents where one must transform the weak analyte signal to one easily interpreted by the forensic analyst. Our previous work with gunshot residue and SERS further illustrated the usefulness of transforming the data so the analyte signal is better viewed.[5] Both the SERS and the data processing will expand our current understanding of forensic ink analysis.

This report will present a case study in Raman used for pen ink analysis. It is recommended that investigators working with inks familiarize themselves with the abilities of Raman spectroscopy in analyzing ink samples in a non-destructive way. After this presentation, attendees will see that Raman technology has the potential to be a tool for transformation when it comes to forensic ink analysis.

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Raman Spectroscopy; Ink Analysis; Forensic Investigation

Y26 A Gas Chromatography/Mass Spectrometry and Pyrolysis Gas Chromatography/Mass Spectrometry Analysis of Lubricant and Spermicide in Condoms and Personal Lubricants

Laura Cross, Indiana University-Indianapolis, Jeffersonville, IN*

Learning Objectives: Attendees of the presentation will gain an understanding of the importance of lubricant analysis, the two main classes of lubricants (water-based and non-water-based), and how an unknown sample should be analyzed for determining its class and presence or absence of spermicide. Topics to be discussed include the primary chemical structures for each class (and how they are represented on a mass spectrum), previous instrumental methods for lubricant analysis, and how lubricant analysis can be improved through the use of Gas Chromatography/Mass Spectrometry (GC/MS) for water-based lubricants and Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) for non-water-based lubricants using one extraction of an unknown.

Impact Statement: My presentation will impact the forensic science community as it provides a solution to improve the efficiency of lubricant analysis. Current published research advises the use of three different solvents for the determination of a lubricant's class and presence/absence of spermicide with the analysis consisting of three instrumental methods spanning two instruments (GC/MS and Py-GC/MS). In this presentation, I am providing a method that could be implemented in forensics laboratories for lubricant analysis that only requires one solvent and two instrumental methods spanning two instruments (one instrumental method for each previously mentioned instrument).

Abstract Text: During the summer of 2024, the Kentucky State Police (KSP) Central Crime Laboratory made it a goal to develop and implement an experimental and instrumental method for lubricant analysis. In sexual assault cases, when DNA evidence is absent from swabs collected using a sexual assault kit, the identification of lubricant and/or spermicide could be beneficial as associative evidence. Such evidence can be used for determining if a condom or lubricant was used, prove whether penetration occurred, and/or explain why DNA evidence was not found. The goal of the research conducted was to simplify and implement specific instrumental and experimental methods for handling lubricant evidence to gain optimal information and results from the sample. Previous analysis of lubricants in the KSP laboratory was conducted with a "general unknown" method that consisted of an extensively long run time using GC/MS and was not specific for lubricant analysis. The research began with a literature review of previously published articles regarding methods of lubricant analysis.

Published research states that water-based lubricants contain Polyethylene Glycol (PEG), non-water-based lubricants contain Polydimethylsiloxane (PDMS) or petrolatum, and nonoxynol-9 was the standard spermicide used in spermicidal condoms.[1-3] Three different solvents (methanol, hexane, and dichloromethane, respectively) have previously been tested and proven to work for the extraction of each, resulting in multiple extractions for an unknown sample. For analysis, three instrumental methods spanned two instruments. GC/MS has previously been used for water-based lubricant analysis under one method and for nonoxynol-9 analysis using a separate method. Py-GC/MS has previously been used for non-water-based analysis. With the required time for each extraction and method, analysis for identification and determination of the presence and class of lubricant in an unknown sample was inefficient and tedious.

To improve efficiency, the literature methods were tested and compared to methods made with modifications. It was discovered that Dichloromethane (DCM) could extract all three compounds of interest effectively from an unknown sample. A single extraction of the sample using DCM could be divided to allow for the analysis of the same sample on two instruments simultaneously without the need for drying the sample in between or extra experimental preparation. One instrumental method on GC/MS could be used to determine the presence or absence of PEG and/or nonoxynol-9, while one instrumental method on Py-GC/MS could be used to determine the presence or absence of PDMS. A proficiency test proved the use of one solvent with two instruments, each with one instrumental method, could accurately be used to determine the class of an unknown lubricant and determine if it matched a standard provided. The KSP Crime Laboratory plans to implement these new experimental methods and procedures in future lubricant analysis case work.

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Lubricants; Spermicides; Pyrolysis

Y27 Evaluating the General Acceptance of Race-Neutral Statistical Analyses of CODIS Loci

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Learning Objectives: After attending this presentation, the attendee should have a clear understanding of the current race-specific statistical model, the proposed race-neutral statistical model, and how the acceptance of a race-neutral model being implemented depends on the popular opinion of forensic science practitioners and other stakeholders.

Impact Statement: This presentation will impact the forensic science community by examining the acceptance of a race-neutral model over a race-specific model. This will allow advancement toward removing perceived bias from the current statistical model. Adopting a single race-neutral statistical model will simplify report conclusions and expert testimony, resulting in fewer misinterpretations when compared to the race-specific model statistic.

Abstract Text: Currently, Random Match Probabilities (RMP) in forensic DNA profiles are reported using a race-specific model that requires DNA analysts to present three different RMP statistics in court. These values are calculated using an allele frequency database separated by race populations provided by the Federal Bureau of Investigation (FBI). In 1996, the National Research Council determined that the use of race-specific RMP values was necessary due to the slight, consistent variations of the allele frequencies within the major race populations. At the time, only 13-core Short Tandem Repeat (STR) loci were examined. In 2015, the FBI increased the number of core STR loci from 13 to 20, which raised the question of whether the race-specific model is still necessary. In 2020, the race-specific RMP values and race-neutral RMP values were analyzed to determine if the difference in uniqueness of values calculated using the two different models is inconsequential or not.[1] This study found that 98% of variation occurs among individuals rather than between races.[1] This result implies that the use of a race-specific model is insignificantly more conservative than the use of a race-neutral model. Furthermore, it has been found that using a race-neutral model is made possible by adjusting the co-ancestry coefficient to a conservative 0.03 value.[2]

This project aimed to create a qualitative study interested in the current opinion of forensic practitioners and other relevant stakeholders regarding the switch from a race-specific to a race-neutral report model. A ten-question survey was created and sent out to forensic DNA analysts, defense and prosecution attorneys, and university-level forensic students. The survey was accompanied by an informational video and infographic to give the participants basic background details regarding the study. A three-month window was given to allow participants sufficient time to record and submit their responses.

The conclusions made by this project will be used to assess if the forensic science and law enforcement communities are ready to use a single statistical model. Any questions or concerns regarding the methodology of the race-neutral model can be identified and used to evaluate if the method needs more research and development. The acceptance of a race-neutral model has the potential to reduce communication errors and remove ethnicity as a consideration in statistical analysis. However, a change to the RMP statistical method may require a *Daubert* hearing to reevaluate the suitability of this method for the courtroom.

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Statistics; Forensic DNA; Public Opinion

Y28 Utilizing Fragmentation of the Gentueri CollectEject Swab for CODIS Upload

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Learning Objectives: This research aims to determine the smallest amount of DNA that is appropriate in developing a DNA profile that provides at least 8 out of the 13 original core loci markers required for Combined DNA Index System (CODIS) upload eligibility. Additionally, it aims to test the efficiency of the Gentueri CollectEject Swab as well as the efficiency of the Harris punch as a coring method for swab fragmentation.

Impact Statement: The standard protocol for DNA extraction requires the entire swab tip. However, since there is a limited amount of DNA recovered at crime scenes, this proves to be an issue for forensic laboratories when testing crime scene DNA. This research permits the retesting of DNA by the application of swab tip fragmentation for extraction via the Harris punch.

Abstract Text: The Gentueri CollectEjectSwab is a DNA collection swab type, that is unique by the means of its ejection method, its desiccant drying technique, its scalpel-like shape, and its material.[1] This swab utilizes a multilayered cellulose material in contrast to the standard cotton swab used in most criminal investigations and collection kits.[1] Since the standard cotton swab contains tightly wound material, it holds onto a large amount of DNA, making it difficult to extract.[1] The Gentueri CollectEject Swab opens as it expands when wet, collecting DNA with the scalpel-like design of the swab tip that promotes increased DNA collection.[1] Placing it in the drying pouch forces the swab to dry with the layers open, promoting full DNA extraction.[1] Furthermore, the swab's drying pouch reduces the risk of contamination from outside environmental factors while the desiccant prevents bacterial growth on the swab tip and speeds up the drying process.[1]

This study examined the smallest number of punches taken from the Gentueri CollectEject Swab to yield DNA profiles suitable for CODIS upload and explored its effectiveness in DNA collection. Typically, the protocol for extraction of DNA requires the entire swab tip. However, since there is a limited amount of DNA recovered at crime scenes, this proves to be an issue for forensic laboratories when testing crime scene DNA. Therefore, utilizing the Harris punch method to collect fragments of the swab tip allows for future testing with the remaining swab tip. Preliminary results suggest three punches are the smallest amount utilized for successful quantification, which provides a suitable profile for CODIS upload. A smaller punch amount would not be suitable for meeting the 1ng/µL threshold, resulting in little to no peaks appearing at loci markers on the electropherogram. As of 2017, CODIS required 20 core loci markers to be met on the DNA profile compared to the original 13 core loci makers that were established in 1997.[2,3] However, a minimum of 8 out of the original core 13 loci must be present on the DNA profile to be eligible to be uploaded in CODIS.[4] Therefore, the absence of peaks due to inadequate amounts of DNA poses an issue for the suitability of upload into CODIS. The impact of this research permits the retesting of DNA by the application of swab tip fragmentation for extraction.

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Fragmentation; CODIS; Gentueri CollectEject Swab

Y29 Evaluating the Reliability of the Megyesi Total Body Scoring Method on People of Color

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Learning Objectives: In this poster presentation, attendees will learn about how the Megyesi Total Body Scoring (TBS) method works for estimating time since death and why it's important to evaluate its reliability when applied to People Of Color (POC). This presentation will explore how factors like skin tone might influence the accuracy of this method and discuss ways to make forensic techniques more representative of the world's population through the inclusion of a diverse sample. This presentation will highlight both the strengths and limitations of the TBS method and incite the development and application of more representative methodologies.

Impact Statement: This presentation will impact the forensic science community by raising awareness about the limitations of widely used forensic methods, like the Megyesi TBS method, when applied to POC. By highlighting these gaps, this research encourages a critical re-evaluation of current practices and promotes the development of more inclusive and reliable forensic tools. The findings from this research aim to contribute to improving the accuracy and fairness of forensic analyses, ensuring that all populations are better represented and served in forensic casework.

Abstract Text: This poster presentation showcases my thesis research, which critically examines the reliability of the TBS method developed by Megyesi et al. for assessing decomposition, with a specific focus on POC.[1] The TBS method was designed to quantify the decomposition of human remains through observable indicators. It assigns scores based on visible changes in decomposition compared to standardized descriptions provided by Megyesi and colleagues, which often include evaluations of skin color changes. While this method provides a structured approach to quantifying decomposition and calculating Accumulated Degree Days (ADD), the original descriptions and criteria were developed with the assumption that the decedents had lighter skin tones. This assumption introduces a potential bias, raising questions about whether the TBS method is equally effective for estimating decomposition in individuals of color.

This study utilizes a semi-experimental design to analyze a comprehensive dataset of decomposition photographs collected from the Texas State University Forensic Anthropology Center. This dataset includes images of five POC and five White donors, providing a balanced representation for comparative analysis. To ensure a robust evaluation, observation packets were distributed to 21 participants, who followed established protocols for TBS scoring. The reliability of these scoring assessments was measured using Fleiss' Kappa and weighted Cohen's Kappa, which help quantify the degree of agreement among raters. Additionally, correlation analysis was performed to explore the relationship between TBS scores for White individuals and POC. This approach helps to identify any potential discrepancies in how decomposition is assessed across different demographic groups.

The research also delves into participants' understanding and application of the TBS methodology, evaluating their performance through the percentage of correct answers. By investigating how rater backgrounds and subconscious biases might influence scoring consistency, the study aims to uncover and address potential biases in forensic assessments. This is crucial for refining forensic anthropology practices to ensure they are equitable and accurate for diverse populations. Overall, this presentation seeks to highlight the need for improved forensic methodologies that account for demographic diversity. By addressing these issues, the research contributes to the broader goal of enhancing the reliability and inclusivity of forensic assessments, ultimately fostering a more just and accurate approach to forensic science.

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Decomposition; Reliability; Diversity

Y30 The Characterization and Quantification of Cell-Free DNA in Touch Samples

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Learning Objectives: Touch DNA has become a fixture in day-to-day trace DNA analysis. Attendees will walk away knowing more about persistence of cell-free DNA in their samples and techniques to assess cell-free DNA.

Impact Statement: This project aids in understanding the dynamics of cell-free DNA in touched samples. Currently, there is little understanding pertaining to the origins and constituents of DNA found in touch samples. Lack of knowledge pertaining to the biological properties of touch DNA makes it hard to know exactly when or how a genetic profile will arise from a touch sample. Quantifying and characterizing the fragments of cell-free DNA in touch samples will allow for better understanding and predictability of how and when analysis can produce genetic profiles. For example, understanding whether the degraded nature of touch samples is due to intrinsic properties such as cell-free DNA fragments or extrinsic properties such as environmental factors will help standardize collection and amplification techniques. Furthermore, understanding the biological components that contribute to touch DNA will provide critical information pertaining to how touch DNA is left at a crime scene, which will help in the utilization of touch DNA evidence in the courtroom. Overall, characterization of cell-free DNA will add to the predictability and reliability of touch sample analysis.

Abstract Text: Advancements in technology pertaining to DNA analysis have enabled the generation of genetic profiles from trace DNA samples, including DNA deposited through touch. Cases involving touch DNA are commonplace; however, despite the frequent analysis of such samples, the composition remains poorly understood. It is generally assumed that touch DNA primarily originates from epithelial cells shed from the skin's outermost layer. However, during the keratinization process, these cells lose their nuclei, rendering them less likely to contribute significant amounts of DNA. Nevertheless, enucleated cells and their surrounding extracellular environments may still harbor residual DNA, known as cell-free DNA, due to incomplete nuclei degradation during keratinization.

This study aims to characterize the components of DNA samples deposited through touch, with a particular focus on cell-free DNA derived from keratinized epithelial cells. Participants handled a computer mouse in a normal fashion over a single day. Two swabs were simultaneously taken from the computer mouse and underwent two separate extraction methods to assess cell-free and total DNA of the mouse. Seventeen cell-free and 17 total DNA samples were collected, extracted, and analyzed. The analysis revealed that genetic profiles generated from cell-free DNA fractions were of comparable or superior quality to those obtained from samples containing both nucleated and non-nucleated DNA. The average proportion of alleles present in the cell-free fraction was 56.0 ± 34.9%, while the total DNA fraction had an average of 49.0 ± 42.9%.

These findings suggest that cell-free DNA may represent a significant proportion of touch DNA samples. As a result, DNA extraction methods tailored to the recovery of smaller cell-free fragments could outperform traditional techniques designed for cell-bound DNA. Improved understanding of the compositions of Touch DNA has the potential to enhance forensic collection and analysis protocols, reducing variability between samples and increasing the reliability of DNA evidence in forensic investigations.

STR Profiles; Trace Analysis; Low DNA

Y31 A Robustness Analysis of High-Dimensional Consensus (HDC) Mass Spectra in Forensic Drug Identification

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Learning Objectives: In our poster presentation, we highlight the limitations of traditional mass spectrometry in identifying seized drugs and suggest HDC as a means to address ambiguity. Attendees will gain insights into how HDC leverages multiple measurements to improve identification accuracy as well as see a demonstration of robustness, and HDC's outperformance of popular traditional machine learning and deep learning models like logistic regression, random forest, XGBoost, and Multilayer Perceptron (MLP) in real-world scenarios. Additionally, we will provide evidence of HDC's robustness in maintaining high performance even when synthetic noise is added, demonstrating its potential for forensic science applications. Our poster will be applicable to a wide range of audiences, including chemists, biologists, mathematicians, computer scientists, and law enforcement officers.

Impact Statement: Our poster aims to be of significant interest to the forensic science community by addressing the importance of seized drug identification and introducing a novel and associated robustness testing in identifying such compounds using mass spectrometry. The HDC mass spectra approach is an improvement over traditional techniques by utilizing multiple measurements, improving accuracy and reliability in drug identification. As HDC outperformed, on real-world data, some popular machine learning models that also require multiple measurements and maintained its robustness in the presence of simulated noise, we believe this method could have a significant impact as an algorithm that ultimately advances the accuracy and efficiency of forensic drug analysis.

Abstract Text: Mass spectrometry is a powerful tool for identifying compounds, yet the complexity of fragmentation and variability in measurements can make it difficult to differentiate compounds with similar mass spectra using a single query mass spectrum. Most forensic laboratories use Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS) as part of their workflow for identifying controlled substances of interest. While a single mass spectrum is typically utilized for analyte identification, we explore a scenario where multiple measurements are taken and introduce a novel identification method called High Dimensional Consensus (HDC) mass spectra.

We tested our method on 160 mass spectra, representing 10 replicate measurements of 16 distinct compounds, all generated by GC/EI/MS. The dataset includes common controlled substances—synthetic opioids (tramadol, fentanyl, acetyl fentanyl, benzyl fentanyl), stimulants (amphetamine, cocaine, methamphetamine), steroids (trenbolone), other drugs (heroin, alprazolam, phenylephrine, xylazine)—and common adulterants such as caffeine and quinine.

Our findings suggest that HDC, which leverages variations in peak measurements across replicate injections, achieves 100% accuracy of compound identification during testing. Notably, HDC performs exceptionally well in distinguishing structurally similar mass spectra profiles. For instance, despite a cosine similarity of 0.997 between methamphetamine and phentermine, HDC produced a similarity score of 3.6092e-34 between methamphetamine and phentermine, effectively differentiating the two. Additionally, the significant gap in HDC similarity scores between the top match and the second match in the generated hitlist, which is a ranked list of potential matches among all possible compounds, further enhances HDC's ability to accurately identify the target analyte among other illicit drugs.

Our experiments showed that 93.75% of results had a gap greater than 0.3, with 81.25% exceeding 0.74. In a comparative study, HDC outperformed several widely used traditional machine learning models, including logistic regression, random forest, and XGBoost, as well as a simple deep learning classifier, achieving up to 12.5% higher accuracy on real-world examples without synthetic noise. In a robustness study, when synthetic noise was introduced to simulate measurement inconsistencies, HDC managed to preserve its remarkable performance at moderate noise levels. Overall, HDC demonstrates strong performance and robustness, showing great potential in seized drug identification by providing reliable, explainable results that forensic scientists can use to fight against drug-related crimes and help people in need.

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Seized Drugs; illicit Drug Identification; Forensic Analysis

Y32 Maximum Likelihood Techniques for Discriminating Mass Spectra of Isomers

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Learning Objectives: In our poster presentation, we highlight limitations of traditional mass spectrometry in discriminating between isomeric compounds. We review several pre-existing techniques involving the use of replicate mass spectra to tackle this problem. We demonstrate the use of likelihood classification methods to improve these algorithms by reducing the number of analyte spectra required for accurate classification. Attendees will gain an understanding of commonly used maximum likelihood classification algorithms as applied to mass spectrometry data. In addition, the audience will understand the quantitative limitations on classification accuracy due to low sample concentrations, as well as the drawbacks of pre-processing algorithms applied to Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS) data. Our poster will be applicable to a wide range of audiences, including chemists, biologists, mathematicians, computer scientists, and law enforcement officers.

Impact Statement: Our poster presents the use of likelihood classification techniques applied to replicate GC/EI/MS mass spectra. Our approach is likely to be of significant interest to the forensic science community, as it demonstrates an accurate method of discriminating between mass spectra of isomeric compounds, while minimizing time and effort on the part of the analyst. Our quantitative analysis on the impact of sample concentration on classification accuracy is likely to help end-users make better informed decisions based on our methods. Finally, we hope that our analysis of the loss of accuracy induced by common pre-processing algorithms will induce a more nuanced understanding of the limitations of such methods.

Abstract Text: Objective discrimination of structurally similar compounds such as isomers is a problem of widespread interest from law enforcement as well as public health perspectives. Mass spectra are common signatures used to discriminate between compounds. Mass spectra obtained from the same compound can have a wide degree of variance, due in part to the inherently stochastic nature of the fragmentation process as well as experimental factors such as sample concentration. Due to this variance, mass spectra of many isomers are often indistinguishable using conventional methods. For "low-resolution" spectra, this problem is often compounded by the loss of information due to commonly used pre-processing algorithms.

Recent work from our group at the National Institute of Standards and Technology (NIST) tackled the problem of isomer discrimination by using replicate mass spectra to construct a "High Dimensional Consensus (HDC) spectrum" and an associated "HDC similarity score."[1] In mathematical terms, an HDC spectrum is a representation of a given set of mass spectra as a probability density function of a Gaussian mixture model. Like other techniques involving the use of replicate mass spectra, the HDC method compares a set of replicate "analyte" mass spectra against a database of library spectra.[2,3] While such methods are promising, they require the analyst to obtain multiple replicate mass spectra of a single sample. This can be challenging in practice, due to limited sample volumes as well as the time and cost required to do so.

In this work, we show how *likelihood classification techniques* can be used in conjunction with the Gaussian mixture model from the HDC method to obtain accurate classifiers using single analyte spectra. We demonstrate the effectiveness of our method on a dataset of GC/EI mass spectra. These spectra were obtained from three sets of isomers of forensically relevant compounds (*ortho*-, *meta*-, *para*-Fluorofentanyl (HCl); 3,4-Methylenedioxy-α-methylaminohexaphenone (HCl), N-Methyl-N-propyl methylone; MDMB-FUBINACA, EMB-FUBINACA, MDMB-FUB7AICA, MDMB-4en-PINACA), serially diluted in methanol at concentrations of 0.33mg/mL, 0.033mg/mL and 0.0033mg/mL.

We will show how our methods outperform traditional similarity scoring techniques while minimizing the number of analyte spectra required in comparison to other methods involving replicate spectra.[1-3] On this dataset, our methods achieve classification accuracies above 95%, as opposed to the sub 80% accuracy we get using conventional scores such as the cosine similarity. Additionally, we will quantify the degradation in classification accuracy due to reduced sample concentrations. Finally, we will show how the use of "raw" (unnormalized) spectra versus traditionally normalized "low-resolution" spectra improves the discriminative power of several classification algorithms, especially for low-concentration samples.

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Mass Spectrometry; Isomers; Algorithms

Y33 The Persistence of Trace DNA Samples in a Carjacked Scenario

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Learning Objectives: After attending this presentation, attendees will understand the principles of how handling time, recovery time, and environmental conditions affect the persistence of DNA samples within and around a vehicle when it has been used by two persons.

Impact Statement: This presentation will impact the forensic science community by improving best practices for law enforcement and forensic experts during the investigation of a carjacking when DNA samples are necessary to recover.

Abstract Text: The complexity of a carjacking scenario can vary on each occasion making the decision of where to look for DNA evidence difficult. The make and model of a car, how long a perpetrator was in a car, and how long until that car was recovered by law enforcement are some general factors that impact the persistence of DNA from any individuals within the vehicle. Furthermore, every vehicle contains specific features that anyone may touch when entering, exiting, or driving the vehicle. These features are of significance for investigators as these locations can collect touch DNA. The duration of contact between an individual and these features may significantly impact the amount of DNA deposited on that surface.[1,2] Other conditions such as the manner in which an item is handled, and an individual's shedder status will also impact DNA deposition.[1,3] In such a scenario, when two individuals touch the same location in succession, it becomes challenging to determine the individuals that comprise the subsequent DNA profile.[4] This study aims to determine how handling time and recovery time affect DNA deposition and recovery in a carjacking scenario.

In this preliminary study, two participants were selected to simulate a carjacking scenario in which one individual (Participant A) enters the driver's side front door of another individual's (Participant B) vehicle for a designated period of time. During this time in the vehicle, Participant A was responsible for holding the steering wheel for 20 minutes before exiting. This experiment was repeated several times with separate recovery intervals of 60, 120, and 240 minutes, before the steering wheel was swabbed for DNA recovery. On each occasion, the vehicle was located in a shaded parking spot, and nobody had access to the vehicle until each simulation was complete. Participant B is the known owner of this vehicle and the only frequent driver of this vehicle. Participant A has never entered this vehicle prior to this preliminary study.

All DNA swabs were subject to the same processes of DNA extraction with the QIAshredder/QIAamp DNA Investigator Kit, quantification with the Quantifiler Trio Kit, amplification with the GlobalFiler Amplification Kit, and capillary electrophoresis on the SeqStudio Genetic Analyzer prior to profile evaluation using GeneMapper ID-X software. The quantity of DNA recovered from the steering wheel across these simulations ranged from 0.45–1.12ng/uL. For each simulation, a mixed DNA profile was examined that included alleles for Participant A, Participant B, and additional alleles not specific to either individual. Participant A was a minor contributor on each occasion and displayed a full Short Tandem Repeat (STR) profile on all but one occasion. Participant B was always the major contributor and displayed a full STR profile every time. Anytime an additional allele appeared in the DNA profile, it was always as a minor contributor. These additional alleles are likely a result of background DNA or were caused by indirect transfer of DNA.

Understanding common modus operandi in a carjacking scenario can prove useful for investigators when determining what areas around a vehicle may contain suitable amounts of persistent DNA to obtain a possible profile under varying circumstances. In this study, it was observed that the steering wheel of a vehicle with a known owner is a suitable location for DNA recovery.

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DNA; Trace; Carjacking

Y34 The Effect of Ethanol on GHB UDP-Glucuronosyltransferase Activity

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Learning Objectives: Attendees will learn of toxic potential of the "club drug" Gamma-Hydroxybutyrate (GHB), an endogenous Gamma-Aminobutyric Acid (GABA) -analog and metabolite. Further, the metabolic pathways for the formation and elimination of the drug are presented. Finally, attendees will learn of the potential for mechanistic inhibition of the formation of the phase II GHB metabolite, GHB-glucuronide by ethanol functioning as a competitive inhibitor of GHB UDP-glucuronosyltransferase, and the implications thereof for "Drug-Drug" interaction.

Impact Statement: This presentation will provide useful information regarding the potential for drug-drug interaction between GHB and ethanol. Such knowledge will allow a more thorough understanding of toxicologic findings by both toxicologic and medical personnel. Understanding the implications of co-ingestion for interpretation of Central Nervous System (CNS) depressant effects will facilitate and enhance the understanding of casework involving both drugs.

Abstract Text: An increased CNS depressant effect as a result of exogenous GHB and ethanol co-ingestion suggests the possibility of an interaction between endogenous GHB metabolic pathways and ethanol.[1] GHB is an endogenous GABA-analog and metabolite in mammalian brains.[2] GHB and ethanol are both substrates for a family of phase II conjugation enzymes, the Uridine-5'-Diphosphate Glucuronosyltransferases (UDP-GTs).[3] These enzymes utilize the co-substrate UDP-Glucuronic Acid (UDP-GA) to produce GHB-glucuronide and ethyl-glucuronide, respectively.[3] Based on the structural similarities between the C1 and C2 of ethanol and C3 and C4 of GHB, we have hypothesized that ethanol would function as a competitive inhibitor of the GHB UDP-glucuronosyl transferase, consequently increasing the internal concentration of the drug, prolonging half-life and increasing AUC.

We have evaluated our hypothesis using a method modified from Foti and Fisher, using a 0.5mL mouse microsomal incubation containing GHB (10mM), 0.75mg of microsomal protein suspended in Tris-HCl buffer (50mM), magnesium chloride (8mM), CHAPS (1.5mg), and UDP-glucuronic acid (4mM) in 1.5 mL Eppendorf tubes.[4] Ethanol was included at concentrations equivalent to 0, 50, 100, and 200mg/dL (0mM, 10.9mM, 21.7mM, 43.4mM). Each concentration was evaluated in triplicate. All samples were pre-incubated at 37°C for 3min., after which the UDP-glucuronic acid was added, and all samples incubated for a further 30min. The reaction was terminated by the addition of 100uL 10 g/mL potassium fluoride. The samples were centrifuged, and the supernatant was extracted using Sep-Pak Aminopropyl (NH_2) 3cc Vacuum Cartridges as per Janda et al.[5] The formation of GHB-glucuronide and ethyl-glucuronide was measured and quantified using an HPLC-mass spectrometer using a HILIC 2.6µm (150mm x 2.1mm) column with a 90/10 Acetonitrile/100mM ammonium formate mobile phase. In our incubations, the amount of GHB-glucuronide formed in mixtures containing 50mg/dL ethanol was not differentiable from the 0-ethanol controls. The mixtures containing ethanol at 100 and 200mg/dL averaged 57 and 50% of the 0-ethanol control, respectively. These data suggest that ethanol acts to some extent as a competitive inhibitor of GHB UDP-glucuronosyl transferase. Further work will focus on the evaluation of the kinetic parameters of the enzyme reaction and the effect of ethanol on those parameters.

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Toxicology; GHB-Glucuronidation; Ethanol-Glucuronidation

Y35 Problematic Exclusionary Differences: The Tyranny of Universal Standards for Determining Exclusionary Differences

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Learning Objectives: Attendees of this presentation will gain an understanding of the statistical issues with the use of American Society for Testing and Materials (ASTM) -style comparison methods for trace element profiles when source-dependent thresholds for declaring an exclusionary difference exist. Attendees will learn about novel developments for testing if multiple thresholds are warranted.

Impact Statement: The use of statistically rigorous methods for characterizing the applicability of standards for identifying exclusionary differences will be explored. The implications of differing rates of false exclusions among sources (resulting from the inappropriate use of a single threshold) will be discussed relative to the concept of algorithmic fairness and the fidelity of evidence interpretation to assist in the administration of justice.

Abstract Text: Within the forensic science community, there is a need for a statistically rigorous determination of whether an exclusionary difference exists. With respect to the ASTM glass interpretation standards, this difference is determined to have occurred if any one of the standardized differences between the measured trace element concentrations is greater than four.[1-3] In this work, we will present strategies for assessing the validity of using a common threshold for all sources when determining if an exclusionary difference exists.

The determination of when an exclusionary difference exists is integral to the Kirkian Two-Stage approach to evidence interpretation.[4] If an exclusionary difference is observed when comparing questioned vs. known source objects, then the known source is excluded as having given rise to the questioned object at the first stage of the approach; however, if the known source is not excluded as the source of the questioned object, then the examiner must determine the rate at which competing alternative sources would be excluded. These methods/algorithms are typically constructed to ensure that the false exclusion rate for each source is equal. However, if the underlying score function that the algorithm is based on has a distribution that varies by source, then the corresponding thresholds for determining an exclusionary difference will need to vary as well.

In this work, we will review various strategies for identifying when the within-source distribution of scores varies between sources; methods for estimating the thresholds; remedial approaches such as pooling subsets of the sources together; and implications of this type of variability among the sources to the classical Kirkian approach to evidence interpretation. Although we will focus on the Kirkian approach, these issues are even more problematic for Likelihood Ratio (LR) -based methods due to the need to estimate a likelihood function from just a few observations from a specified source; the discussed remedial methods will apply equally to the LR paradigm for evidence interpretation.

We will illustrate these methods with trace element concentrations in glass and Al foil as well as micromorphometric data from small arms propellants.

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Exclusionary Difference; Algorithmic Fairness; Evidence Interpretation

Y36 An Investigation of the Impact of the Fire Debris Sample Extraction Process on Latent Fingerprints

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Learning Objectives: This presentation will discuss results from a preliminary study performed to investigate the potential impact of a common, standardized fire debris extraction process on latent fingerprints. After attending this presentation, attendees will have a better understanding of the impact of various extraction conditions on the quality of latent fingerprints.

Impact Statement: This presentation will impact the forensic community by providing preliminary foundational data that addresses and supports best practice recommendations stated in several published standards covering fire debris analysis. Such information is useful to forensic science practitioners performing related casework, as well as stakeholders who depend on their results.

Abstract Text: The chemical analysis of fire debris supports fire investigations by determining if debris samples contain an ignitable liquid.[1] Current American Society for Testing and Materials (ASTM) standards covering sample extraction techniques for fire debris analysis suggest that heating items above room temperature could negatively impact other potential evidence, such as DNA or latent fingerprints; however, no published study directly addresses the extraction conditions recommended in the ASTM standards[2-4]. This study aimed to examine the effects of a commonly used fire debris extraction process on latent fingerprints.[2]

Phase 1 of the study investigated the potential impact of extraction conditions (heating at temperatures ranging from 23–80°C, held for 16 hours) on the quality of latent fingerprints deposited on porous and non-porous substrate materials. Phase 2 of the study examined the effect of the presence of different ignitable liquid vapors on the latent fingerprints during one set of extraction conditions (heating at 60°C, held for 16 hours).

To simulate differences in print patterns and sebaceous material combinations, latent fingerprints were collected from three volunteer donors. Each print deposit was performed in the same manner: the donor wiped their face with their printing finger, then lightly touched the substrate material, allowing their printing finger to remain in contact with the substrate for approximately 2 seconds.

A Full Spectrum Imaging System Color Lab (FSIS 1) from Arrowhead Forensics was used to digitally capture images of the latent fingerprints before and after exposure to the fire debris extraction conditions. Four different substrate materials (glass slides, polyvinyl chloride-based packaging material, paper business cards, and aluminum metal disks) were used to simulate a variety of fire scene-related items on which latent fingerprints might be found. Three ignitable liquids (70% isopropyl alcohol, 50% weathered gasoline, and diesel fuel) were used during Phase 2 of the study.

The data collected during this study (a total of 155 sets of before/after images) were evaluated by an experienced, qualified latent print examiner who determined the amount of degradation present in the final images of the latent fingerprints, and whether they were of comparison value/suitable for forensic analysis.

While results from the Phase 1 and Phase 2 experiments indicate that some latent fingerprints appear to be less affected by the conditions of the fire debris extraction process than others, it is notable that every set of extraction conditions investigated during this preliminary study resulted in at least one final latent print image that was determined to be unsuitable for forensic analysis. Thus, given the possibility of degradation to the extent that a latent fingerprint could become unsuitable during the fire debris extraction process, the results from this study support the recommendations in the ASTM standards regarding the avoidance of heat when extracting items intended to also be examined for latent fingerprints.[2-4]

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Fire Debris Analysis; Latent Prints; Standards

Y37 Using Historical Biocultural Variables and Vitamin D Deficiency to Assist With Biological Profiles

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Learning Objectives: This presentation discusses the potential of using metabolic bone disease and biocultural variables to add more information to biological profiles and assist in Doe identification. After attending this presentation, attendees will recognize variables that can influence vitamin D deficiency, implications for how these individuals lived their lives, and which variables we can use to assist in skeletal identification.

Impact Statement: This presentation can assist when one encounters unknown skeletal remains who are vitamin D deficient. Variables that cause deficiency can help gain more information on that individual and lead to potential identification.

Abstract Text: The biocultural approach uses biological, environmental, and cultural variables to help identify how an individual spent their childhood or their adult life.[1,2] When skeletal remains are identified, DNA may not be sufficient in identification and complete biological profiles are needed. This research examines historic skeletal, genealogical, and biocultural information to determine if there is a correlation with the presence of vitamin D deficiency.[3-5]

One hundred seventy-seven individuals living between the 1830s and 1940s in the United States were analyzed and traced through the historical record using Ancestry and FamilySearch. Skeletal results and biocultural variables were compared via chi-square and Spearman RS correlation. Climactic data were also compared via Analysis of Variance (ANOVA) and t-test. Results indicate that 30 individuals had confirmed vitamin D-deficient lesions and 20 had potential vitamin D-deficient lesions. The average Ultraviolet (UV) index ($p = 1.12E-07$), temperature ($p = 2.58E-08$), and number of clear sky days ($p = 5.93E-05$) are statistically different between the northern and southern United States. When comparing intersectionality of biocultural variables and vitamin D lesions, results indicate that there were trends in determining where an individual spent their childhood (chi-square $p = 0.006$; Spearman RS 0.92, $p = -0.008$), year of death (chi-square 0.005; Spearman RS 0.87, $p = -0.01$), presence of spinal pathology (chi-square $p = 0.05$; Spearman RS 0.72, $p = -0.03$), and age ($p = 0.02$; Spearman RS 0.60; $p = 0.04$). There were also correlations between vitamin D deficiency and sex (0.61; $p = 0.04$), social race (0.74; $p = 0.04$), and occupation (0.50; $p = 0.05$). Individuals with active vitamin D deficiency were more likely to be White males, born in the north, with load-barring spinal pathology, and who worked in blue-collar occupations. More research is warranted but completing biological profiles using biocultural variables may help identify where someone is from and how they lived their lives which in turn will assist with identification.

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Anthropology; Skeletal Remains; Unidentified Persons

Y38 The Impact of Different Environments on DNA Degradation in Decomposing Mammalian Specimens

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Learning Objectives: Attendees will be provided with a better understanding of how DNA breaks down over time and how environmental factors can influence this process. The decomposition process and the variable of heat will be explained in detail to provide an understanding of how these factors affect DNA degradation.

Impact Statement: Due to the random nature of science, human actions are unpredictable. Victims of mass disasters, homicides, and those of ancient descent can benefit from this study. Fragmented DNA aids in identification within these scenarios. By understanding that degraded DNA is time-dependent, the postmortem interval can be estimated providing a potential timeline for investigators.

Abstract Text: Degradation of Deoxyribonucleic Acid (DNA) is influenced by variables like temperature, humidity, and ultraviolet radiation.[1] DNA degradation begins when temperatures exceed 190°C but also during decomposition.[2] Monitoring DNA degradation during decomposition is important as fragmented DNA aids in identification, but degraded DNA poses challenges in forensic casework.[3] DNA has emerged as a power tool in the identification process.

In forensic science, the law of individuality is crucial because a particular subject has not been duplicated in another individual or object.[1] Identifying when DNA is too degraded to serve a reliable profile will help eliminate the compromise of quality genetic samples. The decomposition process results in gradual disappearance to complete loss of long DNA fragments.[4] With gradual disappearance over time, DNA serves as a measure of time.[5] By measuring the degradation of DNA in a unit of time, the postmortem interval can be estimated, providing a potential timeline for a specific case.[5]

The purpose of this project is to quantify DNA during various stages of degradation during decomposition. Mammalian specimens were placed in four different types of sealed containers and monitored for temperature, humidity, and stage of decomposition. Multiple tissue swabs were taken weekly from each specimen. Samples were collected from the rectum and the inside of the mouth from each specimen. Then DNA was extracted using a PrepFiler Forensic DNA Extraction Kit, then underwent Polymerase Chain Reaction (PCR). Double-stranded DNA quantity was recorded via nanodrop before and after PCR. Samples were then diluted and sequenced using a SeqStudios Genetic Analyzer. Preliminary results show that there was a change in the quality of DNA as the specimen decomposed. The genetic analyzer was not able to produce hig-quality sequences for each specimen. More data is needed to determine how far into decomposition one can obtain adequate DNA to produce usable genetic profiles.

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DNA; Decomposition; Animal Remains

Y39 An LC/MS/MS Analysis of Novel Psychoactive Arylcyclohexylamines in Blood

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Learning Objectives: Attendees will learn about a new Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for the detection and quantification of Arylcyclohexylamine (ACH) drugs in human blood samples.

Impact Statement: This method will impact the forensic science community by enhancing the capabilities of forensic toxicologists to quantify human blood concentrations of novel psychoactive substances within the class of ACHs.

Abstract Text: ACHs are a class of hallucinogenic substances that includes drugs such as Phencyclidine (PCP) and ketamine. Non-medical use of these substances has increased significantly since the start of the 2000s.[1-3] Numerous synthetic analogs of ketamine and PCP have entered the black market as they have become increasing in international popularity as recreational drugs, such as Methoxetamine (MXE), Hydroxetamine (HXE), Fluorexetamine (FXE), 2-Fluoro-2-oxo-PCE (2-F-2-oxo-PCE), and 2-Fluorodeschloroketamine (2-FDCK).[4] Therefore, new toxicological methods must be developed to include these novel drugs as they are encountered.

One method by Li et al. has been developed for ACH drugs, which included 36 different hallucinogenic drugs and their detection and quantitation via Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) in human hair.[5] Hair has several advantages as a biological matrix for toxicological analysis, including its long detection window and the non-invasive collection procedures for hair samples. However, hair analysis requires extraction methods that are more expensive and time-consuming than extraction methods used for more commonly used matrices, such as blood.[6,7] Blood is the gold standard for forensic toxicology testing because blood sample analysis provides the concentration of drug(s) circulating in the subject's body without any required conversions. This research focuses on the development and validation of a quantitative method for ACH drugs in blood.

In this study, an LC/MS/MS method was developed by building upon the work of Li et al. to detect and quantitate ACH drug concentrations in human blood. This method is currently undergoing validation in accordance with the AAFS Standard Board (ASB) Standard 036 (REF). Following validation, this method will be applied to authentic human blood samples.

The scope of this method included ketamine, PCP, MXE, HXE, FXE, 2-F-2-oxo-PCE, 2-FDCK, 3-Fluoro-PCP (3-F-PCP), 3-Methyl-PCP (3-Me-PCP), and 4-Methyl-PCP (4-Me-PCP). The internal standard used was a mix of ketamine-D_4, PCP-D_5, and MXE-D_3. Quantitation was performed using external calibration and the quantitative range was assessed from 10 to 1,000ng/mL. This method utilized a basic liquid-liquid extraction method using a 0.1M Borax Buffer, n-butyl chloride, and ethyl acetate to prepare human blood samples for chromatographic analysis. Chromatographic separation was achieved under gradient elution using a HPLC Column Poroshell 120 EC-C18 (3 x 100mm, 2.7µm in particle size) analytical column via an Agilent 6495 tandem quadrupole mass spectrometer coupled with an Agilent 1290 Infinity ultra-high performance liquid chromatograph. The aqueous and organic mobile phases used were 0.1% formic acid in water and 0.1% formic acid in acetonitrile, respectively.

This newly developed LC/MS/MS assay was capable of accurately identifying and quantifying 10 different ACH drugs, including two pairs of isomeric compounds, over a runtime of 13 minutes. Method validation is currently in progress, and authentic human blood samples will be analyzed upon completion of validation.

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Novel Psychoactive Substances; Postmortem; Arylcyclohexylamines

Y40 Investigating the Ability to Generate DNA Profiles From Swabs of the Body Surface Utilizing Rapid DNA Technology: A Pilot Study

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Learning Objectives: Attendees will learn the potential benefit of utilizing rapid DNA technology for identification purposes, using swabs of the body surface in particular. They will also learn about optimal areas on the body surface to collect samples for rapid DNA testing.

Impact Statement: This research contributes to the existing literature about the ideal location for generating single-source profiles with rapid DNA technology. This can be applied to multiple contexts, including but not limited to crime scenes and mass disaster scenarios, especially those where other means of identification (i.e., visual IDs and fingerprints) are not possible. In addition, there is also the potential for faster positive identifications to bring closure to families sooner.

Abstract Text: Rapid DNA analysis is a fully automated process of developing a DNA profile from a biological sample in 90 minutes. However, because the sample undergoes fewer Polymerase Chain Reaction (PCR) cycles, the sample must be high quality (i.e., contain large amounts of DNA) to get consistent results. Rapid DNA technology is typically used at police booking stations to quickly generate an arrestee's DNA profile. Recently, the use of rapid DNA has proved valuable in identifying human remains. For example, it has been used for the identification of human remains after mass casualty events. In these contexts, samples are usually taken via buccal swabs or from bones. The present study seeks to determine if it is possible to generate DNA profiles using rapid DNA technology by swabbing the skin surface of living individuals. If this proves to be successful, then there is a possibility that rapid DNA can generate a profile from the skin surface of deceased individuals. This will potentially broaden the application of rapid DNA technology to human identification at crime scenes, especially if buccal swabs, other body fluids, or fingerprints are unavailable for testing or if noninvasive sampling is required. Compared to traditional DNA analysis, rapid DNA technology has the potential to produce positive identifications faster.

Generation of a DNA profile from a skin surface swab can have varying levels of success even with traditional DNA analysis. Cells from the skin surface are less likely to be nucleated than those from the buccal region or other body fluids. Previous studies suggest the successful generation of a DNA profile is influenced by the place of collection, the tool of collection, and the extraction methods. This study utilized the inner elbow, inner thigh, and forehead. Participants swabbed each of these areas three times over three days. DNA extraction and amplification was accomplished using the GlobalFiler Express amplification kit on a RapidHIT ID system as per manufacturer's instructions. DNA profiles were interpreted using GeneMarker ThermoFisher HID software.

Each sample was evaluated for completeness and whether it was a single-source or mixed profile. There was little to no variability in profile completeness between body locations of individuals. However, in comparing the three body swab locations combining individuals, a Friedman's test and a One-Sample Kolmogorov Test indicated that there is a statistically significant difference in the completeness of the forehead samples compared to the inner elbow and inner thigh samples, with the forehead samples being more complete. Additionally, both tests demonstrated that there is a statistically significant difference in the percentage of mixed profiles of the inner thigh compared to the inner elbow and forehead, with the inner thigh samples being less mixed.

This study demonstrates that it is possible to obtain complete profiles from skin surface swabs using Rapid DNA technology, which in turn can facilitate positive identifications. The skin surface may be a viable swab site when blood or saliva is unavailable or noninvasive sampling is required or recommended. Preliminary results suggest the inner thigh might be the best area for avoiding mixed profiles, but the forehead appears to be the best for complete profiles. More testing across a larger sample size is needed to make a concrete recommendation and assess if that success can be replicated on deceased individuals.

Rapid DNA; Human Identification; Forensic DNA Analysis

Y41 The Development of a Quantitative Method for the Analysis of Illicit and Novel Benzodiazepines in Tablets Using Ultra-Performance Liquid Chromatography Coupled With Triple Quadrupole Mass Spectrometry

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Learning Objectives: After attending this presentation, attendees will be knowledgeable about the process of developing a targeted, quantitative method using a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) and the importance of updated method upkeep, particularly with classes of Novel Psychoactive Substances (NPS) such as illicit benzodiazepines.

Impact Statement: This presentation will contribute to the forensic science community by introducing a newly developed and validated method for the analysis of benzodiazepine in tablet matrices. The implementation of this method will enhance the ability to detect and quantify benzodiazepines in case samples, supporting both forensic and clinical investigations.

Abstract Text: Benzodiazepines, widely prescribed for the treatment of anxiety, seizures, insomnia, and alcohol withdrawal, are also frequently abused due to their central nervous system depressant effects, which include relaxation, sedation, and memory impairment.[1,2] With their growing prevalence in illicit markets, benzodiazepines pose an escalating public health threat when used on their own and in conjunction with other substances. When benzodiazepines are used in combination with other substances such as heroin and cocaine, they can enhance the sedative effects of these substances.[2] When used with stimulants and hallucinogens, they serve as an anxiolytic.[2] Increasingly, benzodiazepines have surfaced as NPS on the black, gray [unscheduled], and even legal markets, further complicating efforts to control their misuse and leading to significant legal and health concerns.[3]

This research focused on the development of a robust analytical method utilizing Ultra-Performance Liquid Chromatography (UPLC) coupled with triple Quadrupole (QqQ) Mass Spectrometry (MS) for the detection, quantification, and recovery of 17 illicit and novel benzodiazepines from tablet matrices. The method employed an Acquity I-Class UPLC system, Waters Xevo TQD mass spectrometer, and Intellistart and MassLynx software to achieve high sensitivity and specificity. MS detection was done in positive electrospray ionization mode for all analytes. The analytes included pyrazolam, clonazolam, clonazepam, flunitrazepam, flualprazolam, triazolam, etizolam, lorazepam, alprazolam, desalkylflurazepam, bromazolam, temazepam, flubromazepam, diclazepam, desalkylquazepam, desalkylgidazepam, and diazepam, with oxazepam used as the internal standard for quantification.

Given the dynamic nature of the drug market, which is characterized by the continuous emergence of NPS, forensic and clinical laboratories face significant challenges in adapting methods for the detection and quantification of these drugs. Timely updates and the development of new analytical methods are essential to keep pace with these evolving threats. Advancements of applied instrumental analysis, such as this research using LC/MS/MS, play a key role in addressing this need.

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Method Development; Liquid Chromatography; Benzodiazepines

Y42 DNA in Drugs: An Interdisciplinary Approach to Identify Drugs and the DNA Within

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Learning Objectives: After attending this presentation, attendees will learn about a novel technique utilizing an optimized extraction method for the dual identification of DNA and drug material from a single extract. They will learn how the different byproducts (layers) of an organic DNA extraction were used for drug identification via Gas Chromatograph/Mass Spectrometry (GC/MS).

Impact Statement: This research will impact the forensic community by introducing a novel approach to detect drugs and DNA from a single extract via the optimization of an extraction method for the simultaneous DNA profiling and drug identification utilizing Excedrin tablets. This approach maximizes the information obtained from a single extract, supporting comprehensive forensic investigations.

Abstract Text: Collaboration among forensic science experts is essential to advancing investigative capabilities. Merging disciplines like forensic biology and drug chemistry have enabled the development of DNA profiles from drug powders, paraphernalia, and packaging materials.[1-3] However, extracting DNA from drug powders poses challenges, such as inhibition on downstream DNA analysis due to residual drug.[4] An effective extraction method needs to be developed to address this issue. By optimizing an organic extraction method that preserves both biological and chemical compounds, dual identification of DNA and drug profiles is possible. Sequestering and analyzing often-discarded portions of the DNA organic extraction allows for the identification of drug substances within them via GC/MS analysis.

The Center for Forensic Science Research and Education (CFSRE) standard organic extraction protocol was assessed utilizing neat saliva. For Phase 1, a sensitivity study was conducted to determine the volume of saliva that provides sufficient DNA recovery. Deidentified male and female saliva were utilized and extracted in triplicate, with saliva volumes of 1µl, 2µl, 5µl, 10µl, 25µl, and 50µl. DNA was extracted following the CFSRE standard organic extraction protocol. Quantification was performed using the Investigator Quantiplex Pro RGQ kit on the Rotor-Gene Q. Results revealed low Degradation Index (DI) values for male and female extracts, with average DI values of 1.56-1.65 (male) and 1.13-1.48 (female). For 2µl of spiked saliva, the average concentration for male extracts was 3.023ng/µl, while female extracts had an average concentration of 0.59ng/µl. The optimal saliva amount for downstream method optimization was determined to be 2µl, which provided enough DNA yield of 0.5ng of DNA input for amplification with the Investigator 24plex.[6]

For Phase 2 of this study, 2µl saliva (one male and female donor) was spiked directly into a fully crushed Excedrin tablet. Variables tested to optimize the organic extraction method included: inclusion of a butanol purification step, incubation volume (1mL–7.5mL), and incubation temperature (37°C and 56°C). After overnight incubation, the crushed drug powder was added to a spin basket and centrifuged at 14,000 RPM for 5 minutes to maximize recovery of overnight lysate for the organic extraction. DNA extraction layers were preserved and analyzed to determine which layer(s) was best suited for identifying Excedrin's active ingredients (acetaminophen, caffeine, and aspirin-related compounds) via GC/MS. The organic layers, aqueous layer, and TE wash layers were subjected to acid-base extraction prior to analysis. Various dilutions were prepared to avoid overload on Agilent's 5975B GC/MS. Additionally, a 1:10 neat injection of the organic layers was also prepared for analysis. Results showed that the organic layers are best to identify Excedrin's active ingredients as they were detected in the extracted and neat layers. Results demonstrated that DNA extraction byproducts can be used for drug identification. Following protocol optimization, the optimized method will be used in Phase 3 to test mock case samples (Excedrin tablets hand-held by donors of various shedder statuses).

The results of this study may be used for the successful extraction of DNA from drug material in an innovative manner. These findings will offer the forensic community a novel approach to identifying drugs and DNA from drug material utilizing a single extract, maximizing information obtained. This method could aid investigators, such as DNA analysts and drug chemists, in processing casework involving drug material. By using a single sample for both DNA and drug analysis, it provides dual benefits, potentially linking drug seizures or other drug-related evidence through the identified drugs and DNA profiles.

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Forensic DNA analysis; Drug Chemistry; Interdisciplinary

Y43 LC/QqQ/MS Method Development and Validation for the Quantitative Analysis of Drugs and Their Metabolites in Postmortem Blood

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Learning Objectives: This presentation highlights an analytical method developed to investigate the effects of Postmortem Redistribution (PMR) on common prescription drugs and their metabolites in paired peripheral and central postmortem blood samples. Attendees will gain insight into the process of method development and validation and will develop a better understanding of PMR or drug metabolites.

Impact Statement: Currently, there is little information regarding the effects of PMR on metabolites and whether they differ from their associated parent drugs. This research aims to contribute to this area of study by presenting a Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) method that is able to identify and quantify drugs across multiple drug classes that are often found in postmortem toxicology cases, as well as their common and/or significant metabolites.

Abstract Text: PMR is a phenomenon that describes the redistribution of drugs into blood from solid organs such as the lungs, liver, and myocardium after death, with the liver and lungs being the primary contributors. PMR has a significant impact on postmortem toxicological analysis as the location from which blood samples are collected may affect the measured concentration of a drug, depending on the extent to which the drug is affected by PMR.[1-5] Cardiac or central blood concentrations of drugs susceptible to PMR are generally greater than concentrations found in peripheral blood due to the release of drugs over time into the central compartment from solid organs.[2] For drugs that undergo PMR, central blood can have 3-5 times higher drug concentrations than peripheral blood obtained at the same time.[6] Information about PMR is largely limited to discussions of parent drugs, but in many cases, drugs are quickly metabolized, leaving little to no parent drug and a significant concentration or variety of drug metabolites. Current research is not only limited by a lack of information on the impact of PMR on the common metabolites of major drugs of abuse, but also by small sample sizes. A lack of antemortem information further complicates studies of PMR.[2]

The overall aim of this research is to identify and quantify commonly encountered and forensically relevant drugs and their metabolites in paired postmortem blood samples in order to investigate the effects of PMR. Following method development and validation, authentic deidentified human peripheral and central blood samples will be analyzed and compared, the results of which will also be discussed depending on availability.

The LC/QqQ/MS method developed for this study separated and identified the drugs of interest by altering chromatographic conditions and tandem mass spectrometer parameters. Mobile Phase A (MPA) consisted of 5mM ammonium formate and Mobile Phase B (MPB) consisted of 0.1% formic acid in acetonitrile. Optimal cone voltage (V), collision energy (V), and product ions for each analyte of interest were determined via direct infusion. The two most relevant ions for each analyte were then used to develop a Multiple Reaction Monitoring (MRM) method, with the optimal dwell time used as determined by the instrument's software. Analytes of interest were ionized using positive Electrospray Ionization (ESI+) and detected via MRM.[7] Spiking solutions of analytes of interest were prepared from standard reference materials via serial dilution for calibration model analysis. Internal standards were prepared and added to each sample. Blood samples will be spiked with the prepared standard solutions containing the multiple analytes of interest and calibration curves were prepared and evaluated, ensuring accurate and reliable quantitation of known concentrations of the analytes of interest.[7] Method validation will be performed according to the standards set forth by the American Academy of Forensic Science (AAFS) Standards Board (ASB) Standard Practices for Method Validation in Forensic Toxicology.[8]

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Postmortem; Metabolite; Method Development

Y44 A 12-Month Study on the Storage Conditions of Ignitable Liquids on Carbon Strips Used for Forensic Fire Debris Analysis

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Learning Objectives: The purpose of this research presentation is to evaluate the storage conditions of carbon strips containing ignitable liquids over a 12-month time frame. Storage parameters are assessed by comparison to Gas Chromatograph/Mass Spectrometry (GC/MS) results of unstored carbon strips to determine the most effective conditions at reducing the loss of volatile compounds.

Impact Statement: The work detailed here will help facilitate guidelines for the long-term storage of the extractions of ignitable liquids found in fire debris. Furthermore, it will allow analysts to assess chromatograms of stored ignitable liquids with more information as to what changes might have occurred due to post-collection evaporative loss.

Abstract Text: Ignitable liquids are an important class of physical evidence because their presence in fire debris can point toward an intentionally set fire in potential arson cases. In the field, fire debris is collected in an airtight metal can and returned to the lab for the analysis and identification of ignitable liquids. The most common method of analysis, passive headspace extraction, utilizes a carbon strip suspended in the can on which volatiles present in the debris can evaporate and adsorb.[1] The volatiles are then extracted from the strip using a simple solvent extraction and analyzed by GC/MS. Due to the nature of forensic analyses, it is often necessary to store samples in the event that future testing is needed; however, the literature on best practices for storage is sparse. In one study on this topic, Baerncopf and Hutches assessed the reproducibility of chromatograms for gasoline and a heavy petroleum distillate as a function of storage time and storage container.[2] While the previous study validates the use of stored charcoal strips for future analysis, the study only varied the storage container type and only assessed storage at room temperature. The work presented here attempts to build on this work by including temperature as a parameter.

The main objective was to assess the practicality and viability of different storage conditions for the carbon strips on which the ignitable liquids have been adsorbed. Temperature, vial type, and storage duration were all factors under study. A Kimwipe placed in a metal fire debris can was spiked with gasoline, and a carbon strip was suspended in the can. The cans were placed in an oven overnight at 60°C to allow complete evaporation, after which the strips were removed and cut in half. The first half of the strip was analyzed immediately by GC/MS while the other half was stored under specified conditions and analyzed at a given time point, ranging from 2 weeks to 12 months. The chromatograms of the stored and unstored strips were compared to determine an optimal set of storage conditions. Preliminary results indicate that snap cap vials, as opposed to screw cap, are most sustainable at reducing the risk of loss of volatile compounds in ignitable liquids. Furthermore, refrigeration did not impact the chromatographic abundance of peaks stored for short and moderate durations of time. Additional results will demonstrate the impact of long-term storage (12 months) on the chromatograms of stored extracts. The culmination of this work will lead to guidelines for how best to store carbon strips utilized for forensic fire debris analysis.

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Fire Debris Analysis; GC/MS; Passive Headspace Extraction

Y45 An Analysis of Locally Purchased Δ8-Tetrahydrocannabinol Products Via Gas and Liquid Chromatography

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Learning Objectives: The objective of this poster is to present qualitative and quantitative data gathered during the analysis of commercial Δ8-Tetrahydrocannabinol (THC) products using Gas and Liquid Chromatography coupled with Mass Spectrometry (GC/MS and LC/MS). The chemical constituents of each commercial product were identified, and relevant cannabinoids of interest were quantified using an internal standard calibration curve.

Impact Statement: The work presented aims to inform the forensic science community of the constituents present in legal hemp-based products as well as to guide characterization methods for both the qualitative and quantitative analysis of various cannabinoids. Δ8-THC products were purchased in a variety of consumption types; however, the research in this poster will focus on preliminary work performed on hemp oils.

Abstract Text: Δ8-THC is an isomer of the psychoactive cannabinoid in marijuana, Δ9-THC. The 2018 United States Farm Bill legalized hemp products, defined as containing less than 0.3% Δ9-THC, at the federal level.[1] Immediately following the passage of this bill, both hemp and hemp-derived products experienced an increase in popularity. Because Δ8-THC can be synthesized from Cannabidiol (CBD), a naturally occurring cannabinoid in hemp, synthetic Δ8-THC products began to flood the market in states where marijuana remained illegal, including Tennessee. With a lack of oversight and regulation, the commercial Δ8-THC products contained many contaminants unknown to the consumer, including synthetic byproducts and other unlisted cannabinoids.[2] This led to an uptick in the number of calls to Poison Control concerning Δ8-THC products, as well as several reports of hospitalizations involving children who unknowingly consumed products marketed to look like candy or snacks. The goal of this project was to analyze locally purchased commercial Δ8-THC products sold in Tennessee to determine the presence and quantity of both cannabinoids and contaminants.

Initial work was conducted using GC/MS, where major constituents were identified and preliminarily quantified. Identifications were made utilizing a National Institute of Standards and Technology (NIST) Mass Spectral Library search. Δ8-THC was confirmed by retention time comparison to a standard. Several quantification methods for Δ8-THC were then compared, including an internal standard method and an external calibration curve. LC-MS was then utilized to specifically quantify cannabinoids present in the commercial Δ8-THC products. An internal standard calibration curve was used to find the weight percent of Δ8-THC, as well as Δ9-THC, CBD, and other cannabinoids identified.

The analysis of the commercial products via GC/MS found the presence of compounds expected for the type of product (i.e., gummies and tinctures) as well as other cannabinoids not advertised on the label. Several products were found to contain Δ9-THC, which will be subsequently quantified to verify that the concentration was less than the allowed 0.3%. The outcome of this project is to be able to inform consumers of the presence of cannabinoids and other contaminants in products being marketed as safe as well as to guide regulations towards a reliable testing method.

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Cannabinoids; GC/MS; LC/MS

Y46 An Investigation of DNA Contribution From Trace Samples Recovered From a Hammer Used in a Simulated Assault Scenario

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Learning Objectives: While attending this presentation, attendees will learn the importance of trace DNA investigations in modern forensic science in blunt force scenarios. Attendees will learn how handling time and sampling time may affect the presence of trace DNA on a weapon.

Impact Statement: This presentation will impact the forensic science community by filling in specific gaps in current literature regarding trace DNA and its recovery from the handle of a weapon. It will offer better insight on the effects of sampling and handling time, as well as surface porosity, on the persistence and recoverability of trace DNA.

Abstract Text: Trace DNA is invisible, or latent, biological material left behind due to a person coming into direct contact with an object or another individual, oftentimes leaving behind skin cells and/or sweat. This term encompasses another form of DNA called "touch DNA." Touch DNA specifically refers to the DNA left behind due to a "touch," or the physical contact made between source and the surface.[1] This is the kind of DNA that would be present after the handling of a weapon and can be extremely useful in a criminal investigation to assist in identification of suspects and victims using DNA profiling. This DNA is commonly found all over a crime scene, particularly if it has not been thoroughly cleaned—Locard Exchange Principle states that "every contact leaves a trace."[2] There are several aspects of DNA evidence that can impact its utility in a criminal investigation, such as background DNA and surface porosity of substrate.[3,4] Background DNA can interfere with the profile recovered: this type of DNA, from an unknown source, can come from primary or secondary transfer.[3] A more porous surface will hold DNA better, making it more difficult to recover the DNA from it.[4] These inherent characteristics of DNA evidence affect the amount of DNA recovered in a criminal investigation. With all these factors, it is important to consider the sampling method to optimize the amount of DNA recovered.

Current literature about the factors impacting DNA persistence and recovery produced conflicting results, and there is a lack of investigations into blunt force scenarios. To investigate how these factors may impact trace DNA persistence on a weapon, two simulations were designed to mimic a blunt force scenario using different hammers, contact, and sampling times. The two different types of simulations include a participant swinging two different hammers either one singular time (Simulation 1) or ten times (Simulation 2). The selected hammers have two different handle types, wood and fiberglass, to investigate how porosity affects the persistence of DNA. These simulations were performed several times each to investigate the impact on sampling time—the hammers were sampled after 6, 18, 24, and 48 hours. Sampling was conducted using the double swab method. The DNA samples are extracted with the QIAshredder/QIAamp following the DNA Investigator protocol, then quantified with the Trio Quantification Kit, Polymerase Chain Reaction (PCR) amplified with the GlobalFiler Amplification Kit, and amplicons separated on the SeqStudio Genetic Analyzer prior to Short Tandem Repeat (STR) profile analysis using GeneMapper ID-X software. Through preliminary analysis, it has been determined that the amount of DNA recovered ranged from 0.006ng to 0.526ng. Preliminary tests indicate that a singular swing of the hammer can produce enough DNA yield to generate an interpretable STR profile. The ten swings—Simulation 2—produced full and interpretable STR profiles. All simulations have produced both single source and mixed STR profiles. For mixed samples, the participant appeared to be the major contributor to the STR profile, but background DNA has also been observed and likely indirectly deposited by the participant. Further simulations are ongoing to generate a large dataset which may be used to gain more insights and knowledge on the transfer, persistence, prevalence, and recovery of DNA in a simulated assault scenario.

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Forensic DNA; STRs; Assault Scenario

Y47 The People Who Do Forensic Anthropology: Inferences From Interviews With Ten Forensic Anthropologists

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Learning Objectives: After attending this presentation, attendees will gain insights into the realities of pursuing a forensic anthropology career through interviews with ten professionals, providing clarity on educational pathways, emotional demands, and professional expectations, along with practical advice for success in this competitive field.

Impact Statement: This presentation will impact the forensic science community by contributing to the conversation on the development of a standard curriculum in Forensic Anthropology.

Abstract Text: As portrayed in popular media, forensic anthropology often presents a different reality than what practitioners face. Aspiring forensic anthropologists may have visions of glamorous and high-tech environments. Yet, the field is rooted in service to law enforcement and the community through specialized knowledge of human skeletal remains in the unglamorous field, laboratory, or medicolegal settings and has lacked a standardized curriculum.[1,2]

Forensic anthropologists work in academic, medicolegal, and military settings, and as the field evolves, forensic anthropologists would benefit from developing a standardized curriculum that incorporates best practices. Standardization is essential for ensuring consistency in methodologies and enhancing the credibility of forensic findings in medicolegal contexts. Additionally, it needs to address the lack of comprehensive information available to students seeking this career path. Without a standardized curriculum, different programs teach varying content, resulting in significant knowledge gaps and inconsistent or incomplete understanding of critical aspects of the field such as skeletal analysis techniques, ethical considerations, and forensic procedures. This variability means that students from different institutions may graduate with vastly different skill sets, lacking exposure to certain best practices or areas of expertise. As a result, aspiring forensic anthropologists face uncertainty regarding expected educational requirements, the possible duration of training, and the realities of the career. Ultimately, this knowledge gap can hinder career preparedness and weaken the overall development of future professionals in the field.

Interviews with ten forensic anthropologists, including professionals from academia, the military, and medical examiner offices, were conducted. This approach was employed to gain a better understanding of the educational requirements, practical experience, professional expectations, and possible career paths for students considering a career in forensic anthropology. This presentation draws four key conclusions about what it means to pursue a career in forensic anthropology in any setting.

First, every forensic anthropologist interviewed emphasized the impact of their work, whether in shaping the next generation of forensic professionals through teaching or helping communities by restoring the identities of the deceased. Forensic anthropology, in every setting, is an act of service to others. Second, flexibility is essential in forensic anthropology. The professionals interviewed stressed that no two days are alike concerning daily activities. Third, hands-on experience, such as internships, is crucial for advancing in forensic anthropology. Finally, the emotional toll of forensic anthropology cases should not be underestimated. Interviewees shared the psychological challenges of working with trauma, death, and the emotional weight of interacting with grieving families.

These findings highlight critical topics that should be integrated into a standardized forensic anthropology curriculum, ensuring that students are academically prepared and equipped with the practical skills and emotional resilience required for this demanding career. This presentation advocates for the development of such a curriculum, providing aspiring forensic anthropologists with the comprehensive knowledge and tools necessary to successfully navigate their career paths.

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Forensic Anthropology; Career Pathways; Professional Development

Y48 Mapping Mortality: A Decade's Review of Drug Usage in Maryland (2014–2024)

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Learning Objectives: This presentation will highlight the prevalence and patterned trends of illicit substance use in Maryland over a decade's time, including historical and emerging trends of concern, possible adulterants, and concurrent usage between substances. This information can be of use in public health initiatives that contribute to evidence-based recommendations for improving health outcomes in Maryland.

Impact Statement: This presentation will also examine how drug use trends vary across demographics and location, as well as the impact of new emerging drugs on the State's drug landscape.

Abstract Text: **Introduction:** Over the past decade, synthetic opioids (and their derivatives) have increasingly infiltrated the drug market, resulting in a significant rise in overdose deaths. Often mixed with other drugs, either intentionally or unknowingly, these adulterants have dramatically enhanced the risk of fatal overdoses. In this study, we examined the epidemiological and toxicological data obtained from autopsies performed by the Maryland Office of the Chief Medical Examiner (OCME) from 2014 to 2023. Our goal was to provide a comprehensive survey surrounding the prevalence and patterns of drug usage and possible adulterants over the first ten years of the current opioid epidemic in order to highlight the critical need for targeted strategies in mitigating the devastating impacts on public health.

Methods: Data was obtained from the Maryland OCME database by reviewing all deaths where the cause of death was classified as substance use-related following examination between 2014 and 2024. The dataset was further evaluated by the different types of illicit substances that were reported on the death certificate. Cases included all demographic categories, encompassing the Statewide jurisdiction of the Maryland OCME. The percent of total cases which underwent examination at the OCME and were certified as a substance use fatality was approximately 25% in 2014 and 2015, with a sharp increase noted to approximately 34% of total examination cases being substance use-related in 2016. A peak was noted in 2020 and 2021, with approximately 39% of total examination cases being certified as substance use-related.

Comprehensive drug testing included volatiles analysis, an acidic neutral drug screen for antiepileptics and barbiturates, an alkaline drug screen for therapeutic and abused drugs, and an Enzyme-Linked Immuno-Sorbent Assay (ELISA) screen for morphine, benzodiazepines, and oxymorphone. The scope of the comprehensive screen remained consistent throughout the study duration. Any additional confirmatory procedures that were added during the study time period were for analytes that were already detected by the initial screening procedures.

Results: Between 2014 and 2024, there were approximately 57,000 autopsies performed (96.22 per 100,000 population) at the OCME for the state of Maryland. Over this period, each year's averaged increases of prevalence of the following illicit substances, as reported on death certificates, were: fentanyl, 196%; cocaine, 109%; methamphetamine, 8.3%; and phencyclidine, 1.9%. An increase in polysubstance use was also revealed, with an annual average increase of 7.4% in concurrent fentanyl and cocaine usage. The data will be extended through 2024 at the conclusion of the year.

Discussion and Conclusion: As highlighted by our study, the rising trends in illicit drug use, particularly the increasing prevalence of polysubstance misuse, poses a grave threat to public health. Over recent years, there has been a troubling escalation in the concurrent use of substances (such as fentanyl and cocaine). This surge in polysubstance use exacerbates the already severe consequences of drug use and misuse, including the heightened risks of overdose, the intensified physical health issues, and the increased strain on health care systems. The additive or synergistic effects of combining drugs often lead to more complex and challenging health crises, making treatment and intervention more difficult. Furthermore, these trends contribute to an immense societal burden, which manifests themselves as increased health care costs, lost economic productivity, and an increasingly overwhelming demand for emergency and rehabilitation services. Addressing this issue requires a multifaceted and multidisciplinary approach, including continued evaluation and recognition of the data trends, enhanced prevention strategies, improved access to treatment, and robust public health initiatives to mitigate the devastating impacts on individuals and communities alike.

Opioids; Drug Abuse; Novel Synthetic Opioids

Y49 Forensic Analysis of PDFs: The Role of Peritus in Standardization and Reproducibility

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Learning Objectives: The goal of this presentation is to train forensic analysts in the use of the Peritus Framework for digital document analysis, covering all stages of the process from data extraction to the validation of the chain of custody. Analysts should be able to efficiently apply the tools provided by the platform, ensuring the integrity and auditability of the results.

Impact Statement: The implementation of the Peritus Framework in digital forensic analysis has the potential to unify forensic procedures in Brazil and worldwide by integrating various specialized tools within a single platform. This provides greater efficiency, standardization, and auditability of processes, ensuring that the chain of custody is maintained and easily audited, thus reinforcing the reliability of digital evidence in forensic investigations.

Abstract Text: **Introduction:** In Brazil, forensic analysis of digital documents involves the use of various software for document analysis and authentication. The Peritus system, developed by the Federal Police, compiles the main programs needed for conducting digital forensics. Created to handle the increasing volume of multimedia evidence and mitigate the lack of uniformity in forensic procedures, it standardizes processes, improves analysis quality, and enhances the auditability and reproducibility of results, while ensuring the integrity of digital evidence.[1]

Objectives: The aim of this article is to verify whether the Peritus system replaces other programs in forensic analysis and to highlight its effectiveness in consolidating tools into a single framework. Additionally, it shows how the system ensures the chain of custody and its auditability.

Methodology: The study followed a descriptive and qualitative approach, which was divided into three stages:

1. Data Import and Extraction: PDF files were imported into Peritus, where the Hash-256, metadata, images, and typographic fonts were extracted. Reports on file size were also generated for forensic analysis.
2. Image and Content Analysis: After extraction, the images were analyzed using filters such as binarization, distortion removal, brightness and contrast adjustments, and the CLAHE filter, allowing for the detection of manipulations.
3. Chain of Custody and Derivation Graph: The chain of custody was recorded in a derivation graph, documenting all actions taken, and ensuring auditability, reproducibility, and transparency throughout the process.

Results and Discussions: The application of the Peritus Framework has proven effective in analyzing PDF files, providing information such as the SHA-256 hash, which preserves the integrity of the file. Metadata, images, fonts, and previous versions are extracted in an integrated manner, eliminating the need for various programs such as PDF Gears, 010 Editor, ImageJ, and JPEG Snoop. The Peritus Framework integrates robust features, such as the Image Analyser, which works alongside the Workspace and native tools like the hexadecimal viewer and metadata reader, optimizing image analysis and authentication. This integration allows analysts to perform checks on geometry and lighting consistency, noise residues, and forgery detection.

As described, the Peritus Framework was developed to increase efficiency and uniformity in multimedia forensic investigations in Brazil, automating processes and ensuring a complete chain of custody record through a derivation graph that documents all stages of the process.[1] However, they also point out some limitations, such as the need for specialized training and the time required for teams to adapt to the system. Additionally, they suggest improvements to the user interface to facilitate broader adoption of the platform and highlight the challenges of integrating with other forensic systems that do not use Peritus, especially in different jurisdictions.

Conclusion:
The Peritus Framework has proven to be an essential tool for modernizing digital forensic analysis, integrating various features into a single platform that provides greater control, auditability, and efficiency. The application of advanced filters ensures a detailed and accurate investigation, facilitating the detection of manipulations and ensuring the integrity of the chain of custody. While the system has shown great potential, challenges remain, such as the need for specialized training for analysts and the time required to adapt to the new system. Furthermore, improvements to the user interface and integration with other forensic systems are recommended to facilitate broader platform adoption. With these improvements, Peritus has the potential to become an international standard in digital forensic analysis, reinforcing its importance in the field of forensic science.

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Document Analysis; Forensic Analysis; Chain of Evidence

Y50 Echoes of a Shotgun: Unraveling the Forensic and Clinical Mystery of Multiple Gunshot Wounds in a 26-Year-Old Male

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Learning Objectives: The learning objectives of this case involve recognizing and accurately documenting the characteristics of gunshot wounds, such as wound size, depth, and the presence of contusion collars, while also noting the absence of close-range firing signs like burning, singeing, blackening, and tattooing. Additionally, the case emphasizes the importance of preserving clothing for forensic analysis, particularly gunpowder residue testing, to support the forensic investigation. The role of advanced imaging techniques, such as Computed Tomography (CT) angiography scans, is highlighted for detecting the presence of shotgun pellets and aiding in medical management. Furthermore, the case illustrates the principles of conservative wound care and stresses the necessity of follow-up to monitor healing and address complications. Lastly, it underscores the significance of interdisciplinary collaboration between clinical teams and forensic experts to ensure comprehensive care and accurate forensic assessment in gunshot injury cases.

Impact Statement: Presenting this case report will have a significant impact on the forensic community by underscoring the critical importance of meticulous clinical documentation and forensic examination in cases involving multiple gunshot injuries. It highlights the value of thorough wound assessment, advanced imaging, and gunshot residue analysis in bridging clinical care and forensic investigation. Additionally, this case reinforces the necessity for collaboration between health care providers and forensic experts to ensure accurate case interpretation, legal clarity, and improved standards of practice in managing firearm-related injuries. The insights from this case will contribute to refining forensic protocols and improving outcomes in both medical and legal contexts.

Abstract Text: A 26-year-old male was brought to the Emergency Department after sustaining multiple gunshot injuries. Upon examination, multiple round-shaped firearm entry wounds, ranging from 6mm to 8mm in diameter, were observed on the left thigh, extending from the upper thigh to the knee. The wounds were muscle-deep with fresh clotted blood and a contusion collar approximately 0.2cm wide around each entry site. No signs of close-range firing, such as burning, singeing, blackening, and tattooing, were present. The patient's clothing, including a shirt, banyan, and trousers, showed corresponding defects with dried blood but no visible soot, gunpowder, or burn marks. The clothing was preserved and sent to the Forensic Science Laboratory (FSL) for gunpowder residue analysis. Additionally, a single round-shaped firearm entry wound, 8mm in size, was located on the abdomen, 12cm above the Anterior Superior Iliac Spine (ASIS) and 11cm left lateral to the midline. The wound was query cavity-deep with fresh clotted blood and a contusion collar, without signs of burning or blackening. His T-shirt also showed corresponding defects and was sent for forensic examination. Three to five round-shaped firearm entry wounds, ranging from 6mm to 8mm in diameter, were also found on the lower and lateral part of the left middle finger, bone-deep, with similar clotted blood and a contusion collar. X-ray and CT imaging confirmed the presence of shotgun pellets in the affected areas.

The patient was managed conservatively with wound care and was advised to follow up for further evaluation. A follow up was also done again after three months and radiological investigations were repeated.

This case also underscores the importance of meticulous clinical and forensic examination in gunshot injury cases, emphasizing the role of gunshot residue analysis and advanced imaging in both medical management and forensic investigation.

Firearms; Gunshot Residue; Forensic Imaging

Y51 An Assessment of Postmortem Interval by Analyzing Cellular Changes in Bone Marrow in Autopsy Cases: An Observational Study

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Learning Objectives: The learning objectives of this study are to understand the significance of Postmortem Interval (PMI) in forensic investigations and the limitations of traditional methods for its estimation. Learners will explore the potential of bone marrow, particularly from the sternum, as a reliable tissue for PMI estimation due to its resistance to postmortem degradation. The methodology for collecting and analyzing bone marrow aspirates, including the use of Giemsa stain and microscopic evaluation of cellular changes such as cell count, morphology, autolysis, and depletion, will be covered. This study aims to highlight bone marrow examination as a promising tool for improving the accuracy of PMI estimation in forensic practice.

Impact Statement: This study provides a significant advancement in forensic science by exploring bone marrow as a reliable tissue for PMI estimation, addressing the limitations of traditional methods. By demonstrating the resilience of bone marrow to postmortem degradation and its potential for more accurate cellular analysis, this research offers a novel approach to improving PMI determination in medicolegal autopsies. The findings have the potential to enhance the precision of forensic investigations, contributing to more accurate criminal and civil case resolutions. Furthermore, the study underscores the importance of ethical considerations in forensic research and could inspire future investigations into alternative tissues for PMI estimation, ultimately improving forensic practices and judicial outcomes.

Abstract Text: The determination of the PMI during medicolegal autopsies remains a critical but challenging task for forensic experts. PMI estimation is essential in resolving civil and criminal cases, as it helps establish the time of death, verify witness statements, and narrow down suspects.[1-3] Traditional methods of assessing PMI, including physical, metabolic, and physicochemical changes in the body, offer limited accuracy due to their sensitivity to environmental factors.[4,5] Additionally, as decomposition progresses, many of these methods lose their reliability, further complicating the estimation process.[5-7]

In this study, we explored the potential of using cellular changes in bone marrow as an alternative method for estimating PMI. Bone marrow, especially from the sternum, exhibits less degradation compared to other organs, even in advanced stages of decomposition. Its resistance to postmortem changes makes it a suitable tissue for analysis in forensic autopsies. Furthermore, the histological and immunological properties of bone marrow allow for better preservation of cellular morphology, offering a more reliable indicator for PMI estimation.

This observational study was conducted in the Department of Forensic Medicine & Toxicology and Pathology at All India Institute of Medical Sciences (AIIMS), Bathinda. After obtaining approval from the Institutional Ethics Committee, bone marrow aspirates were collected from the sternum of deceased individuals brought in for medicolegal autopsies. A total of 34 cases, where the time of death was known and consent for participation was obtained, were included in the study. Neoplastic conditions, pathological bone diseases, traumatic injuries to the sternum, and cases with unknown PMI were excluded. Bone marrow smears were stained using Giemsa stain and analyzed microscopically for cellular changes, including cell count, morphology, autolysis, and depletion.

Statistical analysis was performed using SPSS version 21.0. Continuous variables were expressed as mean and standard deviation, while categorical data were analyzed using Chi-square or Fisher's exact test. A p-value of less than 0.05 was considered statistically significant.

The study findings suggest that cellular changes in bone marrow can provide valuable insights for estimating PMI, particularly in cases where traditional methods prove unreliable. Bone marrow examination offers a promising avenue for improving the accuracy of PMI assessment in forensic practice.

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Postmortem Interval; Bone Marrow; Autopsy

Y52 The Extraction, Amplification, and Analysis of Human DNA Recovered From Carrion Larvae and Adult Flies (*Sarcophaga Bullata*)

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Learning Objectives: Attendees will: (1) discover the extraction protocol regarding the isolation of human DNA from post-feeding insect remains, (2) evaluate the different quantitative and qualitative results of human DNA recovered from adult flies verses the larvae stage of the fly life cycle, and (3) learn the application of the extraction and analysis of DNA recovered and how this can be applied to crime scene investigations along with the various forensic disciplines.

Impact Statement: This research aims to discuss the success rate of extraction and genomic analysis of human DNA from blood flies and their larvae in order to further the development of the forensic science field in identifying unknown human remains and broadening the knowledge of various methodologies that recover human DNA from crime scenes.

Abstract Text: When viable DNA samples cannot be recovered due to environmental and taphonomic conditions of the remains, investigators must rely on non-traditional means of DNA collection.[1] Forensic Entomology plays a crucial role in determining accumulated degree hour, assisting in time of death estimations.[2] However, few studies have combined DNA analysis and entomology. Detritivores ingesting human tissue introduces a new possibility to obtain human DNA from a crime scene. There have been statements pertaining to the relevance of using the larvae and flies for extraction when the body is present. There are numerous studies that indicate that these methods could be used in order to identify suspects and victims at a scene where the quality of evidence is damaged or nonexistent.[1,3]

The purpose of this research was to determine if human DNA can be extracted from carrion flies and their larvae (*Sarcophaga bullata*). Flies and maggots were raised and fed chicken and non-pathogenic human blood, then crushed in a sterilized mortem and pestle that homogenized both subjects' DNA. The human DNA was extracted using PrepFiler BTA Forensic DNA Extraction Kit and amplified through the use of human primers to isolate the Single Nucleotide Polymorphisms (SNPs) of the fractionated human genome apart from the fly genome. The flies and larvae were tested at the different Instar stages to determine if recovery is more successful in larvae or adult flies. Previous research has validated the success of human and model organism DNA extraction from various species of fly larvae; however, this research will bring a new perspective in the analysis of both adults and larvae.[3] The comparison of recovery and concentration of double-stranded DNA via the NanoDrop and SNPs via the genetic analyzer confirm the successful extraction of the DNA and its validation for application within the forensic community. Preliminary results show that human DNA can be extracted, but further research is warranted to determine the quantity and quality.

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Fly Larvae; Forensic DNA; Extraction

Y53 CODIS-Eligible DNA Collection From Sexual Assault Victims

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Learning Objectives: This poster presentation will provide quantitative data comparing the Combined DNA Index System (CODIS) -eligible DNA profile yields between swabs collected from the cervix with use of a speculum vs. "blind" vaginal swabs collected during sexual assault medical forensic exams.

Impact Statement: The aim of this project was to identify whether the use of a speculum to visualize and collect swabs from the cervix results in higher proportions of CODIS-eligible DNA profiles than swabs collected without a speculum or without visualizing the cervix. Through this information, medical staff will be able to properly inform their patients and potentially minimize post-assault trauma that may occur when being examined with a speculum.

Background: During a sexual assault examination, the best practice is to provide patients with as much control as possible of the medical procedures that are performed during the exam and has the option to consent or withdraw from certain collection methods. Among these procedures, for women specifically, a speculum may or may not be utilized to visualize the cervix to assess any injuries and accurately collect potential DNA evidence from the perpetrator. However, some patients prefer to opt out of this procedure due to trauma or pain from the assault. If the victim opts against the speculum procedure, the medical personnel will collect an evidence swab "blindly," without visualization of the cervix. Although there continues to be substantial research being done regarding sexual assaults and the victim experiences when going through a speculum exam post-assault, there is hardly any evidence pertaining to the effectiveness, or lack thereof, of collecting evidence swabs using a speculum to visualize the cervix. Due to this gap in research, medical staff cannot properly educate their patients to make an informed decision. Additionally, DNA linking a suspect to a crime may be considered one of the most valuable pieces of evidence when prosecuting sexual assault cases.

Methods: Data were from a clinic-based forensic nursing program in a large metropolitan city in Texas from January 1, 2020, to December 31, 2023. Exam information was matched with Texas Department of Public Safety (DPS) data on whether a CODIS-eligible DNA profile was obtained. Data were sorted into four groups for comparison: visualization of the cervix within 24hrs of assault, no visualization of the cervix within 24hrs of assault, visualization of the cervix past 24hrs of assault, and no visualization of the cervix past 24hrs of the assault. Proportions were compared between groups.

Results: Analysis of the data reveals that 47.8% of patients who had their cervix visualized yielded CODIS-eligible DNA, and 51.1% of patients who had not had their cervix visualized yielded CODIS-eligible DNA. Additionally, 65.6% of patients examined within 24 hours of the sexual assault yielded CODIS-eligible DNA, and 44.7% of patients examined greater than 24 hours after the sexual assault yielded CODIS-eligible DNA. Preliminary results indicated there were no differences in proportions of CODIS-eligible DNA profiles from samples in which the cervix was visualized vs. "blind" vaginal samples comparing unweighted proportions. Future analyses will use weighted proportions and compare them for statistical significance. Additional research is needed to validate this finding.

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Forensic DNA; Sexual Crimes; Examination

Y54 Age Dynamics in the Violence Reporting and Examination Process for Female Victims in Santa Catarina, Brazil (2021–2024)

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Learning Objectives: Attendees will learn about the quantification and analysis of female victims of violence in Santa Catarina, Brazil, from 2021 to the first half of 2024. The presentation will cover the rate of victims' attendance to forensic examinations by age group and examine the age profile at different stages of the violence reporting and examination process.

Impact Statement: This presentation will enhance the forensic science community's understanding of victim engagement with forensic processes. By identifying age-related trends in attendance rates and victim profiles, forensic professionals can better tailor their approach to improve victim cooperation and outcomes, ultimately leading to more effective prosecution and prevention of violence against women.

Abstract Text: Violence against women is a pervasive issue with significant societal implications. This study focuses on the state of Santa Catarina, Brazil, analyzing data from 2021 to the first half of 2024 to quantify female victims at various stages of the violence reporting and forensic examination process. The stages examined include: (1) initial reporting via phone, (2) formal police report filing (BO), (3) forensic examination attendance, and (4) femicide investigations.[1] The data sources vary, with phone reports obtained from the federal government's open data and police and forensic data sourced from the Scientific Police of Santa Catarina.[1,2]

The primary objectives of this study are to determine the rate of attendance at forensic examinations by age group and to establish the age profile of victims at each stage of the reporting and examination process. The attendance rate is calculated by comparing the number of police reports filed for bodily injuries and sexual crimes to the number of corresponding forensic examinations conducted. During the study period, the overall attendance rate was 46%, with 159,935 police reports filed and 73,062 forensic examinations completed. Notably, this rate varies by age group: victims under 10 years old attended forensic examinations 73% of the time, those aged 10-17 years attended 60% of the time, and young adults aged 18-19 years had the lowest attendance rate at 37%. Beyond 18 years, the attendance rate increases linearly with age, reaching 50% for victims aged 74 years. This trend suggests that younger victims, particularly those accompanied by guardians, are more likely to attend forensic examinations, while young adults are less likely to do so. However, the attendance rate improves with age and experience, albeit remaining below 50% for most adult age groups.

The age profile of victims varies across different stages of the reporting and examination process. The stages of police report filing and forensic examination attendance show two peaks in victim age groups: 11-17 years (approximately 10% of victims) and 20-24 years (approximately 13% of victims). After 25 years, the proportion of victims decreases, with 10% aged 40-44 years and only 1% aged 70-74 years. These trends are consistent between police report filings and forensic examinations, with minor differences, such as a higher attendance rate among children and a lower attendance rate among women aged 20-24 years. In contrast, the stages of phone reporting and femicide investigations show distinct age-related patterns. Phone reports are more concentrated among older women aged 30-59 years, accounting for 74% of all reports. Femicide victims, however, are predominantly aged 20-44 years, with 70% of cases occurring in this age group, peaking at 21% for victims aged 40-44 years.

This study underscores the importance of understanding the age-related dynamics of victim engagement in the forensic process. By highlighting disparities in attendance rates and age profiles at different stages, the findings can inform targeted interventions to improve victim support and participation in forensic examinations, thereby enhancing the effectiveness of the criminal justice response to violence against women.

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Violence Against Women; Forensic Analysis; Victim Age Profile

Y55 The Early Identification of Respiratory Illnesses in Infants Through Medicolegal Death Investigative Practices

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Learning Objectives: This research will examine the existing policies and procedures of various pediatric care organizations in Delaware County, PA. The data reviewed will include an analysis of medical records of children who have died from respiratory illnesses within the county, alongside interviews with health care professionals and surveys completed by staff from health care facilities and the medical examiner's office. These sources will help highlight current practices and identify areas for improvement to reduce mortality rates among children.

Impact Statement: The early detection of respiratory illnesses in children and infants underscores the crucial role of preventive care in reducing mortality rates within this demographic. This project aims to enhance the identification and management of respiratory illnesses in infants and children through the lens of medicolegal death investigation practices to decrease mortality rates from respiratory-related cases in Delaware County, PA.

Abstract Text: Respiratory illnesses remain a significant cause of morbidity and mortality in infants and children. This research aims to enhance the early identification of respiratory illnesses within medicolegal death investigations in Delaware County, Pennsylvania, to reduce pediatric respiratory-related mortality. A retrospective review of case logs from 2019 to 2024 at the Delaware County Office of the Medical Examiner included data from autopsy reports, medical records, immunization and discharge records, and interviews with healthcare professionals. Findings revealed that rhinovirus (HRV) and enterovirus (ENT) were the most frequently detected pathogens, particularly in infants aged 4–5 months, while influenza A, influenza B, and respiratory syncytial virus (RSV) were absent. HRV/ENT infections were associated with more severe disease than RSV or influenza and were often linked to significant comorbidities. These findings emphasize the importance of considering HRV/ENT in cases of severe acute respiratory infections and highlight the need for developing specific antiviral treatments.[1]

Recent advancements within the Delaware County Medical Examiner's Office, including expanded staff, increased funding, and enhanced investigative protocols, have significantly improved the accuracy of respiratory illness investigations in pediatrics. The implementation of expanded respiratory panel testing rose from 63% (2019–2022) to 100% (2023–2024), improving pathogen detection and contributing to more precise determinations of cause of death. These advancements also identified critical gaps in community care practices, including prenatal and pediatric care coordination, adherence to immunization schedules, and continuity of care between healthcare providers and families.

Through sustained public health education, improved access to healthcare resources, and strengthened collaboration among stakeholders, Delaware County aims to reduce infant mortality and improve pediatric outcomes. This study highlights the critical role of medicolegal investigations in identifying factors contributing to respiratory-related deaths and advancing preventative measures.

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Education; Prevention; Infant Death

Y56 Analyzing the Photo-Oxidation Effects of UV on the Physical Structure and Optical Properties of Synthetic Fibers

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Learning Objectives: After attending this presentation, attendees will understand the general scheme for fiber analysis and its complications. Attendees will also learn some physical and optical characteristics of synthetic fibers that have undergone UV photo-oxidation, and how these can be identified through various microscopy techniques.

Impact Statement: This presentation will impact the forensic science community by introducing data that support prior research on UV fiber degradation and present a visual guide of some observable physical and optical changes that synthetic fibers can undergo after extensive durations of UV exposure. It is suggested that research on fiber analysis, specifically synthetic fibers, should be expanded to include the interactions between fiber and dye, other instrumental techniques, and the use of chemometrics.

Abstract Text: Traditional fiber analysis involves a schematic approach using microscopic and spectroscopic techniques that examine fiber and dye pigment compositions. Common analysis conclusions include the association of a questioned fiber to a known fiber, disassociation, and indeterminate results. However, fiber samples found at a crime scene can be subjected to a variety of external factors that contribute to its degradation, and thus, alteration from otherwise being recognized as associated to a collected known sample. One such factor is the exposure to ultraviolet (UV) radiation, which is readily available naturally and easily obtainable commercially. Previous studies have dissected the chemical reactions that underlie UV fiber degradation and outlined various spectral and optical changes that occur. In our research, we provide data characterizing these changes in a more visual approach, specifically focusing on samples of synthetic fiber, which has been less explored.

Our study operated under three distinct conditions: a controlled setting of UV-C light exposure to simulate fiber degradation resulting from isolated high-intensity UV radiation; a controlled setting of indoor grow light exposure to simulate fiber degradation resulting from isolated natural sunlight with a wider range of UV, visible, and infrared wavelengths; and an outdoor, exposed environment in suburban Virginia to simulate fiber degradation affected by UV and a combination of other external factors, similar to what would happen at an outdoor crime scene. Common dark and light synthetic fiber samples of acrylic, polyamide (nylon), and polyester were exposed to the three conditions and periodically collected after 2, 4, 6, 8, and 24 weeks for analysis. Traditional forensic fiber analysis techniques of compound light microscopy (brightfield and darkfield) and polarized light microscopy were used to document and categorize changes in the physical structure and optical properties of the fibers based on their exposure condition and duration. Notable characteristics included various fracture patterns, pigment fading, yellowing, and retardation changes.

Fibers; Statistics; Trace Analysis

Y57 A Comparison of Confiscated Vaping Products From Virginia Schools From 2019–2020 to 2022–2024

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Learning Objectives: Attendees will understand the vaping devices that are most prevalent among middle and high school students in the Commonwealth of Virginia.

Impact Statement: This presentation will impact the forensic science community by increasing their awareness of drug delivery systems (electronic cigarettes) used by middle and high school students, the evolution of these systems, and drugs other than nicotine used by students.

Abstract Text: **Background:** United States Food and Drug Administration (FDA) regulations prohibit the sale of electronic cigarettes (e-cigs) to consumers <21 years old. In 2020, the FDA prohibited the sale of flavors other than menthol and tobacco in pod-based products to deter underage use. Between 2020 and 2024, e-cig use by high school students decreased from 19.6% to 7.8%; middle school student use decreased from 4.7% to 3.5%.[1,2]

Objectives: To evaluate and analyze confiscated e-cigarettes as part of a program to address e-cigarette use in public schools in the Commonwealth of Virginia.

Methods: E-cigs were confiscated from students (2019 to 2020, 2022 to 2024), and 775 e-cigs were submitted to the Laboratory of Forensic Toxicology Research (LFTR) at Virginia Commonwealth University. E-cig liquids (e-liquids) were analyzed using dual column Headspace/Gas Chromatography/Dual Flame Ionization Detection (HS/GC/FID), Gas Chromatography/Mass Spectrometry (GC/MS), and Liquid Chromatography/Tandem Mass spectrometry (LC/MS/MS).

Results: For the 2019-2020 confiscation period, NJoy "Ace" was the most prevalent product (37.7%), followed by Puff Bar disposable types (17.9%); JUUL (16.6%), Vuse "Alto," "Solo," and "Vibe" (9.3%); Smok "Nord," "Novo," and "Trinity Alpha" (7.3%); and all other brands (11.3%). Nicotine was identified in all samples, predominantly as salts (50.3%). Cannabidiol (CBD) was identified in one product. Ethanol was not identified at >1%.

For the 2022-2024 confiscation period, Lost Mary was the most prevalent product (15.2%), followed by Elf Bar products (10.9%), Geek Bar "Pulse" (6.6%), Vuse "Alto" (6.3%), Mr. Fog "Switch" and "Max Pro" (6.3%), and all other brands (54.7%). Nicotine was identified in 85.6% of e-liquids, predominantly as salts (77.4%). Cannabinoids were identified in 14.4% of e-liquids, including Δ8-Tetrahydrocannabinol (THC), Δ8-Tetrahydrocannabinol acetate (THC-O), Δ9-THC, and Hexahydrocannabinol (HHC). Ethanol was identified at >1% in eight e-liquids, up to 3.27% (w/v).

Discussion/Conclusion: The variety of commercially available vaping products used by middle and high school students in Virginia increased significantly between 2019-2020 and 2022-2024. The prevalence of nicotine salts, cannabinoids, and ethanol increased between the two confiscation periods. Despite restrictive regulations, students have access to e-cigarettes across the Commonwealth of Virginia.

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E-Cigarettes; Cannabinoids; Mass Spectrometry

Y58 Fetal Pig Decomposition in Western Pennsylvania: A Characterization of Burial Environment and the Decomposition Process in a Western Pennsylvania Non-Human Outdoor Decomposition Facility

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Learning Objectives: Attendees will learn how to characterize the general decomposition process and identify differences in burial vs. surface environmental influences of fetal domestic pigs (*Sus scrofa*) in western Pennsylvania. Attendees will learn how to validate the decomposition scoring methods of Keough et al. (2017) and Megeysi et al. (2005) using fetal domestic pigs (*Sus scrofa*) in western Pennsylvania. Attendees will learn how the results of the fetal pig (*Sus scrofa*) decomposition from the Indiana University of Pennsylvania (IUP) Outdoor Decomposition Facility compare to other decomposition studies regionally.

Impact Statement: Western Pennsylvania is an understudied region for decomposition and as the rates of decomposition vary regionally, this information is valuable in assisting forensic time-since-death estimations to become more accurate. Using domestic fetal pigs (*Sus scrofa*) as proxy for human remains, this research characterizes the general decomposition process, including any environmental factors that may disturb the remains in western Pennsylvania, the validity of Keough et al. and Megysei et al.'s Postmortem Interval (PMI) scoring methods, and comparing them to other regional decomposition analyzes.

Abstract Text: Decomposition rates are crucial in accurately estimating the PMI of remains in forensic investigations. This research advances the knowledge of forensic decomposition and taphonomy studies regarding buried and surface remains in western Pennsylvania, an understudied region. As decomposition rates vary regionally, understanding environmental factors that affect decomposition such as burial placement, scavenger activity, and climate are essential.[1-3] Nine domestic pigs (*Sus scrofa*) are used as a proxy for humans in collecting decomposition rates due to their physiological similarities to that of a human.[4,5] The PMI scoring methods derived from Megyesi et al. and amended by Keough et al. will be used during the decomposition analysis to determine their applicability in a western Pennsylvania environment.[6]

The thawed fetal pigs, obtained from the Penn State Swine Facility, will be organized 2 meters apart in rows of three on 50x50cm squares, placed on their left side with legs pointing west. Each pig will be spatially recorded using Total Station technology in their southwest corner. Additionally, each section of the pig, including the head/neck, trunk, and limbs, will be spatially recorded to monitor scavenger activity. Scored daily using the Megyesi and Keough methods at the station, this data is supplemented by progress photos and trail camera footage.

This research examines the duration of the decomposition process for pigs placed on the surface versus those that are buried, investigates whether the Keough or Megyesi PMI scoring methods are more appropriate for fetal pigs in western Pennsylvania, and compares these results to similar studies in diverse ecological areas. Three buried pigs will be exhumed after 30 days, and another set of three buried pigs will be exhumed after 60 days to assess their rates of decomposition. By addressing these questions, the study aims to enhance the accuracy of PMI estimations in forensic contexts, ultimately contributing to the overall knowledge of decomposition for western Pennsylvania.

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Decomposition; Pigs; Pennsylvania

Y59 A Novel Technique to Identify Impurities Recovered With DNA Swabbing From Unfired Brass Ammunition

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Learning Objectives: The goals of this presentation are to: (1) identify the impacts of transition metals on DNA recovery and amplification, (2) explore the use of Energy Dispersive Spectroscopy (EDS) for forensic applications, (3) interpret EDS maps and their implications, (4) understand the co-recovery of zinc and copper with DNA swabbing techniques, and (5) analyze the effects of substrate on DNA amplification success.

Impact Statement: This research addresses a significant gap in forensic DNA recovery from bullet casings, emphasizing the critical interference of metal impurities on DNA amplification. By identifying copper and zinc as metal impurities in DNA recovery and the impact on DNA amplification, this work paves the way for innovative strategies to enhance DNA recovery in firearms-related investigations. Ultimately, improving the reliability of forensic evidence can lead to more accurate links between suspects and crimes, thus bolstering the integrity of the justice system.

Abstract Text: Low copy number DNA (commonly called touch DNA or transferred DNA) on bullet casings recovered at a crime scene or retrieved from a loaded gun has potential to be credible evidence in firearms crimes. These recovered bullet casings, having touch DNA evidence, can be used to link the weapon with the person who loaded it. However, brass is a commonly used alloy in the production of ammunition and is composed of the transition metals Copper (Cu^{2+}) and Zinc (Zn^{2+}). These transition metals can associate with the touch DNA from bullet casing and could affect the DNA Taq polymerase amplification performance.[1,2] Therefore, the identification and quantification of these Cu^{2+} and Zn^{2+} impurities is a critical first step in the mitigation of these transition metals.

In this exploration, control human male DNA (AmpFlSTR Yfiler 0.1ng/µL) in a gradient of amounts (30.0µL–3ng, 50.0µL–5ng, and 100.0µL–10ng) was deposited at marked locations on the sterilized unfired brass ammunition casings. Once the deposited DNA was air dried, a double swabbing (wet/dry) technique was conducted using 50.0µL Rapid Stain Identification (RSID) kit universal buffer. The RSID buffer along with the deposited DNA was recouped in Eppendorf tubes by centrifugation at 6,000 rpm for one minute. This recouped solution was subjected to determine the recovery of the deposited DNA and co-recovery of substrate associated metals. Eight (8.0µL) microliters of each sample were analyzed by Energy Dispersive Spectroscopy (EDS) for the identification and mapping of the metallic impurities as well as their atomic quantities. Additionally, 2.0µL of each sample was used to quantify human male DNA recovery efficiency by real-time Polymerase Chain Reaction (qPCR) method.

The EDS analysis showed that the atomic quantities of Zn^{2+} (0.01–0.10%) and Cu^{2+} (0.02–0.85%) metals increased proportionally with the increased amount of deposited DNA on the bullet casings. It is observed that Zn^{2+} and Cu^{2+} were both recovered in the swabbing solution but that the proportions favored Cu^{2+} more than Zn^{2+} when compared to the brass casings.However, the deposited (3ng, 5ng, and 10ng) human male DNA was undetected by qPCR. It seems that the deposited controlled human male DNA was undetected due to either combined effect of Zn and Cu or Cu or Zn independently as follows: (1) direct inhibition of the Taq polymerase activity, (2) quenching or interfering with the detectible fluorescence required for analysis, or (3) breaking the template DNA into smaller fragments unsuitable for primer annealing with the template DNA and hence template DNA undetected by qPCR.[1,2] The development of effective techniques to remove these transition (Cu and/or Zn) metals is vital for the recovery of forensically viable DNA profiles and necessary for the identification of the person who loaded the firearm.

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Touch Sample; Brass Ammunition; Energy Dispersive Spectroscopy

Y60 A Comprehensive Study of 3D Scanning and Mapping Technology in Forensic Investigations: Accuracy, Efficiency, and Volume

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Learning Objectives: After this presentation, attendees will understand the impact that three-dimensional (3D) scanning and mapping technology has on the investigative process as perceived by current law enforcement professionals. This presentation will provide attendees with insights into how 3D scanning and mapping technology benefits investigations in terms of increased accuracy, efficiency, and volume of data collected.

Impact Statement: This presentation will enhance forensic competence by outlining the forensic benefits of 3D technology in relation to accuracy, efficiency, and the volume of data collected during investigations. Because this study highlights the benefits of 3D scanning and mapping as perceived by law enforcement officials, attendees will be able to apply what they've learned to improve their own departments' investigations.

Abstract Text: The role of technology within law enforcement settings is growing and changing rapidly, with the implementation of 3D scanning and mapping technology more recently allowing for improvements in several aspects of the investigative process. Most of the existing literature regarding this topic focuses on using this technology in experimental settings as well as on specific aspects of 3D scanning technology. This research is intended to fill a gap in the literature by offering a more general overview of this technology with insights from experienced professionals who work real-world cases using 3D technology. This study utilizes a mixed-methods approach, combining qualitative interviews and survey data to provide a more comprehensive documentation of how 3D scanning and mapping systems impact the field of forensics. It focuses on the broad enhancements of accuracy, efficiency, and the volume of data collected as observed by law enforcement professionals who regularly use this technology in official cases.

This study hypothesizes that current law enforcement officials feel that the implementation of 3D scanning and mapping technology significantly enhances the investigative process in comparison to traditional forensic methods. Regarding 3D technology, Norris noted that, "investigators must avail themselves of all the tools at their disposal for identifying and interpreting the facts so they may arrive … at the truth."[1] Additionally, a statement made by Wang et al. concluded that "non-invasive, high-resolution measurement and increased insights are always the goal of forensic crime scene documentation," which is precisely what the integration of 3D scanning and mapping systems provide for law enforcement.[2]

This study's use of mixed methods will help to provide a more comprehensive understanding of how 3D scanning and mapping technology impacts the investigative process. Quantitative surveys aim to provide numerical data that can be analyzed to determine some of the impact that 3D scanning and mapping technology has had on the work that these law enforcement officials do daily. The information gathered by quantitative surveys is important because it is quantifiable data that can be shown to the audience in manners such as graphs, tables, and charts to convey the importance of 3D scanning and mapping technology in forensic investigations. The addition of qualitative interviews will take the research to a much deeper level with more information that a survey simply cannot capture. Interviews will allow for subjects to respond with a more open-ended interpretation of the questions asked than surveys. Because this research is so heavily focused on the accuracy, efficiency, and volume of data collected by this technology, it is important to gather the most detailed analysis of the technology straight from the professionals who use it the most, instead of simply diluting their responses down to multiple choice number responses in a survey.

The results of this study are anticipated to highlight how current law enforcement professionals perceive 3D scanning and mapping technology to benefit their investigations in terms of accuracy, efficiency, and volume. From this study, readers will gain a more general understanding of the benefits of 3D technology from the perspective of current law enforcement professionals, which they can implement into their own departments' investigative practices.

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3D Technology; Forensic Investigation; YFSF

Y61 Using the Truncated Normal Distribution for Value of Evidence in the Forensic Identification of Source Problems

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WITHDRAWN

Y62 A Qualitative and Quantitative Analysis of Heavy Metals in Topical Cannabis Pain Relief Products

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Learning Objectives: After attending this presentation, attendees will learn about the increase of Cannabidiol (CBD) products on the market and the lack of regulations surrounding these products. Attendees will gain a better understanding of the possible presence of heavy metals in CBD products and the health risks these metals could cause for consumers.

Impact Statement: This presentation will explore the extent to which CBD products meet current legal requirements, set forth by the Food and Drug Administration (FDA), regarding the allowed limits of heavy metal contaminants in products. It proposes the use of a lower-cost method for analysis, which could save money and resources for analyses of these products.

Abstract Text: In 2018, the United States passed the Farm Bill, which removed CBD from the list of controlled substances, where it was previously considered a Schedule 1 drug, and legalized any cannabis-derived products containing less than 0.3% Tetrahydrocannabinol (THC).[1] As a result of this bill, there has been an increase in sales and an influx of matrices for cannabis-based products. One subsection of cannabis products that has gained interest in recent years is the topical use of CBD. Research has shown that topical CBD products may be useful to stimulate wound healing, treat non-melanoma skin cancer, and reduce inflammation, itching, and pain.[2] However, since CBD has no accepted medical or recreational use, with the expectation of CBD to treat epilepsy, any cannabis-based products on the market are largely unregulated by FDA.[3]

Due to the lack of regulations and limited analysis of CBD products before they enter the market, there are concerns about label accuracy and potential product contamination. Heavy metal contamination in CBD products is an area of concern, partially due to processing cross-contamination and post-processing adulteration, but also due to the natural bioaccumulative capacity of the cannabis plant, which removes heavy metals from substrate soils and deposits them in the plant tissues.[4] Heavy metals are classified as carcinogens and have been shown to aid cancer progression or reduce sensitivity to treatment.[5] Topical exposure to heavy metals was previously thought to only have local effects on the body; however, recent studies have shown that they are capable of penetrating deep into the skin, reaching the blood circulatory system, and causing exposure to internal organs.[6]

In this study, 20 topical CBD products sold online were analyzed for the potential presence of lead and cadmium. Samples consisted of cream, gel, or balm topicals that were classified as isolate, broad spectrum, or full spectrum CBD. A method using Atomic Absorption Spectroscopy (AAS) was developed to investigate the presence of heavy metals in lotion. Standard addition calibration curves were created for each sample and analyzed with the AAS to determine lead or cadmium concentrations.

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Cannabinoids; Heavy Metals; Topical Skincare

Y63 The Identification and Quantification of Illicit Drugs in Blood Using Stir Bar Sorptive Extraction and LC/QqQ/MS

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Learning Objectives: This presentation will utilize laboratory data to demonstrate the importance of Stir Bar Sorptive Extraction (SBSE)as a potential extraction method for whole blood samples spiked with illicit drugs. After attending this presentation, attendees will better understand the benefits of stir bar sorptive extraction to forensic toxicology. It is an extraction method that, when coupled with Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), can aid in the identification and quantification of a wide range illicit drug classes.

Impact Statement: This presentation will impact the forensic science community by expanding the possibilities for extraction when it comes to whole blood toxicological samples. Forensic toxicologists will learn that SBSE is just as efficient as alternative methods while being a simplistic procedure that is more sustainable and more cost and time effective. It also has the potential to promote collaboration with police through the development of an on-site test kit.

Abstract Text: The evolving drug epidemic has not only increased the risk of injury and death in people struggling with addiction but has also strongly affected the surrounding communities. The danger proposed by illicit drug use has led to further research into more sensitive substance detection and identification techniques for biological samples. SBSE is used in environmental and pharmaceutical chemistry to detect and quantify organic pollutants in water reserves and dietary supplements. This extraction method utilizes a polymer-coated magnetic stir bar to absorb analytes of interest that can later be desorbed based on temperature or solvent change for separation. SBSE has been shown to have higher analyte recoveries with less solvent use as compared to the more common solid phase and liquid-liquid extraction methods. It is an extraction method that, when coupled with LC/MS/MS, can aid in the identification and quantification of a wide range illicit drug classes. Very little research has been done using this method with biological samples such as blood, let alone in a forensic toxicology setting. In this study, polydimethylsiloxane-coated magnetic stir bars were used to extract analytes correlating to a 15-drug panel from human whole blood samples. Each blood sample was spiked with a known analyte concentration. The drug panel included illicit substances such as fentanyl and heroin and medical prescriptions like oxycodone and methadone. Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) with a biphenyl column was used for separation. A five-drug panel of 6-acetylmorphine, benzoylecgonine, EDDP perchlorate, fentanyl, and noryoxycodone HCl were quantified utilizing internal standard calibration curves. Thirteen out of 15 drugs were able to be extracted upon the addition of 0.25 M NaCl. Preliminary quantitation results were achieved. Victims and their grieving families would receive justice and closure by furthering research in drug extraction techniques that are more efficient, more sustainable, and more cost and time effective.

Forensic Toxicology; Stir Bar Sorptive Extraction; LC/MS/MS

Y64 Optimizing Simultaneous DNA, RNA, and Protein Extraction From Human Teeth: The Effects of Incubation Time and Environmental Factors

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Learning Objectives: Attendees of this presentation will gain insights into how to improve the simultaneous extraction technique of DNA, RNA, and proteins, as well as the impact of chemical treatments on the yield of these biomolecules. The initial step of extraction is essential for obtaining sufficient quality and quantity of nucleic acids and proteins for forensic analysis.

Impact Statement: This presentation will impact the forensic community by presenting a study that enables the simultaneous extraction of DNA, RNA, and proteins for applications in both human identification as well as the estimation of the postmortem interval.

Abstract Text: DNA isolation from human remains is of utmost importance to be able to identify a victim. However, there are situations in which, apart from the Short Tandem Repeat (STR) profile, other parameters need to be estimated, like the time-since-death. Recent advances in this field point to "-omics" technologies to improve these estimates, particularly in skeletonized remains. Among them, transcriptomics and proteomics are emerging as promising techniques for this purpose. However, this requires the extraction of RNA and proteins from the remains along with the DNA, which is particularly challenging in this kind of sample exposed to different environmental conditions.

This study aimed to assess the efficiency of simultaneous DNA, RNA, and protein extraction from human teeth, the hardest and most decomposition-resistant tissues in the human body. Furthermore, it investigated the effects of environmental factors, such as exposure to sodium hydroxide and hydrochloric acid, on the extraction process, simulating conditions relevant to actual forensic cases. A total of 22 teeth, including control and chemically treated samples, were processed. The control samples were studied at different incubation times (2, 5, 12, and 24 hours), while all treated samples were incubated for 24 hours. For each tooth, dentin and pulp were separated, and 200ng aliquots of dentin were prepared for further analysis.

The Zymo Quick-DNA/RNA Microprep Plus Kit was used for the simultaneous DNA/RNA/protein extraction. Different incubation times on the digestion buffer and proteinase K were evaluated. DNA/RNA/protein concentrations were assessed through Qubit fluorescent quantification, and human-specific quantification was carried out by applying the Promega PowerQuant kit.

According to our results, both Qubit and PowerQuant quantification showed an increase in DNA yield in the control samples after 24 hours of incubation, although not significant, obtaining average concentrations around 30ng/ul. Respective to RNA, time did not impact the RNA yield, obtaining average concentrations around 50ng/ul. In contrast, for proteins, 2 hours of incubation increased the protein yield, obtaining average concentrations around 1ug/ul. Regarding the treated samples, the average concentrations of DNA, RNA, and proteins were comparable to those of the control samples at the 24-hour incubation period. However, the DNA yield in the samples treated with sodium hydroxide was significantly lower, measuring only 10ng/µL.

To the best of our knowledge, this is the first study assessing the efficiency of simultaneous extraction of DNA, RNA, and proteins from human remains. This research specifically includes samples subjected to various environmental conditions, which are key to study for forensic contexts. Our findings demonstrate that it is feasible to isolate these three molecules with appropriate yields, applicable not only to human identification but also to estimate the time since death.

Teeth; Forensic DNA; Mass Disaster

Y65 The Influence of Heat on Animal Fiber Transformation: Macroscopical and Microscopical Approaches

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Learning Objectives: Attendees will gain an understanding of the microscopic features of animal fibers and learn how to differentiate between animal fibers using three microscopy techniques. Additionally, they will explore the effects of heat on the structural and optical properties of animal fibers.

Impact Statement: This presentation explored the influence of heat on animal fibers, broadening the forensic knowledge on animal fiber evidence in cases of wildfires or arson. Additionally, it will expand the understanding of animal fibers, and the effects of heat on these fibers, offering new insights that can enhance forensic investigations involving trace evidence.

Abstract Text: Animal fibers are widely used in textiles and are frequently found in households, especially in environments with pets. As a result, animal fibers often appear in everyday environments and can be potential evidence at crime scenes. In a previous study, Henson and Rowe explored the influence of heat on human head hair and observed an increase in fiber diameter as well as a formation of bubbling structures in the medulla and/or cortex.[1] This study aims to examine similar changes under various heat conditions stemming from their structural similarity, including the presence of the medulla, cortex, and cuticle. The effects of heat on various animal fibers are analyzed by using three microscopical techniques: bright-field microscopy, dark-field microscopy, and polarized light microscopy.

A Zeiss microscope photomicrograph 2 was utilized to perform three microscopy techniques. Dog fibers were obtained from a male Maltese with white fur. Additional animal fibers, including boar, camel, deer, rabbit, horse, and Mongolian horse fibers, were purchased from Amazon. All fiber samples were subjected to temperatures between 100°C and 200°C using a hair straightener and between 100°C and 300°C using an oven. For the hair straightener, all possible combinations of two distinct temperatures (100°C and 200°C) and two time durations of 20 seconds and 2 minutes were applied to the samples. For the oven, all possible combinations of three distinct temperatures (100°C, 200°C, and 300°C) and three time durations of 20 seconds, 3 minutes, and 5 minutes were applied to the samples.

Macroscopically, all the animal fibers showed a color change to darker colors and exhibited brittleness under severe heat conditions when using both a hair straightener and an oven. The higher the temperature and the longer the exposure time, the more severe the changes were.

Microscopically, all the animal fibers showed an increase in fiber diameter and the formation of bubbling structures under severe heat conditions caused by both a hair straightener and an oven. As with the macroscopic changes, the higher the temperature and the longer the exposure time, the more pronounced the differences were. These findings correspond to those of a previous study conducted on human head hair by Henson and Rowe.[1]

The bubbling structures likely resulted from the evolution of gases from the medulla and/or cortex, as the air or fluids inside the medulla expand under heat, contributing to the increase in fiber diameter.

Results from this study will help broaden the understanding about animal hair evidence that is exposed to heat in cases of wildfires or arson.

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Microscopy; Animal Fibers; Heat

Y66 A Portable Microfluidic Biochip for the Simultaneous On-Site Detection of *Streptococcus Pneumoniae* and *Bordetella Pertussis* for Forensic Pathology Investigation

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Learning Objectives: This presentation is intended to provide attendees with a deeper understanding of microfluidic devices and how they can be applied to forensics to reduce the required time it takes to detect pathogens postmortem. On this topic, attendees will learn about the microfluidic device our laboratory has developed as an innovative approach for the simultaneous identification of *Streptococcus pneumoniae* and *Bordetella pertussis*.

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 related to death in patients. Therefore, developing technologies to improve the detection of pathogens that may have contributed to the patient's death is essential to aid forensic pathologists in reaching a proper diagnosis.

Our lab has been working on the development of an innovative microfluidic device that will help forensic pathologists reach this goal. Our microfluidic devices can be used to detect multiple pathogens faster and accurately than the commonly used detection methods, providing more reliable results. This can reduce the amount of resources needed to investigate a criminal case and provide better results.

Abstract Text: *Streptococcus pneumoniae* (*S. pneumoniae*) as well as *Bordetella pertussis* (*B. pertussis*), are common human pathogens that can lead to different diseases such as rhinosinusitis, otitis media, whooping cough, meningitis, and pneumonia. Combined, more than 24.1 million new cases and 1 million *S. pneumoniae* and *B. pertussis*-related 1 million deaths are reported worldwide.[1-3] 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-causing pathogens during autopsy is important for forensic pathologists' postmortem diagnosis, as these can help in the prevention or understanding of the spread of disease. Therefore, we have developed a polymer/paper hybrid microfluidic biochip integrated with Loop-Mediated Isothermal Amplification (LAMP) to aid in the multiplex detection of *S. pneumoniae* and *B. pertussis*. 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 might 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 both *S. pneumoniae* and *B. pertussis*. With its efficient design, materials, and size, this detection device can be used in both well-equipped laboratories and places with limited resources. This device will be an advancement in the detection methods used today for pathogenic detection.

It is expected that the use of this device will aid in the rapid identification of both life-threatening bacteria, 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 in crime sciences and the morgue. Furthermore, this device will help researchers better understand the spread of infectious diseases and expand the use of microfluidic devices in a variety of scientific fields.

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Microfluidic Device; LAMP (Loop-Mediated Isothermal Amplification); *Streptococcus pneumoniae* and *Bordetella pertussis*

Y67 Investigating Without A Badge: How Civilian Detectives Influence Forensic Science and Law Enforcement

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Learning Objectives: The learning objectives of this research are to gain insight and knowledge on the evolution of the true crime community and how it has changed form fascination to involvement. This research aims to begin the conversation of how civilians can be utilized by law enforcement regarding cases by using real cases where they have positively contributed to solving investigations but also how they have negatively impacted cases as well.

Impact Statement: This research defines the emerging role of civilian detectives and explores their growing involvement in modern crime-solving. By tracing the evolution of the true crime community and analyzing the underlying theories that explain the public's fascination with crime media, the study highlights how civilian input has positively contributed to real investigations. Semi-structured interviews will be conducted with police departments that have firsthand experience regarding civilian detective involvement, allowing for qualitative data to be produced through the transcriptions. Through case analysis and theoretical application, the research proposes potential solutions for law enforcement investigations by incorporating civilian detectives. The findings could lead to more collaborative and efficient investigative processes, ultimately strengthening law enforcement.

Abstract Text: Civilian detectives, keyboard warriors, internet sleuths, or true crime enthusiasts have been around since the true crime community gained popularity in the 1900s but have since increased in traction due to the overwhelming and accelerated pace that information moves around due to social media. The true crime community has been fostering a fandom that has recently started to take investigative matters into their own hands and want to physically pursue cases on their own. This study aims to find how civilian detectives are interacting with cases by interviewing police agencies that have the highest probability of interaction with this group.

Currently, no academic studies have proposed to study what civilian detectives do, how cases are interfered, and how police agencies are currently using information provided from this group to push forward an investigation. This proposal aims to mend the bridge between civilian detective work and law enforcement. This is aimed specifically toward police departments and detectives that have had experience with citizen detectives and to prepare other departments for future encounters with citizen detectives and the general public that aim to interfere with investigations by trying to solve the crime or just have curiosity about what happened.

This proposal utilizes theories such as the social network theory to explain how social media can influence a person's interest and involvement in the true crime community and what type of person is most likely to become a civilian detective. Psychology journals aim to explain that women are most likely to become interested in the true crime community because, "the victims in true crime books are often women, it may be the case that women simply have more to gain from reading these books in terms of understanding survival strategies and defense tactics."[1] The media has been an influential part in growing this obsession with all things associated with crime like over-producing podcasts, tv shows, news articles, blogs, and other related social media posts that go viral on a certain case, spinning it from reality into entertainment. People also play detective through video games or game rooms as a real-life Sherlock Holmes. "As online sleuths and podcasters become more integrated in the crime-solving space, there has been a renewed interest in how media coverage and police investigations are reported and conducted."[2]

By explaining this new phenomenon that has continued to be highlighted in the media, police agencies can begin to utilize these civilian detectives or gain a better understanding of what they are capable of to determine what a proper usage might be. By defining the role of civilian detectives, tracing the evolution of the true crime community, applying relevant theories to explain the growing public fascination with crime media, and analyzing real cases where civilian involvement has positively contributed to investigations, this research aims to propose potential solutions for enhancing investigation processes within law enforcement.

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Forensic Investigation; Social Media; Research

Y68 The Effects of Anxiety and Depression on Adolescent Offender Behavior

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Learning Objectives: After attending the poster session, attendees will understand the effect of anxiety and depression on the behaviors of adolescent offenders.

Impact Statement: This presentation will impact the forensic science community by aiding case workers with the knowledge to recognize how anxiety and depression have impacted adolescent behavior and help build treatment programs for adolescent offenders, which will in turn help in reducing repeated offenses. With the ability to recognize symptoms, the deeper understanding of the effects of anxiety and depression on adolescent offenders, and knowledge about effective treatment, it is possible that the recidivism rates in adolescent offenders who have depression and anxiety can be reduced.

Abstract Text: Adolescence is marked by many physical, cognitive, moral, social, and emotional changes, some of which consist of a change in behaviors in an individual that are seen as risk-taking or unsettling. These risky behaviors, however, may be taken to a different level when an individual has a mental disorder or disorders, like anxiety and/or depression; these same behaviors may also lead to some adolescents getting involved with the law. Such behaviors may include taking drugs, drinking alcohol, stealing, damaging property, etc. Some adolescents get involved with such tasks because they find that it is what peers are doing, while others get involved because they have a compulsion to do so, especially adolescents who are experiencing symptoms of anxiety and/or depression. Going further into the compulsory behaviors of adolescents that become offenders would be the categorization of life-course persistent pathways and adolescence-limited pathways

Approximately 60-70% of adolescents that become involved with the law meet the criteria for at least one mental disorder, which consists of anxiety and depression. With this statistic being significant, there has been an increased need for the juvenile justice systems to be more able to help assist adolescents with mental disorders. The goal of having juvenile justice systems is then to focus on the prevention of adolescent offenders committing more crimes in the future, but also as adults, which then becomes another statistic, with an estimated 43% of state and 23% of federal prisoners having reported having a history of mental health problems.[1-9]

This poster will be a literature review to bring up the issue that there is a need for the effects of mental disorders, like anxiety and depression, to be studied in adolescents and how that impacts youth offender behavior. This literature review will also cover the prevalence of adolescents that will then become offenders in their adulthood due to the lack of treatment currently available.

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Anxiety; Depression; Development

Y69 Posthumous Sperm Viability: Exploring the Impact of Time Since Death for Forensic and Reproductive Applications in India

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Learning Objectives: Attendees will understand the relationship between time since death and sperm viability, methods for assessing postmortem sperm viability, recognize the forensic applications of posthumous sperm retrieval, and identify optimal timeframes for postmortem sperm retrieval.

Impact Statement: This presentation will make an impact by identifying the relationship between time since death and sperm viability, time-sensitive protocols for the retrieval and use of sperm in Assisted Reproductive Technologies (ART), offering new possibilities for grieving families seeking to preserve genetic continuity. Possible correlations of posthumous sperm viability with time since death in context of environmental considerations has the potential to influence forensic science.

Abstract Text: **Background:** Postmortem Sperm Retrieval (PMSR) is a growing area of interest with significant applications in both forensic and reproductive medicine. In reproductive medicine, posthumous sperm retrieval is increasingly sought by families and spouses, often in cases of sudden or unexpected death, as a means of preserving the possibility of biological offspring. However, despite the demand, little is known about how the time elapsed after death affects sperm viability and function, especially in tropical and subtropical environmental conditions found in regions like India. This study aims to fill that knowledge gap by examining the relationship between postmortem intervals and the viability of spermatozoa.

Objective: The primary goal of this study is to assess the viability of spermatozoa retrieved from deceased individuals brought to the mortuary of All India Institute of Medical Sciences (AIIMS) Bhopal, with varying time intervals since death. By correlating sperm viability with time since death, this research seeks to establish evidence-based guidelines for PMSR in Indian conditions.

Methods: The study was conducted on 97 adult male decedents brought to the mortuary of AIIMS Bhopal during 2021-2023, grouped based on postmortem intervals ranging from less than 6 hours to over 48 hours. With permission of the institutional ethics committee, samples of spermatozoa were collected posthumously from the testes or vas deferens during medicolegal autopsy, minimizing contamination of the sample. The viability of the spermatozoa was assessed through a range of methods, including light microscopy, and advanced technologies such as computer-Assisted Sperm Analysis (CASA) used to quantify sperm motility and morphology. The samples were then discarded following institutional protocols. The data was analyzed using SPSS version 25 to determine how sperm viability correlates with the time since death and other factors such as cause of death, temperature, and environmental conditions.

Results: Sperm viability related to the time elapsed since death with motility declining significantly within 6 hours. Within the first 12-18 hours, viable spermatozoa were consistently retrieved. Refrigeration or cool environmental conditions can help extend sperm viability slightly beyond the 24-hour window; however, normal morphology is significantly decreased due to refrigeration. There was significant decrease in success of PMSR attempt in older individuals. Posthumous sperm was most vital in cases of sudden death of younger individuals (18-36y) where the body had not undergone significant decomposition; however, vitality was still observed after 32 hours postmortem, indicating that this timeframe is feasible for PMSR for reproductive purposes.

Conclusion: This study establishes that posthumous sperm retrieval is possible in a standard mortuary setting in India, at least up to 36 hours of death. In reproductive medicine, this can inform the practice of posthumous sperm retrieval and its use in ART. While the study documents time-sensitive sperm vitality, further research is needed to assess the long-term viability during storage of posthumous sperm, which has an added risk of contamination due to postmortem changes.

Autopsy; Time of Death; Reproductive

Y70 Isolating Novel DNA Methylomic Bone Signatures for Age Estimation in Fresh and Forensic Samples

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Learning Objectives: After this poster presentation, attendees will learn about the novel potential with estimating Age-At-Death (AAD) from bone in DNA methylomics successfully in forensic and fresh bone.

Impact Statement: This presentation will impact the forensic anthropological community by providing an update on the current research investigating age-associated DNA methylation changes in bone using genome-wide approaches. The hope with the presented prediction models is the opportunity to develop a forensic assay with a diverse pool of previously unpublished signatures specific to bone.

Abstract Text: Accurate estimation of chronological age is hugely important in forensic science, with applications including cadaver identification for medicolegal and disaster victim identification. Current methods that rely on physical measurement of bones/teeth are invasive and imprecise. By contrast, molecular methods that use signatures of age-dependent biomolecules can accurately predict age.[1-5]. Particularly, epigenetic clocks that use CpG methylation at specific loci to predict age have been widely used in bioscience. However, defining epigenetic signatures to predict age from bone has been difficult, due to the scarcity of DNA within bone; most assays rely on bisulfite conversion to induce a methylation-dependent Single Nucleotide Polymorphisms (SNP), a destructive process that further impedes interrogation of the bone methylome.[6] Consequently, most studies to date have focused on fresh bones, using the Illumina methylation microarray platform, which assesses 450-850,000 CpG loci and has stringent DNA quality/quantity requirements.[7] The sequencing-based TWIST Bioscience Human Methylome Panel (HMP) offers potential to measure 3.8M CpG loci using smaller amounts of degraded input DNA. We aimed to compare bone methylome data obtained from methylation microarray with methylome readouts from the TWIST HMP applied to forensic bone samples and assess the potential of TWIST HMP for derivation of CpG signatures to predict age from taphonomic samples.

A methylation microarray (*in silico*) cohort was collated from previously published articles (n=173; range 0.25-92 years; average age 65 years; 161/173 were fresh bone). Bone samples (n=69; Postmortem Interval (PMI) = 0-2236 days; AAD = 28 – 93 years) were collected from the Southeast Texas Applied Forensic Science (STAFS) facility and Forensic Anthropology Center at Texas State (FACTS). DNA was extracted using a modified Dabney protocol starting from 200mg (bone powder). Between 6.5ng - 200ng ($\bar{x}$ = 174.1) of DNA was run on the TWIST Bioscience HMP and complete methylation data obtained using standard workflows. Previously published CpG signatures to predict age were tested. Novel cross-validated signatures to predict age were generated using GLMnet.

All 69 samples ran on the TWIST HMP passed sequence-based QC and yielded usable methylome data, despite low-quality DNA inputs. For the *in silico* dataset, the previously published Elastic Net (EN) blood and saliva CpG clock performed best (514 CpGs; RMSE 8.5 years).[8] However, previously published molecular clocks performed poorly on the TWIST dataset (RMSE >21 years). When comparing shared loci, global methylation patterns were comparable between *in silico* and TWIST, being bimodal; however, there was a notable shift in the methylated peak for TWIST (~85% methylation) compared to *in silico* (~95% methylation). We trained a 20 CpG cross-platform model that had an RMSE of 12.1 years. When considering datasets separately, optimal models comprising 20 CpG loci had an RMSE of 4.8 years for *in silico* and 5.9 years for TWIST, respectively.

We have pioneered the assessment of the methylome in forensically relevant samples. The TWIST HMP assay was suitable for methylome assessment in challenging samples. Global methylation patterns are similar to array-based cohorts from fresh bone; however, there is a taphonomic-dependent shift toward demethylation in methylated loci. Additionally, signatures of aging derived from methylation microarray perform poorly on TWIST data. This may be due to platform differences, taphonomic processes, and the relatively small change in methylation from age-predictive loci. The TWIST aging signatures described perform well, have not been identified from array-based studies, and offer potential for developing Polymerase Chain Reaction (PCR) -based forensic assays to predict age with greater accuracy and applicable to taphonomically impacted tissues. Therefore, we show a novel successful sequencing-based approach isolating new bone-specific CpG markers for improved AAD estimations in forensic caseworks.

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Methylation; Age-at-Death; Machine Learning

Y71 The Variation of Aerosolized Nicotine as Ethanol Concentration Increases in E-Cigarette Formulations

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Learning Objectives: Audiences will understand how e-cigarette formulations impact aerosolization of nicotine leading to an increase in dosages and the effect ethanol has on drug concentrations in an e-cigarette generated aerosol.

Impact Statement: This presentation will inform the forensic science community of the impact of varying ethanol and humectants (propylene glycol and vegetable glycerin) in e-cigarette formulations on the nicotine aerosolization.

Abstract Text: **Introduction:** Electronic cigarette (e-cig) liquids (e-liquids) are typically formulated as a mixture of Propylene Glycol (PG) and Vegetable Glycerin (VG), flavorants, and pharmacologically active compounds such as nicotine. Technological advancements have enabled e-cigs to be co-opted for use with Drugs Other Than Nicotine (DOTNs), such as ethanol, herbal products, or illicit substances. The interaction between compounds can promote aerosolization. Previous studies have demonstrated that nicotine concentrations in the aerosol from an e-cigarette may vary depending on the ratio of PG to VG present.[1]

Objectives: To determine the effects of varying ethanol and humectants PG and VG on the aerosolization of nicotine generated by an e-cigarette formulation using an in-house aerosol trapping system.

Methods: E-cigarette formulations were created in-house using PG:VG ratios of 100:0, 70:30, 50:50, 30:70, and 0:100 (w/v) with 12mg/mL nicotine and ethanol concentrations of 0 and 25% (v/v) ethanol. A flask trapping system was used to capture aerosols generated using a Kanger SUBOX Mini e-cigarette device (resistance (Ω=0.65), power (W=28.4,) and voltage (V=4.3)). Each trial consisted of ten puffs for a duration of four seconds. The e-cigarette tank was weighed before and after each puffing session. Concentrations of ethanol and nicotine in the aerosol were determined by headspace/gas chromatography/flame ionization detection or liquid chromatograph/tandem mass spectrometry.

Results: The concentration of nicotine aerosolized did not significantly change due to PG:VG ratios but did have a statistically significant ($p = < 0.017$) increase with the higher ethanol concentration. Aerosolized nicotine at 0% ethanol ranged from 3.7±0.5mg to 5.7±0.4mg. Aerosolized nicotine at 25% ethanol ranged from 4.8±0.3mg to 7.5±0.6mg.

Discussion/Conclusions: The matrix composition was not found to change the aerosolization of nicotine, supporting the results of previous studies. Results indicate that ethanol in e-cigarette formulations will promote greater aerosolization of nicotine. Further studies will investigate differing concentrations of ethanol to compare effects on drug aerosolization. Increased drug availability can lead to untoward effects. While experienced consumers self-titrate the amount of drug consumed to manage effects, the increased dose delivered can facilitate substance addiction.

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E-Cigarettes; Nicotine; Ethanol

Y72 An Analysis of the Incidence of Selected Opioids Seized Across Canada, 2018–2022

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Learning Objectives: Attendees will learn about the trends being observed across Canada from 2018 to 2022 for key opioids, related compounds, and fentanyl analogs. An understanding of the impact of opioid frequency surrounding specific periods of the COVID-19 pandemic (pre-, during-, and post-) will also be established. Attendees will also learn how geography in Canada plays a role in the frequency of opioids, namely provincial trends and the impact of opioids on rural communities. Finally, the co-occurrence of fentanyl with prominent cutting compounds (xylazine, etizolam, and methamphetamine) will also be noted.

Impact Statement: Analyzing trends regarding opioid frequency serves to provide a better picture of what is being distributed and consumed illicitly and can be used to infer drug trafficking patterns for different compounds or locations. It also shows new emerging compounds and helps to understand what is being cut with prominent opioids to create designer compounds. This knowledge can be utilized to help address the current opioid crisis. Increased efforts to reduce the impact of opioid overdoses are greatly needed.

Abstract Text: This study presents a comprehensive analysis of selected commonly abused opioids and related compounds within Health Canada's Drug Analysis Service (DAS) database for seized substances by law enforcement, including fentanyl, carfentanil, heroin, oxycodone, morphine, and codeine.[1] Other compounds examined included methadone, xylazine, and fentanyl analogs.

Statistical analysis focused on the frequency of samples containing the selected compounds, incidence of detection between 2018 and 2022, and geographical distribution across Canada. Trends before, during, and after the COVID-19 pandemic were examined. Trends in high opioid activity provinces (Ontario, Alberta, and British Columbia) were noted. Geographical trends relating to rural/northern communities were also examined. Comparisons were made between the five most populated cities in Northern and Southern Ontario. Additionally, the co-occurrence of fentanyl and three compounds of interest (xylazine, etizolam, and methamphetamine) was examined.

The incidence of fentanyl-positive and xylazine-positive samples rose steadily from 2018-2022. Fentanyl-positive sample incidence grew from 8,063 samples in 2018 to 16,553 in 2021 and measured 12,074 samples in 2022. Heroin-positive samples decreased in incidence, falling from 3,881 in 2018 to 1,415 samples in 2021, and measured 941 samples in 2022. Importantly, the available data showed both sample receipt and release dates. Consequently, the data reported here from 2022 did not reflect all submissions from that year, and a drop in detection incidence was observed, as an artifact.

The incidence of carfentanil, oxycodone, morphine, codeine, and methadone presented minor changes across the same five-year window. The 2022 data artifact likely impacted that data as well, to a much lower to degree than with respect to the compounds under investigation.

Detection of fentanyl analogs showed some fluctuation from 2018-2022. Incomplete data from 2023 and 2024 show that there may be a sharp increase in the incidence of fentanyl analogs in those years. In 2018, 2019, 2020, 2021, and 2022, the percentages of fentanyl analogs found in all seized drugs for those years were 0.73%, 0.37%, 0.53%, 0.15%, 0.72%, 5.86%, 8.87%, respectively.

Notably, all compounds investigated had reduced positive detections during the COVID-19 group, with the exception of fentanyl, xylazine, and methadone. Fentanyl and xylazine both saw increases in detection. Fentanyl-positive samples in the pre-COVID-19 group were 19,474 and rose to 31,843 in the during-COVID-19 group. Xylazine-positive samples in the pre-COVID-19 group were 221 and rose to 965 in the during-COVID-19 group. Methadone detection varied from 252 positive samples in the pre-COVID-19 group and 256 positive samples in the during-COVID-19 group. There was not enough data available to make any conclusions for the post-COVID-19 groups. Ontario presented a greater number of positive samples than British Columbia and Alberta, however, the latter two presented larger numbers of positive samples per capita. British Columbia had the highest number of positive detections per capita (0.57%), followed by Alberta (0.35%), and then Ontario (0.33%). Cities in Northern Ontario were also found to have a disproportionately high number of positive detections per capita relative to cities in Southern Ontario. It was found that the number of fentanyl-etizolam-positive samples was the most abundant, while fentanyl-xylazine-positive samples increased significantly over the time window examined. The results from this study help to provide key insights into the overall trends in illicit opioid activity in Canada.

Reference:

1. Health Canada Drug Analysis Service Database, 2018-2022.

Opioids; Drug Analysis; Adulterants

Y73 The Unsupervised Learning of Individuals in Keystroke Dynamics Via Semi-Supervised Multilayer Mixture Models

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Learning Objectives: Attendees will learn about the use of keystroke dynamics as a method for identifying the user of computer systems. This research will introduce a statistical method, namely semi-supervised multi-layer finite mixture model for identifying individual instances with similar (same) keystroke dynamics that can be used for digital forensics.

Impact Statement: If keystroke dynamics are being monitored on a system, multiple people may have access to said system. Being able to parse which keystrokes belong to which person will be important when conducting digital forensics on the system. This is a challenging problem and must be approached using unsupervised methods.

Abstract Text: **Hypothesis:** Keystroke dynamics have been used in recent years to identify the user of a system.[1] Current research has mainly been framed in the context of authentication and assuming a single system user. This, in turn, leads to the use of supervised learning methods such as linear discriminant analysis, nearest neighbor classifiers, support vector machines, and others.[2] Less focus has been placed on situations when supervised methods are no longer applicable such as when multiple people have access to the same system and it is unknown which person is using the system at a given time. Interest is then placed on being able to cluster the keystrokes by user in which unsupervised methods must be used.

Methods: The task of clustering keystroke dynamics is particularly challenging as the speed of people's keystrokes often changes across different sessions of using the system and even within the same session. Thus, not only are there clusters created by users, but also clusters within each user. This research introduces the semi-supervised multi-layer finite mixture model, which is able to not only find clusters that separate the keystrokes by users but also models the clustering that can occur within a user. This method is contrasted with the semi-supervised finite mixture model, which has previously been applied to forensic datasets and is not able to account for the fact that clusters exist within clusters.[3] This model leverages the fact that keystrokes registered from around the same time probably came from the same individual. Therefore, we have groups of observations that came from the same yet unknown user. This information is incorporated into the model, which is why this is a semi-supervised algorithm.

Results: We found that by leveraging this information into the model, we were able to achieve greater accuracy in terms of clustering keystrokes by user as well as simultaneously provide a flexible model for each user found.

Concluding Remarks: A semi-supervised multi-layer finite mixture model is introduced that can cluster the keystroke dynamics of a system by users. Once the keystrokes have been parsed according to user, other methods such as likelihood ratios may be employed to further aid in the digital investigation.

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Digital Forensics; Unsupervised Learning; Clustering

Y74 An Analysis of Human DNA Extraction From Plants in a Mock Burial

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Learning Objectives: This presentation will demonstrate the analysis of human DNA recovery when extracting from plants grown in a mock burial. After attending this presentation, attendees will have a better understanding of the collection process from plants using DNA sample cards, a comparison of different quantitative methods, and the amount of human DNA recovered from plants.

Impact Statement: This presentation will impact the forensic science community by showing results that human DNA recovery is possible from plants in a mock burial. The recovered human DNA that is extracted from the plant's leaves could then be used for developing a profile for cases where no human remains were found.

Abstract Text: Previous studies have shown that the decomposition of a human body in a shallow grave has caused differences in the carbon footprint for the plants above the burial site with higher concentrations of carbon and nitrogen.[1,2] The purpose of this study was to determine whether human DNA can be extracted and quantified from plants grown in blood-infused and human flesh-infused soil. Prior research indicates that human DNA can be extracted from soil, but there are microbial activities in the soil that cause DNA degradation.[3,4] This project focuses on DNA extraction from plants to determine if recovery is possible with less degradation in order to develop a human profile.

In the first phase of the experiment, lima beans were planted individually into soil with either 100uL or 1,000uL of blood, with additional lima beans that were planted in clean soil to serve as control samples. In phase two, lima beans were planted individually into soil with 1,000uL of blood or a piece of human flesh (approximately 4cm x 2cm). Additional lima beans were planted in clean soil to serve as control samples. After growing phase one plants for one month, the leaf samples were collected from the experimental and control plants. The leaves were crushed with the Mini Leaf Crusher onto DNA sample cards prior to DNA extraction and quantitation. Five samples yielded DNA quantities with two having a degradation index above 1.00ng/uL. All five samples were analyzed by the NanoDrop Microvolume Spectrophotometer to compare total DNA concentration to quantity of human DNA. However, no profiles were developed from the five samples using the SeqStudio Genetic Analyzer.

Phase two will follow the same DNA processing methods as phase one. It is hypothesized that during phase two, the plants that grow in the human flesh-infused soil will yield a higher quantity with less degradation. Given the results so far, further research is needed to obtain a higher quantity and quality of human DNA that will be used to develop human profiles from plants in a mock burial.

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DNA; Lima Beans; Quantitation

Y75 Examining the Presence of Foreign DNA on Neck Swabs

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Learning Objectives: This poster will 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 results of genotyped samples to examine the presence of extraneous DNA in the profiles.

Impact Statement: This poster will impact the forensic science community by determining the validity of neck swabs as forensic evidence when strangulation events are suspected. 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.

Abstract Text: 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. A study by Rutty et al. looked into the presence of foreign DNA when not exposed to any direct contact with 24 volunteers and found that 23% of swabs contained non-self DNA.[7]

Reference and neck swabs were obtained from numerous volunteers across three separate visits to a DNA lab. DNA samples were collected using cotton swabs and COPAN FLOQSwabs; these were compared to determine what swabs yield a higher quantity of DNA. 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 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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Forensic DNA; Low DNA; Strangulation

Y76 The Persistence of Human eDNA in Air

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Learning Objectives: This presentation will focus on the ability to obtain human DNA profiles through air filtration of a small enclosure containing human remains. After attending this presentation, attendees will better understand the efficiency of air filtration in the forensic science discipline.

Impact Statement: This presentation addresses the possibility of obtaining genetic profiles from air filtration. This will impact the forensic science community as it provides a tool for cases in which no DNA evidence is seemingly available.

Abstract Text: Environmental DNA (eDNA) is genetic material that organisms shed into their surrounding environment. The eDNA deposited in soil, water, and air has been analyzed to track locations and travel patterns of live animals and determine the presence of species living in an environment. However, this application regarding eDNA obtained from air has not yet been studied in the forensic science discipline. The purpose of this study is to determine how effective air filtration is in obtaining eDNA from human remains stored in contained spaces over time. This study varies from the previously mentioned studies due to the samples being of human remains rather than living organisms that are breathing eDNA into the air. In addition, this study focuses on obtaining a genetic profile to identify an individual rather than the species as a whole.

For the starting trial, the frozen sample was placed into the box for 48 hours. The air was then filtered with a peristaltic filter containing grade 1 Whatman filter paper for two hours to capture any genetic material that was dispersed into the air. The genetic material was then extracted, quantified, amplified, and genotyped. The DNA profiles collected were compared to reference profiles developed from the tissue samples. Further trials will alter the amount of time the samples are left in the box as well as the amount of time air is filtered depending on the results from trial 1.

The ability to generate a genetic profile from air would allow forensic scientists to gain another tool to obtain evidence in cases in which no trace was seemingly left behind, such as the case of Casey Anthony. In this case, a toddler went missing and the main piece of evidence was the trunk of a car. This trunk had no physical evidence present, except a smell that witnesses described as "death." The suspect was not convicted for this crime due to lack of evidence. The ability to analyze DNA from air could provide physical evidence in cases such as this.

Forensic DNA; Human Remains; Air Filtration

Y77 The Development of a Multilocus Sequence Typing Scheme for Characterizing Geographically Distributed *Yarrowia Lipolytica* Strains

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Learning Objectives: From this poster, the attendees will understand the principles of Multilocus Sequence Typing (MLST) and its applications, the importance of MLST in microbial forensics and biotechnology, and the significance of developing an MLST scheme for characterizing *Y. lipolytica* strains.

Impact Statement: The development of an MLST scheme for *Yarrowia lipolytica* has significant implications for the forensic science community. As it relates to microbial forensics, an MLST will allow for strain-level identification, which will be crucial for tracing the sources of *Y. lipolytica* contamination in forensic samples. Developing a centralized forensic MLST database for *Y. lipolytica* will enhance comparative analysis and intelligence sharing. Additionally, there are potential applications in biodefense, where the MLST scheme can be used for *Y. lipolytica* tracking and detection in bioterrorism-related scenarios.

Abstract Text: Yarrowia lipolytica is an ascomycetous, oleaginous, dimorphic yeast that has garnered significant attention as a non-conventional yeast species with broad industrial, biotechnological, medical, and ecological relevance.[1] Its remarkable ability to metabolize a wide range of hydrocarbons has made it particularly noteworthy. However, its genetic diversity and geographical distribution have been relatively understudied.[2] This study aims to fill this gap by developing an MLST scheme for *Yarrowia lipolytica* using seven housekeeping genes. The genetic diversity and population structure of *Yarrowia lipolytica* strains from diverse geographical locations will be characterized. This will involve collecting isolates from various geographical locations, extracting their DNA, and sequencing seven select housekeeping genes, such as Cytochrome c Oxidase Subunit I (COI), Internal Transcribed Spacer (ITS), Cytochrome b (CYTB), ATP Synthase Subunit 6 (ATP6), RNA Polymerase II (RPBI), and Beta-tubulin (TUBB), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using Whole Genome Sequencing (WGS) technologies. The sequence data will then be analyzed using bioinformatics tools to develop the MLST scheme. MLST is a method for characterizing (typing) bacterial or fungal isolates into known groups by identifying their alleles from a set of established housekeeping genes and assigning isolates to "sequence types" accordingly. This takes advantage of the fact that there are thousands of alleles for any given gene that a particular type of organism can possess, and by assigning an allele number and looking at the combinations of the numbers obtained across several genes, one can have unique sequence types.

As a growing prominent organism in the industrial and academic spaces, the genetic diversity and ecology of *Yarrowia lipolytica* must be understood.[3] Hence, the necessity of this study. This project's findings will help improve our understanding of the genetic diversity, population dynamics, and geographical distribution of *Yarrowia lipolytica*, enhance strain selection and optimization for industrial processes, and contribute to developing innovative biotechnological and microbial forensic applications.

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Genetics; Sequencing; Microbial

Y78 Subjective to Objective: An Investigation of Manual and Digital Qualitative Color Change Tools

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Learning Objectives: After this presentation, attendees will have learned about the consistency between visual and digital Munsell tools.

Impact Statement: This research impacts the forensic science community by investigating the consistency of the digital Munsell's color analysis and visual assessment.

Abstract Text: Technology-assisted analyses are expanding in forensic anthropology contexts. For example, the *Munsell Color Book*, originally used to qualitatively evaluate color change on porous (e.g., bone) elements has since been updated as a digital Munsell X-Rite Capsure spectrocolorimeter.[1] This digital form of color analysis employs the Munsell nomenclature and was developed to limit subjectivity used in the distinguishment of color change.[2] Both the book and digital reference tools quantify otherwise qualitative values of hue, value, and chroma that correspond to a specific color represented in Munsell's original book.[2] While most research on the digital Munsell emphasized its use in the archaeological context with photographic imagery, the Munsell Capsure device has practical utility in forensic anthropological contexts and the non-destructive nature of this tool allows it to be used directly on bones.[3] The goal of this study is to investigate the consistency of the digital Munsell device in comparison to visual qualitative inspection across observers.

This study comprised ten private anatomical gift donation crania from Florida Gulf Coast University's Human Identity and Trauma Analysis Laboratory. The discoloration of all crania was evaluated at bregma, or the intersection of the sagittal and coronal sutures, for consistency. The digital spectrocolorimeter's evaluation was then compared to the *Munsell Color Book* student findings to identify if differences in color, hue, value, or chroma existed between the scoring methods. Analyses were considered a match only if the color, hue, value, and chroma were identical. We found that 10% of the results were an overall match of color, hue, value, and chroma; 40% of the color results of the digital Munsell matched the manual results, 50% of the chroma matched; 40% of the hue matched; and 40% of the value matched. Overall, the results indicated the Capsure device was consistent with 10% of the student participants' visual inspection on a 1:1 comparison ratio (such that both analyses were identical). In 60% of the crania that did not identically match, there was a partial match. Ten percent of these cases matched the chroma, 10% matched the hue, and 10% matched the value.

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Munsell Soil Color Analysis; Comparison; Skeletal Remains

Y79 The Application of ATR/FTIR Spectroscopy for Differentiation of Liquid Cosmetic Foundations

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Learning Objectives: After attending this presentation attendees will learn how Attenuated Total Reflectance (ATR) Fourier Transform Infrared (FTIR) spectroscopy can be used to differentiate liquid cosmetic foundations. They will also learn about the impacts of aging on samples on the recorded infrared spectra.

Impact Statement: This presentation will impact the forensic science community by demonstrating class and individual characterization of cosmetic foundations. Cosmetics are worn by the majority of women in the United States as well as many men and thus having tools and approaches to identify and classify cosmetic foundations would be useful to forensic scientists as another point of individualization of crime scene evidence.

Abstract Text: We evaluated the differentiability of silicone and water-based foundations as well as different shades of foundations from several manufacturers and consumer brands using ATR FTIR spectroscopy. We are also examining the effects of aging on the ATR FTIR spectra of silicone and water-based foundations applied to white cotton cloth swatches both with and without sun protection factor (SPF) additives. Evidentiary samples in a criminal case may be collected and analyzed within varying time frames and as such it is important to understand how collected spectra may change over time in order to ensure the proper interpretation of results. Foundations from several different popular makeup brands were examined as well as different shades of foundations (light, medium, dark) within a single product line. An ATR FTIR instrument was used to record the spectra over the range of 400-4000 cm-1 averaged from 64 scans collected at a resolution of 4 cm-1. Air was used as the background when analyzing the foundation directly from the bottle and a clean piece of cotton fabric was used as the background when analyzing the foundations applied to cloth swatches. The cloth swatches were allowed to sit on a shelf in the laboratory under ambient conditions for up to 45 days and IR spectra were collected at specific time intervals. The analysis is being performed using discriminate function analysis (DFA) and several selected IR peaks including O-H stretches.

Cosmetic; ATR/FTIR Spectroscopy; DFA

Y80 The Effect the Distance Between the Sample and Charcoal Strip and the Type of Passive Headspace System Have on Overall Chromatographic Patterns of Ignitable Liquid Residues

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Learning Objectives: Attendees will learn any quantitative effects the type of headspace system has on the chromatographic patterns of Ignitable Liquid Residues (ILRs) from the light to heavy range. Attendees will also learn whether the placement of the sorbent material has any significant effect on the patterns of ILRs.

Impact Statement: This research will aid in the optimization of the extraction of ILRs from fire debris evidence by examining variables that typically are not held constant in real-world applications of these months. The goal of this research is to provide quantitative data on how these variables may or may not have an impact on the chromatographic patterns of ignitable liquids when compared against standard reference materials.

Abstract Text: Fire debris analysis utilizes the volatility of compounds found in ignitable liquids to extract the Volatile Organic Compounds (VOCs) by using a sorbet material to collect the components of the liquid for further extraction. American Society for Testing and Materials (ASTM) E1412 established guidelines for the passive headspace method of extracting VOCs from fire debris samples through the use of activated charcoal strips.[1] While many variables associated with the passive headspace method of extraction such as strip size, time, and temperature have been examined and optimized in studies conducted by Newman et al., there were other variables that had not been held constant in the literature.[2]

This study examined the effect produced by altering the distance between the carbon strip and the sample as well as the type of system in which the passive headspace occurred across the light, medium, and heavy ranges of ignitable liquids. This was accomplished by placing charcoal strips of the same size a distance of 5cm, 7.5cm, and 10cm from the sample in either a nylon arson bag or a paint can. Nylon carpet was used as the matrix and spiked with either gasoline, charcoal lighter fluid, or kerosene to allow for comparison across a wider carbon range. Gas Chromatography/Mass Spectrometry (GC/MS) was used to analyze the fingerprint region of the Total Ion Chromatograms (TICs) for each of the ignitable liquids analyzed. The extent of the distortion observed in the TICs were determined by analyzing the peak ratios of selected target compounds, chosen using the target compounds listed in ASTM E1618, across each of the selected variables followed in the experiment.[3] The data gathered was compared by observing the TICs, the Extracted Ion Chromatograms (EICs), the normalized TICs, and quantitatively comparing the peak ratios between ten selected target compounds in each range of distillates.

With the preliminary data gathered thus far, the ring aromatic profile of gasoline characterized by naphthalene, 2-methylnaphthalene, and 1-methylnaphthalene were much more prominent if the sample was analyzed in a paint can system rather than an arson bag system. The distance from the sample at which the charcoal strip was placed did not seem to have any noticeable effect on either the peak ratios or relative abundance of the compounds in the sample.

Understanding the ways in which specific variables alter the expected patterns of ignitable liquids can help analysts better select a passive headspace system for extraction or help an analyst to better understand differences in the TIC when compared to the standards selected. Optimization of accepted methodologies is significant to providing not only a more complete understanding of the method used to analyze fire debris, but also to provide comprehensive data that encompasses changes in key portions of ignitable liquid TICs while coming from the same source. For future data analysis, learning and recognizing these trends can provide better training data for both recognition of and attribution to a single source of ignitable liquid.

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Fire Debris Analysis; Gasoline; Extraction

Y81 Volatile Organic Compounds (VOC) of Decomposition of Tissues Exposed to Diphenhydramine and Ibuprofen Influence on Fly Attraction

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Learning Objectives: After attending this presentation, attendees will understand variation in attraction of necrophagous flies to decomposing remains with the presence of abused over-the-counter medications and associated changes in VOC profiles.

Impact Statement: This presentation will impact the forensic science community by discussing volatile profiles of decomposing tissues with forensically relevant doses of abused over-the-counter medications on necrophagous fly attraction. This information informs entomologists' roles in establishing forensically relevant timelines with cases involving overdoses of diphenhydramine and ibuprofen.

Abstract Text: Forensic entomology utilizes the presence of flies and other insects to help generate forensically important timelines, such as inferring time of death, neglect, or abuse. Necrophagous flies are attracted to remains through VOCs produced by microbial metabolomics. Changes in VOCs can influence necrophagous fly attraction to or avoidance of remains. Secondarily, drugs can also alter the development of flies consuming these tissues. This, in turn, can affect finding important timelines of a decedent.

Between 2019 and 2020, 10.5% of overdoses included the presence of diphenhydramine and 15% included ibuprofen. In cases where these drugs are present, potential changes in microbial communities may alter VOC profiles, influencing insect attraction, warranting investigation. To do this, 45 grams of beef liver were treated with 0.141g diphenhydramine and 0.024g crushed ibuprofen mixed with 1ml of bovine blood respectively. Diphenhydramine and ibuprofen treatments were placed alongside one control inside vertebrate exclusion cages respectively for 600 accumulated degree hours. Additional samples were enclosed in sanitized jars in the laboratory and allowed to accumulate headspace for two hours. Headspace was then collected through a VOC trap. Sample lids were then replaced with a Wypall lid before being allowed to accumulate for 600 accumulated degree hours before repeating headspace extraction. All samples were processed utilizing a Gas Chromatograph/Mass Spectrometry (GC/MS). Insects were identified to family and by function (i.e., necrophagous, fungivores, etc.) as well as sex and reproductive status (gravid and non-gravid).

Necrophagous flies were generally more attracted to controls than to samples with diphenhydramine. Amines in samples with diphenhydramine increased over time, where the control saw an increase in aromatics, likely driving these differences. Necrophagous flies were generally more attracted to the control than to the samples with ibuprofen. Additionally, gravid females were more attracted to controls, influencing colonizers of remains with ibuprofen. A variety of compounds in the initial sample containing ibuprofen and over time there was an increase in aromatic compounds, acids, and ketones. Volatile profiles between ibuprofen and controls became closer in profile over time, where controls were initially composed of sulfides, increasing aromatics, acids, and ketones over time. Ibuprofen had a variety of compounds initially, increasing in aromatics, acids, and ketones, similarly to the control. Suggesting differences in insect activity may have been due to earlier compounds initiating attraction, rather than in later periods of exposure. This presentation will discuss these variations and influence on casework in forensic entomology.

Entomology; Toxicology; Entomotoxicology

Y82 How Do Muzzle Velocity, Bullet Mass, and Overall Kinetic Energy Influence Skeletal Fracture Initiation and Propagation Patterns?

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Learning Objectives: Attendees will learn about the relationships between velocity, mass, kinetic energy, and various fracture characteristics obtained in a series of gunshot experiments.

Impact Statement: This presentation will impact the forensic science community by providing preliminary baseline data on the presence, quantity, and length of various fracture characteristics and their possible relationships with bullet velocity, mass, and kinetic energy. This data may inform variable selection in future trauma studies.

Abstract Text: In 2022, approximately 79% of homicides and 55% of suicides in the United States involved a firearm.[1] Gunshots frequently impact bone, with skeletal defects in over 90% of gunshot homicides.[2] Research is needed to improve understanding of the variables that influence fracture formation in gunshot trauma.

Bullet kinetic energy affects energy transfer and is therefore an important variable influencing injury production. A recent study found that higher-energy bullets produced more radial cracks, crack branching points, and longer fractures than lower-energy bullets in flat bones.[3] While kinetic energy was primarily increased by increasing velocity, bullet mass also varied across experiments. As such, the individual effects of these variables remained unclear.

The goals of this research were to investigate the influence of bullet mass, velocity, and kinetic energy on experimentally generated gunshot fractures in flat bones.

Thirty-nine bone-in pork shoulders comprising the scapula and approximately 2.5cm of soft tissue were acquired from a local butcher. Gunshots were delivered by an expert shooter from 1.1m with shots targeting the supraspinous fossa. Full metal jacketed 9mm bullets were used in all experiments to standardize caliber and minimize the effects of bullet expansion.

A 9mm pistol and a 9mm carbine were selected as they were expected to produce different muzzle velocities. A chronograph measured bullet velocity for each experiment. Three bullet grains (95gr/6.16g, 124gr/8.04g, and 147gr/9.53g) represented a range of masses. Kinetic energy was calculated as $KE = 1/2mv^2$.

Following soft tissue removal, several fracture characteristics were documented, including: presence of cone cracks, presence and number of radial cracks, crack branching points, and circumferential cracks, minimum and maximum fracture lengths, and Total Fracture Lengths (TFLs). All characteristics were documented on both the entrance and exit side.

Main and interaction effects of mass and velocity on continuous fracture characteristics were evaluated using two-way Analysis of Variance (ANOVA). One-way ANOVA was used to evaluate kinetic energy and location (entrance vs. exit) effects. The Kruskal-Wallis test was used for non-normally distributed variables and the Wilcoxon Signed-Rank test was applied for paired comparisons. Categorical variables were analyzed using Fisher's exact test. Pearson's R was applied to explore correlations between continuous fracture characteristics and velocity and kinetic energy. Spearman's rank correlation was used for mass due to its non-normal distribution.

Measured bullet velocity ranged from 282.25 to 391.67m/s (mean = 337.98±30.02m/s) and kinetic energy ranged from 276.31 to 550.08J (mean = 450.01±59.32J). The results indicate no significant relationships or correlations between velocity, mass, or kinetic energy and any of the investigated fracture characteristics. However, the number of radiating cracks and the presence and number of circumferential cracks and crack branches differed significantly between entrance and exit defects, appearing in higher frequencies at exits. Minimum radial crack length was significantly smaller at exits (5.50±4.53mm) than entrances (9.78±7.32mm), while maximum radial crack length was longer at exits (24.73±23.52mm) than entrances (21.13±9.63 mm). TFL was significantly longer at exits (66.19±34.45mm) than entrances (entry TFL = 41.91±25.23mm).

This study builds on previous research and contributes baseline data on the relationships between bullet velocity, mass, kinetic energy, and fracture morphology.[3] That no significant results were found may suggest that mass and velocity have no effect on fracture patterns within the ranges investigated. A significant negative correlation between mass and velocity ($\rho = -0.78$, $p = 6.13e\text{-}09$) suggests heavier bullets traveled slower, which likely affected results. Future research should investigate more extreme differences in mass and velocity. While not the original research focus, results highlight quantifiable differences in entrance vs. exit defects that should be further investigated in human bone.

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Forensic Anthropology; Trauma Analysis; Gunshot Trauma

PROGRAM COMMITTEE FINANCIAL DISCLOSURE

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I

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K

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Krane, Dan – G15, G23
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Kunkler, Kimberly – Y36
Arrowhead Forensics: Our university received donated equipment.

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N

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S

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V

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W

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Cayman Chemical: Employee / Owner
Williams, Karl – L49
American Kratom Association: Consultant/Advisory Board

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PRESENTING AUTHOR INDEX

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E	Forensic Nursing Science	CB	Case Break Session
F	General	DD	Deep-Dive Session
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